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JUHA HEIJARI

Seed Origin, Forest Fertilization and Chemical Elicitor Influencing Wood Characteristics and Biotic Resistance of Scots Pine

Doctoral dissertation

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Department of Ecology and Environmental Science
University of Kuopio

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ABSTRACT

Scots pine (*Pinus sylvestris*) is the dominant species in Finnish forests and is an important conifer species since it is extensively used by industry. One of the main goals in forest improvement is to increase timber production and forest fertilization has been used quite extensively to enhance stand wood volume growth. The quality of wood is also an important aspect in forestry and different Scots pine origins can be employed to explore the variation in wood characteristics and their biotic resistance. The use of a chemical elicitor represents a novel way to study modification of plant defence and to influence on plant-herbivore interactions.

The main objective of the present study was to gather information from the effects of long-term forest fertilization and the variation attributable to seed origins of the Scots pines on wood secondary chemistry and performance of wood-borer *Hylotrupes bajulus* and wood decaying fungi *Coniophora puteana*. Another aim was to investigate whether defence responses of Scots pine could be induced by exogenous application of a chemical elicitor, methyl jasmonate and what are the possible consequences of this action on the performance of the large pine weevil (*Hylobius abietis*).

The forest fertilization of old Scot pine trees increased wood production and tree volume growth but had no effects on the level of defence compounds in wood or in the extent of destruction induced by wood-boring larva or wood decaying fungi. The young Scots pine trees from different origins exhibited some variability in the levels of juvenile wood terpenoids but this did not explain the damage intensity by wood-boring larva or wood decaying fungi. A low concentration of monoterpenes in wood, especially a low β to α-pinene ratio, seemed to make some seed origins preferable to oviposition of *H. bajulus* females. The exogenous application of a plant elicitor, methyl jasmonate, evoked the induction of chemical and anatomical defence responses in Scots pine trees. It is hypothesized that the increased terpenoid concentration and number of resin ducts in wood contributed to the decreased level of bark gnawing by large pine weevils.

In conclusion, these results increase our knowledge on chemical defence in Scots pine wood and how this impacts on the interactions with insects and fungi. These results may provide a better understanding of how to induce chemical defence to combat herbivore attacks. According to the results, increased timber production achieved by long-term forest fertilization has only negligible effects on wood characteristics and wood biotic resistance. Variation in the wood chemical defence is not a primary explanation for the wood biotic resistance of young Scots pine trees but modification of defence by application of elicitors may increase the resistance of the trees by inducing defence responses.

Universal Decimal Classification: 582.47, 630*81, 631.531, 631.8, 632.4, 632.7, 632.934
CAB Thesaurus: forests; forestry; silviculture; *Pinus sylvestris*; insects; wood borers; wood destroying fungi; *Hylotrupes bajulus*; *Coniophora puteana*; *Hylobius abietis*; pest resistance; wood; decay; wood properties; wood anatomy; wood chemistry; tracheids; xylem; cellulose; seeds; defence mechanisms; elicitors; methyl jasmonate; resin acids; terpenoids; monoterpenes; plant nutrition; nutrients; provenance

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Finally, warmest thanks to my wife Suvi.

Kuopio, May 2006

Juha Heijari

ABBREVIATIONS

C	current year tissue
C+1	second year tissue
CaNP	calcium, nitrogen and phosphorous
CBSC	carbon-based secondary compound
CWCL-ratio	cell wall to cell lumen ratio
GDBH	growth-differentiation balance hypothesis
GLM	general linear model
GRH	growth rate hypothesis
H1	breast height
H2	canopy height
I1L	neonatal larvae
I2L	second instar larvae
I3L	third instar larvae
MJ	methyl jasmonate
MRGR	mean relative growth rate
N	nitrogen
PVH	plant vigour hypothesis
RH	relative humidity

LIST OF ORIGINAL PAPERS

This thesis is based on the following articles which are referred in the text by their Roman numerals I-IV and on unpublished results:

I Nerg A-M, Heijari J, Noldt U, Viitanen H, Vuorinen M, Kainulainen P, Holopainen JK (2004) Significance of wood terpenoids in the resistance of Scots pine provenances against the old house borer, *Hylotrupes bajulus*, and Brown-rot fungus, *Coniophora puteana*. Journal of Chemical Ecology 30: 125-142

II Heijari J, Nerg A-M, Kaakinen S, Vapaavuori E, Raitio H, Levula T, Viitanen H, Holopainen JK, Kainulainen P (2005) Resistance of Scots pine wood to Brown-rot fungi after long-term forest fertilization. Trees 19: 728-734

III Heijari J, Nerg A-M, Kainulainen P, Noldt U, Levula T, Raitio H, Holopainen JK. Wood borer performance on Scots pine xylem in relation to nutrient availability of host plants. Manuscript

IV Heijari J, Nerg A-M, Kainulainen P, Viiri H, Vuorinen M, Holopainen JK (2005) Application of methyl jasmonate reduces growth, but increases chemical defence and resistance against *Hylobius abietis* in Scots pine seedlings. Entomologia Experimentalis et Applicata 115: 117-124

CONTENTS

1 INTRODUCTION

Scots pine (*Pinus sylvestris* L.) is an important tree species in the boreal forests which cover 65 % of the Finnish forest area (Finnish Statistical Yearbook of Forestry 2004). Scots pine is a tree with has a major economical importance, producing timber for sawmilling, the pulp and paper industries and great quantities are used also in house building. In general, the wood of conifers is a very heterogeneous material and with many variations e.g. in tracheid properties, stem form, branchiness, heartwood content, and chemical components. The most important of the chemical components are cellulose, hemicellulose, lignin and extractives (Zobel and van Buijtenen 1989). Silviculture has focused on finding ways to improve tree characteristics, such as growth rate, volume, yield, and timber quality, also taking into account the ecological aspects. However, if one or a few desired wood characteristics are altered by the methods of silviculture, some other characteristic may change in an unfavourable direction e.g. biotic susceptibility of wood may be increased. Conifer resistance against biotic damage is associated with constitutive and inducible defence compounds, i.e. a mixture of mono-, sesqui- and diterpenes in oleoresin (Phillips and Croteau 1999; Trapp and Croteau 2001), phenolics (Rennerfelt and Nacht 1955; Hart and Shrimpton 1979) and also with structural defences e.g. resin canals (Berryman 1988). The defence compounds in conifers have been found to deter insect pests and fungal pathogens (Gershenzon and Croteau 1991; Phillips and Croteau 1999; Larsson et al. 2000; Harju et al. 2003).

In silvicultural practices, provenance experiments have been used to study the possibility to utilize differentially adapted Scots pine seed origins to enhance tree volume growth or to produce wood with specific characteristics, such as tracheid dimensions or percentage of heartwood (Ståhl 1998). However, in those management practices, wood chemical defence and general resistance against biotic damage have received less attention. There is known to be geographical variation in the types and levels of defence chemicals, in particularly mono- and diterpenes, in several *Pinus* species (Zavarin et al. 1993; Tognetti et al. 1997; Manninen et al. 1998; Naydenov et al. 2002). Differences in the chemotype determined from needles, shoots or wood have been related to latitudinal or longitudinal distance between seed origins (Nerg et al. 1994; Tognetti et al. 2000; Naydenov et al. 2005). Provenance experiments conducted with Scots pine have shown that the relative quantity of α-pinene and concentration of α-pinene, β-pinene, limonene and total monoterpenes of shoots and needles are higher in the northern seed origins than in southern seed origins whereas the proportion of high 3-carene trees has decreased in trees originating from more northern sites (Muona et al. 1986; Nerg et al. 1994; Manninen et al. 1998). Differences in biotic damage between Scots pine origins have been found to occur in their susceptibility to fungal diseases (Hanson 1998), their vulnerability to defoliating sawflies (Lyytikäinen 1993) and between specialist and generalist insect herbivores (Manninen et al. 1998). However, the relationship between the wood defence chemicals and the resistance of Scots pine against wood biotic damage is not yet clear.

In forestry, substantial amounts of nutrient supplements have been commonly used to increase wood production. In the 1950's, large-scale nutritional management of forest stands was initiated in Finland encouraged by state forest improvement funds. However, trends in forest improvement changed at the end of 1980's, virtually stopping forest fertilization with the focus changing to management of nutrient imbalances. The need for increased wood production (e.g. for industry and bioenergy) is again increasing the areas of forest being fertilized. The intention is to increase forest stand volume growth by fertilization, mainly with nitrogen which is the growth limiting nutrient. These techniques have been shown to increase wood production of Scots pine and Norway spruce stands in Finland (Saarsalmi and Mälkönen 2001), results confirmed in similar fertilization experiments throughout the Scandinavia (Nilsen 2001; Nohrstedt 2001). Fertilizers consisting one or several other nutrients except nitrogen, such as phosphorous, calcium or potassium has shown minor effects on Scots pine volume growth (Saarsalmi and Mälkönen 2001; Nilsen 2001; Nohrstedt 2001). In general, forest fertilization of Scots pine has improved not only the growth but also altered the characteristics of the wood, e.g. decreased basic density (Mäkinen and Uusvaara 1992), increased annual ring diameter growth (Hirvelä and Hynynen 1990), increased nitrogen and phosphorous concentrations in wood (Finér and Kaunisto 2000) and in other conifers nutrient supplementation has lead to decreased tracheid length (Yang et al. 1988; Mäkinen et al. 2002). In addition, the effects of fertilization on pest resistance (defoliators and bark beetles) and type and levels of defence chemicals in conifers after fertilization have been somewhat contradictory (Björkman et al. 1991; Kainulainen et al. 1996; Kytö et al. 1996, 1998; Anttonen et al. 2002; Herms 2002; Klepzig et al. 2005) indicating that the effects of forest fertilization on Scots pine wood defence chemicals and biotic resistance in general are still poorly understood.

Brown-rot fungus (*Coniophora puteana* Schumacher ex Fries) and old house borer (*Hylotrupes bajulus* L., Coleoptera, Cerambycidae) larvae have been used to test wood preservatives or natural decay resistance of wood as stipulated in the procedures for European standards (European standard EN 113 1996; European standard EN 47 1988). Brown-rot fungus causes structural damage to construction timber in situations where the wood is exposed to high moisture levels (Viitanen 1996). Scots pine heartwood is known to be more resistant to decay than sapwood (Rydell et al. 2005) and defence chemicals e.g. phenolics have been found to play a major role in the decay resistance of trees (Hart and Shrimpton 1979; Harju et al. 2003). Also old house borer larvae appear to avoid heartwood of Scots pine (Holm and Ekbom 1958) and generally the larvae feed on the sapwood of pine and other softwoods e.g. fir and spruce (Robinson and Cannon 1979). The larvae of old house borer feed on the wood from a few to many years (up to 12-years) depending on temperature, wood moisture and nutritive value after which the larvae pupate and the emerging adults mate and the females lay batches of eggs in cracks in the wood (Robinson and Cannon 1979). Old house borer larva feed inside the wood, producing oval tunnels of 6-12 mm diameter size, and thus they cause severe structural damage in the infested wood, especially to construction timber i.e. this species has a low abundance in living forests (Ljungkvist 1983) but is found in old wooden buildings. More information is needed on how silviculture may modify the resistance of Scots pine wood against biotic damage since this will determine the economic value of the wood in the future.

Recent advances in the transgenic modification of wood characteristics have raised questions about the potential application possibilities in tree breeding (Walter et al. 1998; Peña and Séquin 2001; Pasonen et al. 2004; Zhao et al. 2004). Before we embark on genetic engineering to modify terpenoid biosynthesis to increase biotic resistance (Mahmoud and Croteau 2002), the ecological impacts of the increased defence need to be studied (Thaler 1999). One possible way to modify the biosynthesis of terpenoids is to use elicitors, such as jasmonates which are present in plants and act as signalling compounds e.g. released after wounding, herbivore and pathogen attacks and in response to environmental stress situations (Farmer and Ryan 1990; Cheong and Choi 2003). Methyl jasmonate has been found to induce a variety of defence responses in conifers e.g. increase in the levels of needle and wood terpenoids (Martin et al. 2002; Martin et al. 2003), increased phloem phenolic content (Franceschi et al 2002), induction of the anatomical defence responses in xylem (Hudgins et al. 2003; Hudgins et al. 2004), and increased insect (Thaler 1999) and pathogen (Kozlowski et al. 1999) resistance.

There are now many restrictions in the use of insecticides. The limits to the use of permethrin, which was extensively used against the large pine weevil (*Hylobius abietis* L., Coleoptera, Curculionidae) which feed on Scots pine seedlings in the Scandinavia, have created the need for alternatives (e.g. chemical or mechanical) to restrict damage done by adult pine weevils to cultivated conifer seedlings (Petersson et al. 2004). The large pine weevil is a major insect pest and a problem for forest regeneration in wide parts of Europe, where the adult weevils kill conifer seedlings by feeding on the bark (Leather et al. 1999; Wallertz et al. 2005). As the adults emerge, they cause serious damage especially to newly planted pine seedling on forest plantations (Pitkänen et al. 2005; Wainhouse et al. 2004). The large pine weevil adults are generally attracted to host plant volatiles, such as α-pinene and adult females oviposit near to tree stumps of dead or dying coniferous trees which are commonly found in clear-cut locations. The larvae of the large pine weevil develop under the bark, a process which can last for 1-3 years depending on temperature and host quality. Finally, the pupated larva emerge as adults in the late spring or early summer (Nordenhem 1989; Leather et al. 1999). It is possible that plant modification e.g. by using elicitors or genetic engineering to improve biotic resistance or terpenoid production may cause detrimental effects on plant primary metabolism or may influence plant physiology and lead to unanticipated ecological interactions as a consequence of the increased biotic resistance (Thaler 1999; Hristova and Popova 2002).

2 AIMS OF THE STUDY

The aims of this study were to investigate how seed origin, long-term forest fertilization and chemical modification with exogenous elicitor affect the characteristics of Scots pine wood and wood biotic resistance against insects and wood decaying fungi.

Three main topics were addressed: Do different Scots pine seed origins vary in terms of concentration and composition of wood mono- and diterpenes and if this variation can be related to the resistance of the wood against wood destroying old house borer (*Hylotrupes bajulus*) and wood decaying fungi (*Coniophora puteana*)? (I)

What are the effects of long-term forest fertilization on Scots pine growth, wood anatomy and wood primary chemistry, wood mono- and diterpenes and phenolics and resistance against wood destroying old house borer (*Hylotrupes bajulus*) and wood decaying fungi (*Coniophora puteana*) (II, III)?

Does the exogenous chemical elicitor, methyl jasmonate, affect needle-, bark- and wood defence chemistry, plant growth, physiology and resistance against the bark feeding large pine weevil (*Hylobius abietis*) in Scots pine (IV, unpublished results)?

3 MATERIALS AND METHODS

3.1 Experimental design and plant material

The provenance experiment was established by the Finnish Forest Research Institute in spring 1991. Nine Scots pine (*Pinus sylvestris* L.) seed origins ranging from Estonia (lat 58°22') to Northern Finland (lat 67°56') were planted at the Suonenjoki Research Station (lat 62°64') (Figure 1). The differences between the seed origins in wood chemical defence and biotic resistance against wood-borers and decaying fungi was studied (Table 1, I).

Several long-term forest fertilization experiments were established by the Finnish Forest Research Institute in the 1950's to study the effects of forest fertilization on Scots pine. Experimental sites were chosen from Vilppula, Padasjoki and Punkaharju locations (Figure 1) which were fertilized at ten (Punkaharju) or five (Padasjoki and Vilppula) year intervals during the 40-year-long experiment. Non-fertilized control plots and plots fertilized with either nitrogen (N) alone or with calcium, nitrogen and phosphorus (CaNP) in combination were chosen to study the effects on wood characteristics and biotic resistance against wood-borers and decaying fungi (Table 1, II, III).

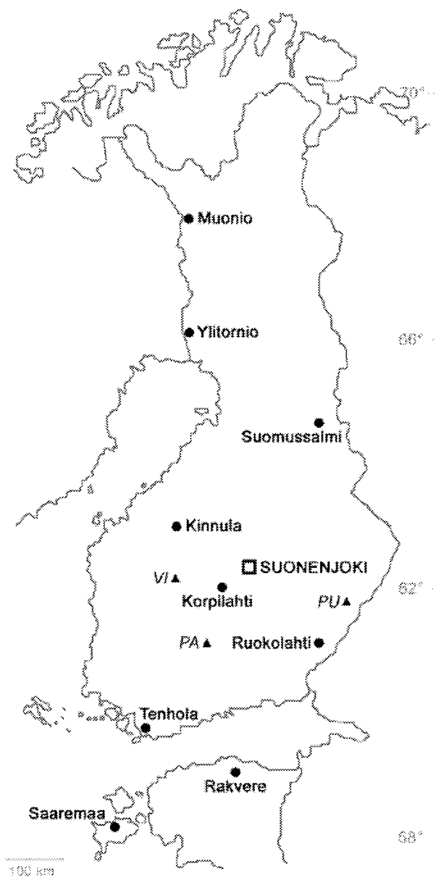

Figure 1. Provenance experiment with Scots pine seed origins (black dots) selected in the study from Estonia and Finland which were planted in the Finnish Forest Research Institute Suonenjoki experimental site (open square) in central Finland (I, unpublished results). Long-term forest fertilization experimental sites (black triangles) of Scots pine: *VI*; Vilppula, *PA*; Padasjoki and *PU*; Punkaharju (II, III).

Table 1 Summary and details of the experiments described in the thesis

Experiment	Plant age (yr)	Analyses/parameters	Plant material	Original publication
Wood chemical defence of Scots pine from different seed origins	7	Wood mono- and diterpenes, performance (MRGR / wood consumption / oviposition) of *H. bajulus* and wood decaying by *C. puteana*	Juvenile wood	I
Long-term forest fertilization of Scots pine	50	Height- and diameter growth, wood primary chemistry, mono- and diterpenes, total phenolics, wood anatomy, performance (MRGR / wood comsumption) of *H. bajulus* and wood decaying by *C. puteana*	Sap- and heartwood, breast- and canopy height	II, III
Modification of Scots pine seedling defence	2	Height- and diameter growth, biomass, physiology, mono- and diterpenes from needles, bark and wood, wood anatomy, performance (gnawed bark area) of *H. abietis*	Needles, bark, wood and roots	IV
Modification of chemical defence of Scots pine from different seed origins	12-14	Height growth and stem diameter, needle total mono- and sesquiterpenes and diterpenes	Needles	Unpublished results

Two different experimental designs were initiated to study the effects of an exogenous plant elicitor, methyl jasmonate (MJ), on Scots pine. In 2003, a field experiment was established in the University of Kuopio Research Garden to study the effects of MJ on chemical defence and biotic resistance of 2-years-old Scots pine seedlings (Table 1, IV). Seedlings were treated once (spraying a volume of 30 ml) in spring with 0 mM (control), 10 mM (total dose 0.067 ml of pure MJ per plant) or 100 mM (total dose 0.67 ml of pure MJ per plant) methyl jasmonate solutions applied with a handheld sprayer.

In 2003-2005, trees from Saaremaa, Korpilahti, Suomussalmi and Muonio (Figure 1) seed origins (same experimental design as in I) were used to study the effects of MJ on plant growth and needle chemical defence (Table 1, unpublished results). From each seed origin three trees from five blocks were randomly selected for MJ treatments with three trees receiving control solution treatments. The MJ treatment solution contained MJ in the concentrations and doses described in Table 2, (v/v) 5% ethanol and 0.1% Tween 20 detergent and distilled water. Control solution contained (v/v) 5% ethanol and 0.1% Tween 20 detergent and distilled water. Each tree received 800 ml of treatment solution according to the timetable described in Table 2 applied with a handheld sprayer. The treatment solution was spread to cover the whole tree including needles and bark of the branches and trunk. Treatments were done two or three times during the growing seasons in the experimental years 2003-2005.

Table 2 Methyl jasmonate (MJ) concentrations, timetable for treatments, total dose of pure MJ per plant and annual sampling dates during the experimental years 2003-2005. Control trees were sprayed with (v/v) 5% ethanol and 0.1% detergent mixed in distilled water

	Year – methyl jasmonate concentration					
	2003 – 3 mM		2004 – 6 mM		2005 – 9 mM	
Treatments	Control	MJ	Control	MJ	Control	MJ
First	5 Jun	4 Jun	8 Jul	7 Jul	14 Jul	12 Jul
Second	21 Jul	17 Jul	7 Sept	3 Sept	16 Aug	18 Aug
Third	22 Aug	21 Aug	nt	nt	nt	nt
Total dose of pure MJ (ml) per plant	*1.6*		*2.2*		*3.2*	
Annual sampling	*29 Sept*		*13 Oct*		*4 Oct*	

nt = not treated.

3.2 Analyses of plant growth, physiology and anatomy

Height and diameter growth of Scots pine trees/seedlings were measured during and at the end of the experiments (II, III, IV, unpublished results). Physiological parameters (net photosynthesis and stomatal conductance) were measured (IV) three times during the growing season with a CI-510 Ultra-light Portable Photosynthesis System (CID Inc., Camas, WA, USA). The anatomical properties of wood, tracheid length (II, III) and tracheid diameter (II), were measured with a light microscope. Tracheids were separated from each other by macerating the wood samples with glacial acetic acid:hydrogen peroxide (1:1, v/v) solution at 60°C overnight. Wood cell wall and cell lumen dimensions from cross-sections (cut with cryo-microtome to thickness of 16 μm stained and mounted on glass slides) were analyzed from photographs with Scion Image 1.0 program (Scion Corporation, Maryland, USA) and PC ImageJ v1.30 (NIH, Bethesda, MD, USA) taken with a digital camera connected to a light microscope (II, IV).

3.3 Chemical analysis

Analysis of mono- and sesquiterpenes (I, II, III, IV, unpublished results), diterpenes (I, II, III, IV, unpublished results) and total phenolics (II) from different plant material, i.e. needles, bark and wood were analyzed in the University of Kuopio. For the mono- and sesquiterpene analyses, samples were extracted with n–hexane. Diterpenes were extracted from freeze–dried and powdered samples with petroleum ether–diethyl ether (Manninen et al. 2002). Mono- and sesquiterpene and diterpene extracts were analyzed with gas chromatography-mass spectrometry (Figure 2). For quantification of mono- and sesquiterpenes and diterpenes, calibrations were made using known amounts of available pure compounds relative to known amounts of the internal standard (1-chloro-octane for monoterpenes and heptadecanoic acid for diterpenes) (Manninen et al. 2002). Total phenolics from heartwood were analysed with the Folin–Ciocalteu technique from the same xylem powder as the diterpenes (II).

Primary chemistry samples were dried (at 60 °C), milled and extracted with acetone for the gravimetric measurement of acetone-soluble extractives and to yield extractive-free samples (II). Alpha-cellulose, hemicellulose, gravimetric lignin and acid-soluble lignin were analysed from the extractive-free samples. Soluble sugars and starch were measured by the anthrone method (II). Nitrogen and carbon concentrations (% from dry weight) from breast- and canopy height milled sapwood samples were determined with the LECO CHN–2000 analyzer at the Finnish Forest Research Institute (III).

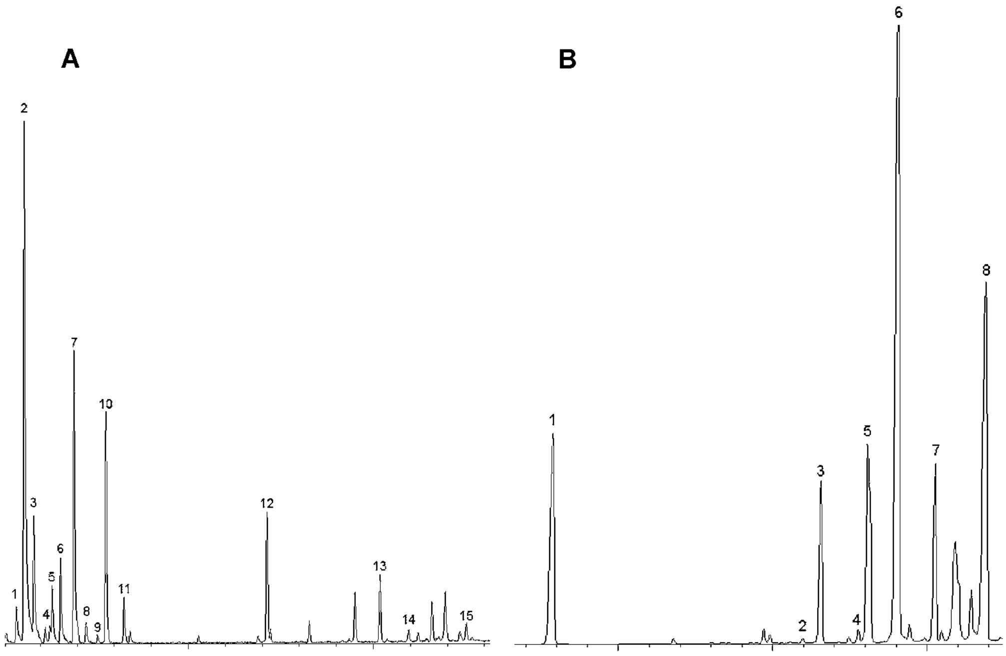

Figure 2. Characteristics of gas chromatography-mass spectrometry chromatograms of needle monoterpenes (A) 1, tricyclene; 2, α-pinene; 3, camphene; 4, sabinene; 5, β-pinene; 6, myrcene; 7, 3-carene; 8, limonene; 9, β-ocimene; 10, 1-chloro-octane (internal standard); 11, α-terpinolene; 12, bornyl acetate; sesquiterpenes 13, β-caryophyllene; 14, α-humulene; 15, δ-cadiene and diterpenes (B) 1, heptadecanoic acid (internal standard); 2, pimaric acid; 3, sandaracopimaric acid; 4, isopimaric acid; 5, palustric + levopimaric acid; 6, dehydroabietic acid; 7, abietic acid; 8, neoabietic acid.

3.4 Performance of insects and decaying fungi

The performance of the old house borer (*Hylotrupes bajulus* L.) larvae was studied with juvenile wood (I) and sapwood of Scots pine (III). Mean relative growth rate for neonatal, second-instar and third-instar size larvae was calculated $[lnW_2 - lnW_1)/(t_1 - t_2)]$, where W_1 and W_2 are the fresh biomass at the beginning (t_1) and at the end (t_2) of the test period (I, III). The amount of wood consumed by second- and third instar larva was determined by filling the empty feeding galleries with fine sand and the weight of the sand corresponded to a certain volume. Oviposition behaviour of *H. bajulus* adults was studied in the experiment with different Scots pine seed origins (I). Adults were allowed to lay eggs under wood disks of six selected Scots pine seed origins (Saaremaa, Tenhola, Korpilahti, Kinnula, Suomussalmi and Muonio) placed on Petri dishes. The number of eggs laid under each wood disk was counted on the following day. Performance (consumed bark area) of the large pine weevil (*Hylobius abietis* L.) was studied in laboratory tests as an indicator of the palatability of Scots pine seedlings modified by exogenous elicitor, methyl jasmonate (IV). Resistance of Scots pine juvenile wood (I), sap- and heartwood (II) against brown-rot fungi (*Coniophora puteana* Schumacher ex Fries) was studied in the VTT Building and Transport Unit using a malt agar plate decay test modified from the standardised EN 113 test (I, II). The wood blocks were placed on a pure culture of a brown-rot fungus and incubated for 6 weeks. The weight loss of the wood blocks during the incubation was expressed in relative mass loss [(loss in mass/initial dry mass) × 100%].

3.5 Statistical analysis

Statistical analyses were conducted with SPSS for Windows statistical software package (SPSS Inc., USA). An independent-samples T-test was used to compare variables within two groups (III, IV, unpublished results). One-way analyses of variance (ANOVA) followed by Tukey's test was used to study the effects of between-subject factors (seed origin, fertilization and methyl jasmonate) on continuous variables (I, II, III, IV, unpublished results). If the parameters were not normally distributed, then Kruskall-Wallis test followed by a Mann-Whitney test was used (I, IV). The general Linear Model, univariate and repeated measure procedures were used in analysis of covariate data (I) and for comparing physiological data (IV), respectively. Factor analysis was used to explain biotic resistance of fertilized wood according to various wood characteristics (II, III). Correlation coefficients were tested by Pearson (II, III, IV) or non-parametric Spearman correlations (I). Chi-Square test was used to determine variables to different categories (III). Hierarchical cluster analysis was used to separate seed origins on the basis of the diterpene concentration in wood (I).

4 RESULTS

4.1 Variation in the wood chemical defence and biotic resistance of Scots pine seed origins

Among the nine Scots pine seed origins, three clearly different clusters based on wood diterpene concentration could be formed; 1) seed origins Muonio and Ylitornio as the low diterpene concentration type, 2) seed origins Saaremaa and Korpilahti as the high diterpene concentration type and 3) other seed origins as the medium diterpene concentration type (I). The wood monoterpene concentration was observed to be highest in the most southern Saaremaa trees and lowest in the Korpilahti trees compared to the trees of other seed origins (I).

Significant differences between seed origins were noted in the juvenile wood decay resistance to *Coniophora puteana*. The wood of Kinnula trees was more susceptible than wood from Saaremaa, Tenhola, Ruokolahti and Suomussalmi seed origins. The wood decay resistance was not associated with the wood diterpene concentration since the more decay resistant seed origins were found in the same diterpene based cluster along with decay susceptible seed origins (I) nonetheless, a few individual seed origins containing high mono- (Rakvere and Kinnula) or diterpene (Saaremaa and Korpilahti) concentrations did correlate negatively with the extent of wood decay. In general, the variation in the wood decay resistance of the different Scots pine seed origins was poorly explained by differences in their mono- and diterpene concentrations.

The Scots pine seed origins did not differ in the performance (mean relative growth rate) of old house borer (*Hylotrupes bajulus*) second- or third instar larvae. The level of mono- or diterpene concentration of wood did not correlated with larval performance (I). In comparison, wood consumption of both larvae instars was higher in one of the low diterpene concentration type (Ylitornio) seed origin than in medium or high diterpene type seed origins (I). The elevated feeding (wood consumption) of second- and third instar larvae was associated with a low total diterpene concentration in wood and a low relative amount of abietic acid and a high relative amount of palustric + levopimaric acid, which account for approx. 42% and 37% of total diterpenes in wood, respectively.

Oviposition (number of eggs laid) behaviour of female *H. bajulus* adults was significantly different between the six selected Scots pine seed origins (I). The most preferred wood for oviposition was the northernmost Muonio seed origin. In general, a high number of eggs was associated with low total monoterpene and β-pinene concentrations and low β to α-pinene ratio in the wood (I). The diterpene concentration of wood did not explain the oviposition behaviour of adult *H. bajulus* females (I).

4.2 Effects of forest fertilization on Scots pine wood characteristics and biotic resistance

The 40-year-long forest fertilization with N or CaNP increased the total volume growth of Scots pine in the two of the studied sites, Vilppula and Punkaharju (Turtola et al. 2002). The increase in the wood volume growth was somewhat higher in the nitrogen fertilized plots than in CaNP plots at Vilppula but these were not duplicated in Punkaharju. However, the extent of the significant increase in the diameter growth of Vilppula trees was observed to be similar with the both fertilization treatments (II).

Forest fertilization in order to increase the growth of Scots pine had only subtle influence on sapwood chemical characteristics, sapwood decay as well as on the performance (mean relative growth rate and wood consumption) of wood-boring *H. bajulus* neonatal-, second- and third instar larvae (II, III). At the breast height of Vilppula trees, both N and CaNP fertilization treatments had an impact on wood anatomy mainly increasing early- and latewood cell wall size in the heartwood boundary (II). Also an increase in the sapwood nitrogen concentration was observed in the fertilized trees of Padasjoki and Vilppula trees (III). The only observed effect from the analyzed parameters of canopy height sapwood was an increase in the nitrogen concentration of N-fertilized trees at the Vilppula site (III). Cell wall chemical composition (e.g. cellulose, hemicellulose, and lignin) varied between trees, treatments and sites, revealing no consistent pattern in allocation of resources to wood primary chemistry (II).

The constitutive chemical defence level (mono- and diterpenes) of breast height sap- and heartwood after long-term forest fertilization was unchanged, but a significantly lower concentration of total phenolics was found in the Padasjoki trees compared to trees at the other sites (Turtola et al. 2002). Furthermore, the heartwood total phenolic concentration was observed to correlate negatively with outer heartwood decay in the Padasjoki and Vilppula trees but not in the Punkaharju trees (II). Factor analysis indicated that total phenolic concentration and to some extent the concentration of the diterpenes negatively affected heartwood decay by *Coniophora puteana* (II) but in the sapwood, the levels of mono- or diterpenes did not explain wood decay. Furthermore, none of the other sap- or heartwood properties examined had any influence on the extent of wood decay.

None of the sapwood properties had any influence on the performance (mean relative growth rate or wood consumption) of the *Hylotrupes bajulus* larvae but when individually examined, the total monoterpene concentration of sapwood exhibited a negative correlation with the neonatal *H. bajulus* larval growth rate (III). Tree stem diameter was observed to correlate positively with the *H. bajulus* second instar larvae growth rate in the Vilppula and Punkaharju trees (III). Also tracheid length correlated positively with the growth rate of *H. bajulus* second instar larvae and wood consumption of third instar larvae (III).

Significant differences were observed in the wood properties and resistance within trees; nitrogen concentration and neonatal larvae survival were higher in canopy- than breast height wood, whereas older instar larvae performed better on the breast height wood (III). In addition, the small diameter trees in Punkaharju had a significantly higher diterpene concentration in their wood, longer and thinner tracheids and higher wood-borer consumption compared to the larger diameter Vilppula and Padasjoki trees (II, III). In comparison, the sapwood of Vilppula trees exhibited less wood decay than Padasjoki and Punkaharju trees (II).

4.3 Effects of exogenous plant elicitor on Scots pine defence responses and biotic resistance

Application of Scots pine seedlings (2-years-old) with plant exogenous elicitor, methyl jasmonate (MJ), affected strongly the growth, physiology, wood anatomy and mono- and diterpene concentrations and herbivore performance (IV). Annual height growth was suppressed after two weeks of both elicitor treatments and continued to be lower the whole growing season (IV). The higher (100 mM) MJ treatment also suppressed seedling diameter growth, wood cell lumen area and in particular, increased the number of wood axial resin ducts. The increase in the wood mono- and wood and needle diterpene concentration was very notable (IV). Net photosynthesis decreased in the needles of the seedlings at the higher level MJ treatment towards the end of the growing season but stomatal conductance was unaffected (IV). The bark area gnawed by the large pine weevil (*Hylobius abietis*) adults was significantly smaller in the higher MJ treatment Scots pine seedlings than in the control and lower-concentration MJ-treated seedlings. Furthermore, *H. abietis* gnawing possibly increased the diterpene concentration in the bark and decreased the level in the wood.

The spraying of 12-years-old Scots pine trees from different seed origins in 2003 with MJ solution led to a significant ($P<0.05$) increase in the concentration of total mono- and sesquiterpenes in the needles from the seed origins nearest to the experimental site (Table 3, unpublished results). The differences in total mono- and sesquiterpene concentration between control and elicitor treated needles in Korpilahti and Suomussalmi were 18% and 15%, respectively. In comparison, the concentration of total diterpenes in the needles of different seed origins did not change after elicitor treatments in 2003. However, when trees of Korpilahti and Suomussalmi seed origins were examined in more detail, the needle concentration of two monoterpenes, β-pinene and limonene were increased by approx. 2.7- and 2.8-fold in the MJ treated trees compared to control trees.

The application of a higher (6 mM) MJ solution in 2004 had a clearer influence on needle total mono- and sesquiterpenes than the lower (3 mM) concentration (Table 3). The increase in the total mono- and sesquiterpene concentration in needles of Saaremaa and Suomussalmi seed origins was 25% and 29% compared to control, respectively. In most seed origins, the increase in two individual needle monoterpenes, β-pinene and limonene was 2- to 4-fold compared to the corresponding levels in the control trees.

When the different seed origins were examined the highest concentration of total mono- and sesquiterpenes and diterpenes was found in the Muonio and the lowest in the most southern Saaremaa seed origin. If all seed origins were combined, the total mono- and sesquiterpene concentration in the needles did not significantly differ between treatments in 2003 but in 2004, the total mono- and sesquiterpene concentration was significantly (15%) higher in elicitor treated needles than in control needles. Furthermore, the total needle mono- and sesquiterpene concentration in 2004 was 24% and 32% higher in control and elicitior treated needles, compared to the levels measured in 2003. If all seed origins were combined, diterpene concentration of needles in 2003 was 38% higher in the elicitor treated needles than in control needles.

Both MJ treatments, i.e. 3 mM and 6 mM applied to the above ground plant parts had no effects on the plant height growth in the first or second experimental year but a significant growth decrease occured after application of 9 mM MJ in 2005 (Table 3). In general, the tree height and diameter were significantly ($P<0.05$) lowest in Muonio seed origin and highest in the Korpilahti seed origin.

Table 3 Significant increases (↑), decreases (↓) or non-significant (ns) effects of an exogenous methyl jasmonate treatments on total needle mono- and sesquiterpene and diterpene (resin acid) concentrations in 2003 and 2004 and total diterpene concentration in 2003, annual height growth and stem diameter growth at 1.3 m compared to control of Scots pine seed origins (unpublished data)

		Seed origin			
		Saaremaa	Korpilahti	Suomussalmi	Muonio
Parameter		*2003*			
Total mono and sesquiterpenes		ns	↑	↑	ns
Total diterpenes		ns	ns	ns	ns
		2004			
Total mono- and sesquiterpenes		↑	ns	↑	ns
Height growth					
	2003	ns	ns	ns	ns
	2004	ns	ns	ns	ns
	2005	↓	ns	ns	ns
Diameter growth at 1.3m	*2005*	↓	ns	ns	ns

5 DISCUSSION

5.1 Wood chemical defence and biotic resistance of Scots pine seed origins

Some variability in the wood defence chemicals was found between Scots pine seed origins. The total wood mono- and diterpene concentration was lowest in the Korpilahti and Muonio seed origins, respectively and highest in the wood of most southern Saaremaa seed origin.

These differences in defence chemical profile of seed origins did not explain their resistance against wood-boring insect *Hylotrupes bajulus* and wood decaying fungi *Coniophora puteana*. The insignificant effect of diterpenes on decay induced by *C. puteana* was in accordance with the previous studies with Venäläinen et al. (2003). In addition, total monoterpene concentration in the wood cannot be used to distinguish which seed origins will be susceptible or resistant to biotic damage. The oviposition behaviour of adult *H. bajulus* females was oriented towards those seed origins with a low total monoterpene concentration in the wood whereas the oviposition behaviour was not associated with the diterpene concentration of wood. Individual monoterpenes, especially α-, β-pinene and limonene, are known to attract *H. bajulus* females and to affect the selection of the oviposition site (Mares et al. 1986; Fettköther et al. 2000) which implies that single monoterpene-related odours or their ratios might be important in the oviposition behaviour of *H. bajulus* females.

The degree of wood destruction assessed by the consumed wood by *H. bajulus* larvae was dependent on relative amount of certain wood diterpenes (low relative amount of abietic acid or high relative amount of palustric+levopimaric acid). The negative effect of the relative amounts of abietic acid and positive effect of palustric+levopimaric acid on wood consumption of *H. bajulus* larvae indicate that analysis of the relative amounts of these diterpenes might be useful in assessing the susceptibility of Scots pine wood to this species. It should be noted that the wood of 7-year-old Scots pine is juvenile wood which differs in its characteristics (e.g. tracheid length, basic density) from the more mature wood properties (Zobel and van Buijtenen 1989) and this may influence wood-borer performance and wood decaying fungi. Furthermore, wood from different seed origins may contain different nutrient, protein, cellulose or lignin contents that can also affect wood biotic resistance (Graf et al. 1989; Hanks 1999; Harju et al. 2001)

Previous studies have shown Scots pine seed origins to differ in needle pathogen and defoliating insect susceptibilities between southern and northern seed origins (Lyytikäinen 1993; Hansson 1998; Manninen et al. 1998). However, the susceptibility of wood to biotic destruction by wood-borers and wood decaying fungi has not been previously studied from different Scots pine seed origins. This is important since insects and decay can have a major impact on the economic value of the wood.

The present results imply that there are variations in the susceptibility of Scots pine juvenile wood from different sources to the destructive impact of biotic factors (*H. bajulus* and *C. puteana*) but the resistance is not clearly linked to the levels of mono- and diterpenes in the wood.

5.2 Scots pine wood characteristics and biotic resistance after forest fertilization

The repeated forest fertilization (N dose of 82-180 kg ha^{-1}) of Scots pine stands increased stem wood total volume growth (Turtola et al. 2002) and had some significant but also inconsistent effects on wood characteristics and wood resistance of the Scots pines (II, III). An increase in volume growth was achieved at the Vilppula site and a slight volume growth also in the Punkaharju site. In previous studies, repeated fertilizations (N dose of 120 kg ha^{-1}) at intervals of 6 yrs have increased Scots pine volume growth by approx. 30–34 m^3 ha^{-1} over 18 yrs (Saarsalmi and Mälkönen 2001). The effect of the 40-year-long N- and CaNP fertilizations was somewhat higher in Vilppula trees and relatively smaller in Punkaharju trees than in the study of Saarsalmi and Mälkönen (2001). The fertilization interval in the Punkaharju site might have affected the growth response but the non-significant changes in Padasjoki site might be associated with already high growth potential at that site.

Scots pine heartwood is known to be more durable than sapwood against brown-rot fungus (Venäläinen et al. 2003, II). The durability of heartwood, especially outer heartwood, is mainly attributable to the phenolic compounds (e.g. stilbenes) that are the best, but not the only factor, explaining differences in wood decaying within the stem (Venäläinen et al. 2003, 2004). Wood diterpenes are known to have only a minor effect on heartwood decay (Harju et al. 2003). In this study the negative effect of wood diterpenes was found to be concentration dependent, however in the previous studies trees with a high diterpene concentration were found to produce also high concentrations of other defence chemicals e.g. phenolics (Venäläinen et al. 2003). In comparison, the wood mono- and diterpene concentration in sapwood was observed to exhibit no correlation with wood decay, although in some cases some evidence for a positive correlation with diterpenes has been found (Venäläinen et al. 2003). In term of the analyzed wood characteristics (nitrogen and carbon, primary chemistry, secondary compounds and anatomy), it seemed that the total phenolic (and to some extent also diterpene) concentration was the best trait to explain wood decay in the heartwood. None of those wood characteristics explained sapwood decay although it has to be remembered that sapwood is devoid of phenolics (Venäläinen et al. 2003). These results indicate that the outer heartwood of Scots pine is most durable against decay followed by inner heartwood and sapwood is the least durable. The difference in amounts of phenolics between outer- and inner heartwood may be one significant factor accounting for heartwood decay (Venäläinen et al. 2003). In general, the degree of decay seems to be affected by the levels of diterpenes and phenolics present in the wood but long-term forest fertilization does not have any major impact on wood decay susceptibility of Scots pine sap- and heartwood.

The sapwood of pine, spruce and fir is the food source for wood-boring larvae, old house borer *Hylotrupes bajulus*. As the name "old house borer" indicates, *H. bajulus* larvae are found commonly in old wooden buildings. The importance of sapwood terpenoids in the resistance of wood against feeding of *H. bajulus* was negligible as it was in the case of sapwood decay by brown-rot fungi. However, monoterpenes exhibited a weak negative correlation with neonatal larvae growth rate. In comparison, the monoterpene concentration of the juvenile wood of Scots pine seed origins showed a slight positive correlation with the second- and third *H. bajulus* larval instars pointing to differences in the performance between larval developmental stages. Thus, this complicated response of various larval stages to wood terpenoids needs to be noted when assessing wood biotic resistance against *H. bajulus*. Furthermore, it was observed that the performance of neonatal larvae was better in the canopy- than breast height sapwood which might be linked to its higher sapwood nitrogen concentration (Mattson 1980; Holopainen et al. 1995). In general, these results imply that the sum of many wood characteristics influence wood-borer performance.

The constitutive diterpene concentration of Scots pine sapwood at the time of tree felling was lowest in the trees with large diameters and highest in the small diameter trees. This is in accordance with the growth rate hypothesis (GRH) and plant vigour hypothesis (PVH) as proposed by Coley et al. (1985) and Price (1991). Basically, these hypotheses suggest that as the plant growth rate (vigour) increases, the defence level decreases thus affecting positively on herbivore performance. Furthermore, in this study, stem diameter in general showed a positive correlation with the performance of late instar larvae in agreement with the GRH and PVH hypotheses. However, the contradictory results between tree diameter and neonatal larval growth rate which is generally considered to be sensitive suggests that those hypotheses may not be very specific ways to assess the relationship between tree growth and wood-borer performance on 50-years-old Scots pine trees though they might be more suitable for fast-growing deciduous trees (Coley et al. 1985; Price 1991).

In addition, the above hypotheses were not clearly supported within the studied sites, since no trade-off between growth and defence was observed in the fertilized trees. It might be that fertilization increased factors other than stem growth e.g. growth of needles, branches or roots and ground vegetation as well as nutrient leaching may have been one explanation in negligible stem growth and defence chemical responses (Saarsalmi and Mälkönen 2001). Therefore, differences in the defence chemicals of wood between the studied sites appears to be more related to differences in seed origins than to forest fertilization treatments. Additionally, the wood consumption of wood-boring *H. bajulus* was higher in the small diameter trees in Punkaharju compared to the large diameter trees in Vilppula and Padasjoki. One explanation for the different wood consumption by the larvae might be the higher wood terpenoid concentration which meant that the larvae had to consume larger amounts to provide them with the energy needed to allow them to undertake detoxifying reactions. Another reason might be the tracheid properties, i.e. wood with long and thin tracheids, as found in the Punkaharju trees was more consumed than wood with short and wide tracheids at the other sites, although the fertilization treatments per se did not affect tracheid properties. Thirdly, it has been proposed that living sapwood is not attacked by *H. bajulus* larvae because of its high content of non-structural carbohydrates (e.g. starch) and buildings are only infested some years after their construction (Höll et al. 2002). In this study, the levels of structural- (cellulose and hemicellulose) and non-structural carbohydrates (starch and soluble sugars) appeared to have no influence on the performance of *H. bajulus* larvae. However, the non-structural carbohydrates may have had impact on generally negative growth rate of second- and third instar larvae or it may be that the second- and third instar larvae possess such large fat storages that they do not need to consume the wood (Berry 1972). In addition, terpenoids were not analyzed at the end of the experiment from the air-dried sapwood, which may have influenced our assesment of the terpenoid concentration and how this level affects wood-borer performance.

In summary, the constitutive defence level (terpenoids and phenolics) as well as the primary chemistry and tracheid length of fertilized Scots pine trees do not seem to change even if stands have been managed for very long periods. Therefore forest fertilization does not alter biotic resistance against wood-boring *H. bajulus* larvae and wood decaying fungi *C. puteana* and thus is not a major threat to the quality of construction timber made from Scots pine.

5.3 Elicitor-induced defence responses in Scots pine

Application of exogenous elicitor methyl jasmonate (MJ) at high concentrations (100 mM) caused a significant decrease in annual height and diameter growth of Scots pine seedlings (IV). The elicitor evoked mono- and diterpene production turning these processes into allocation priorities in Scots pine needles and wood. An increase in the number of wood axial resin ducts was an apparent symptom of MJ treatment. The need to store the increased levels of mono- and diterpenoids in wood (Martin et al. 2002) might have been one reason for the increased resin duct number. In other studies, increases in foliage, stem and root terpenoids and induction of anatomical defence responses have been also observed in conifers after MJ treatments (Hudgins et al. 2003; Huber et al. 2005). In comparison, the Scots pine bark terpenoid defences were not changed by methyl jasmonate treatment as observed also with Norway spruce (Martin et al. 2002), suggesting that in bark, other defence pathways (e.g. production of phenolics) may be induced in response to methyl jasmonate (Hudgins et al. 2004).

The induction of Scots pine terpenoids after the exogenous elicitor treatment led to an increase in the resistance against bark gnawing *Hylobius abietis* adults at the highest MJ concentration. When the *H. abietis* weevils wounded the seedling stem, resin probably deterred further infestation by flushing and sealing the gnawed site. However, the feeding trial was carried out in the laboratory which may not duplicate all of the conditions present in the natural environments e.g. in tree plantations. Furthermore, it may also be that the low elongation growth after high MJ application may decrease seedling vitality and survival which may mean that the adoption of this method may be problematic. Proper timing of elicitor treatment may be crucial. However, these results do indicate that chemical and anatomical defence responses are induced in the Scots pine seedlings and these may increase the resistance of the plant against bark gnawing large pine weevil *H. abietis* damage. The changes in the needle and wood terpenoids might also increase resistance against defoliating sawflies (Larsson et al. 2000), bark beetles or bark-beetle associated fungus (Zeneli et al. 2006), but this needs to be confirmed in future studies.

In the 12-year-old Scots pine trees, rather low (3 mM) methyl jasmonate treatment (total dose of 1.6 ml of pure MJ per plant) evoked an increase in the total concentrations of mono- and sesquiterpenes in the needles of Scots pine seed origins from the vicinity of the experimental site. In the following year, a higher elicitor concentration (total dose of 2.2 ml of pure MJ per plant) increased concentration of needle total mono- and sesquiterpenes in the most southern Saaremaa and Suomussalmi seed origins. Interestingly, diterpene production was increased in the needles after 100 mM methyl jasmonate treatment whereas several (3 mM) applications increased only needle total mono- and sesquiterpenes concentration. However, the total diterpene concentration was not analyzed from the year 2004 needles or from wood where the diterpene levels may be even better induced after MJ treatments. In general, high MJ concentrations per plant have evoked significantly stronger responses (even needle and shoot browning) than seen with lower levels as found also with *Picea abies* (Martin et al. 2002). It seems that the effect of MJ on growth and needle and wood terpenoid chemistry is associated with the applied elicitor concentration and that the MJ dosage needs to be related to the plant biomass. Furthermore, the effect of a high (100 mM) concentration of the elicitor was pronounced during the annual height growth spurt in spring, but elicitor application (3, 6 and 9 mM) during the three consecutive years may have lead to some cumulative effects on the plant height and diameter growth as well as on terpenoid chemistry.

Changes in the growth and needle defence chemicals were noted between the different Scots pine seed origins. The results imply that some seed origins are able to produce increased amounts of needle mono- and sesquiterpenes after stimulation by a chemical elicitor. Furthermore, the MJ treatments were applied in a way that the sprayed compound mist fell down to ground which may have affected root growth, terpenoids and anatomical defence responses (Huber et al. 2005). It remains to be determined how Scots pine wood defence responses (chemical and anatomical) of different seed origins respond to modification with an exogenous elicitor and what are the impacts of this treatment on the biotic damage caused by insects (e.g. bark beetles, wood-borers) and fungi. The assumption is that chemical and anatomical defence responses will be induced in wood, leading to increased biotic resistance. However, the induction of these defence responses may decrease wood production by reducing height- and diameter growth in compensation for the elevated defence capabilities. This was observed in the trees of the most southern Saaremaa seed origin (Table 3). In addition, the positive response on growth achieved as the Scots pine seed origin are transferred southwards (Ståhl 1998) may be diminished by exogenous elicitor treatments.

In general, the results of the chemical elicitor, methyl jasmonate, on growth and defence responses applied in the field with seedlings and young trees are important in evaluating the function of induced defence in Scots pine and the possible use of this compound to increase the resistance of growing trees against insects and diseases. The use of plant chemical elicitors in order to induce terpenoid production and biotic resistance represents a significantly lower risk than that posed by genetic engineering where the impacts of the transgenic plants on ecological interactions are virtually unexplored.

6 CONCLUSIONS

Variation was found in the juvenile wood chemical defence and decay resistance between nine Scots pine (*Pinus sylvestris*) seed origins, but the mono- and diterpene concentrations present in the wood did not explain the extent of wood destruction by wood-boring larva (*Hylotrupes bajulus*) and wood decaying fungi (*Coniophora puteana*). A low monoterpene concentration, especially a low β to α-pinene ratio seems to make wood preferable for oviposition of *H. bajulus* females.

The long-term forest fertilization of Scots pine trees increased stand wood volume growth and individual tree volume growth, depending on the site. The forest fertilization treatments had negligible effects on wood characteristics and damage by wood-boring larva and wood decaying fungi. In general, an increase in wood volume growth of Scots pine may be achieved by forest fertilization, with no change in the wood characteristics or its resistance against biotic factors (*Hylotrupes bajulus* and *Coniophora puteana*). Thus forest fertilization is not a threat to the quality of Scots pine construction timber.

The application of Scots pine trees with an exogenous elicitor, methyl jasmonate, evoked the induction of chemical defence responses in trees of different ages. The increased amounts of mono- and diterpenes were stored in a higher number of resin ducts in wood which contributed to the decrease in the damage caused by the bark gnawing large pine weevil (*Hylobius abietis*). Defence chemicals were induced in the foliage which may increase Scots pine resistance against sawflies and pathogens, but the balance between primary- and secondary production and timing of elicitor applications will need to be refined in the management practices. Further studies are needed to provide more comprehensive knowledge of the effects of methyl jasmonate on the trade-off between growth and defence (e.g. terpenoids, phenolics) as well as its impacts on plant-herbivore interactions in natural environments.

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