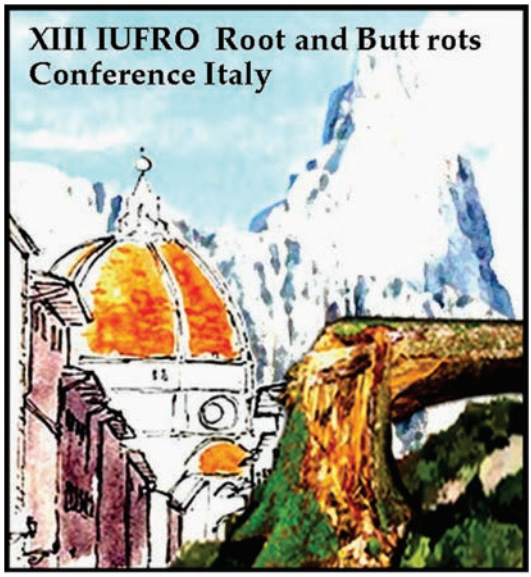
Proceedings e report

93



XIII Conference
"Root and Butt Rot of Forest Trees"
IUFRO Working Party 7.02.01

September 4th – 10th 2011
Firenze – Auditorium di S. Apollonia
S. Martino di Castrozza (TN) – Palazzo Sass Maor, Italy

XIII Conference Root and Butt Rot of Forest Trees
IUFRO Working Party 7.02.01 / edited by P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini. –
Firenze : Firenze University Press, 2013.
(Proceedings e report; 93)

http://digital.casalini.it/9788866553533

ISBN 978-88-6655-352-6 (print)
ISBN 978-88-6655-353-3 (online)

Progetto grafico di Alberto Pizarro Fernández, Pagina Maestra snc

This book was printed with contribution of Fondazione Edmund Mach and Italian Ministry of Education, University and Research, PRIN program number 2008SBCC9S
Cover photos and photos on pages 1, 65, 77, 115, 141, 173, 233 by Kari Korhonen

Università degli Studi di Firenze
Firenze University Press
Borgo Albizi, 28, 50122 Firenze, Italy
http://www.fupress.com/
Printed in Italy

Edited by

Paolo Capretti, Cecilia Comparini, Matteo Garbelotto, Nicola La Porta, Alberto Santini.

Paolo Capretti
Università di Firenze
Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente
Piazzale delle Cascine 28, 50144 Firenze, Italy
paolo.capretti@unifi.it

Cecilia Comparini
Università di Firenze
Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente
Piazzale delle Cascine 28, 50144 Firenze, Italy
cecilia.comparini@unifi.it

Matteo Garbelotto
University of California, Berkeley
Department of Environmental Science, Police and Management – Ecosystem Science
137 Mulford Hall, 3114, Berkeley, CA, USA, 94720
matteog@berkeley.edu

Nicola La Porta
IASMA Centro per la Ricerca e l'Innovazione, Fondazione Edmund Mach
Dipartimento Agrosistemi Sostenibili e Biorisorse
Via E. Mach 1, 38010, S. Michele all'Adige (TN), Italy
nicola.laporta@iasma.it

Alberto Santini
Consiglio Nazionale delle Ricerche – CNR – Istituto per la Protezione delle Piante
Via Madonna del Piano 10, 50019, Sesto Fiorentino (FI), Italy
a.santini@ipp.cnr.it

To cite this book:

Capretti P., Comparini C., Garbelotto M., La Porta N., Santini A., (eds.). Proceeding of the XIII International Conference on Root and Butt Root of Forest Trees. Firenze (FI) – S. Martino di Castrozza (TN), Italy, 4th – 10th September 2012. University Press, Firenze, 2012. 280 pp.

Organizing Committee

Paolo Capretti
Università di Firenze
Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente
Piazzale delle Cascine 28, 50144 Firenze, Italy
paolo.capretti@unifi.it

Cecilia Comparini
Università di Firenze
Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente
Piazzale delle Cascine 28, 50144 Firenze, Italy
cecilia.comparini@unifi.it

Alberto Santini
Consiglio Nazionale delle Ricerche – CNR
Istituto per la Protezione delle Piante
Via Madonna del Piano 10, 50019, Sesto Fiorentino (FI), Italy
a.santini@ipp.cnr.it

Nicola La Porta
IASMA Centro per la Ricerca e l'Innovazione, Fondazione Edmund Mach
Dipartimento Agrosistemi Sostenibili e Biorisorse
Via E. Mach 1, 38010, S. Michele all'Adige (TN), Italy
nicola.laporta@fmach.it

Matteo Garbelotto
University of California, Berkeley
Department of Environmental Science, Police and Management – Ecosystem Science
137 Mulford Hall, 3114, Berkeley, CA, USA, 94720
matteog@berkeley.edu

Scientific committee

Paolo Capretti
Matteo Garbelotto
Nicola La Porta
Alberto Santini

Technical Committee

Università di Firenze
Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente
Piazzale delle Cascine 28, 50144, Firenze, Italy

Matteo Feducci
Beatrice Ginetti
Elisa Locandro
Duccio Migliorini
Alessia Pepori

The XIII Conference "Root and Butt Rot of Forest Trees"
IUFRO Working Party 7.02.01, Firenze – Auditorium di S. Apollonia
S. Martino di Castrozza (TN) – Palazzo Sass Maor, Italy
was kindly supported by

Table of contents

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Preface

In accordance with recent custom established in the IUFRO WP 07.02.01, the 13th International Conference on Root and But Rot was held as an itinerary conference in Italy from September 4 to 10, 2011.
The conference was attended by eighty-five pathologists, mycologists and biologists from nineteen countries. The participants first attended a pre-conference tour to Castel Fusano (the estate of the President of the Italian Republic in Rome) where many *Pinus pinea* trees have been damaged by *Heterobasidion irregulare*. Then they moved to Florence and, after that, to the Trentino Administrative Region.

The conference consisted of three days of scientific presentations in Florence, where the conference participants also had an opportunity to visit several cultural sites in the city. The participants then travelled to the Trentino, passing through conifer woods, Alpine villages and the magnificent landscape of the Dolomites on the way. In San Michele all'Adige (Trento), where the main building of the Fondazione Edmund Mach is located, a scientific session was held. Finally the conference reached San Martino di Castrozza, for two days of scientific sessions.
A field trip to the Natural Park of Paneveggio, Pale di S. Martino (Dolomites) offered a spectacular view of the Val Venegia and of the forest of violins "Stradivari wood". The conference participants visited some conifer forests and saw some areas where *Heterobasidion* and *Armillaria* infection was widespread. It appeared that traditional silvicultural practices relying on natural regeneration are more likely to lower the spread of the fungi causing root and but rot than are the intensive practices common today.

The proceedings were organized under seven headings: Genomics and plant-pathogen interactions; Systematics, taxonomy and phylogeography; Ecology; Population genetics; Etiology and epidemiology; Disease management and control; New reports, diagnostics and research on the application of new diagnostic methods.

Following the custom of the last Conferences of IUFRO WP 07.02.01, most presentations dealt with the root rot caused by *Heterobasidion annosum*, the main agent of root rot in Europe. Molecular studies on the *H. annosum* species complex, its pathogenicity, the host resistance response, the production of secondary metabolites by various host species, and the use of biochemical markers in selection and breeding for resistance, were the subject of extensive discussion.

Participants also directed their attention to other, related topics, such as the new species of *Heterobasidion* that have been found in Europe, and their relationship with other fungal pathogens. and they discussed climate change.

We should like to express our gratitude to all contributors, who made the conference a success and who created a friendly atmosphere. The organizing committee also warmly thanks all the people and the institutions involved in the organization of the conference.

Paolo Capretti
Conference organizer

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Memories from XIII IUFRO Working Party

XIII IUFRO-RBR group picture 6 september 2011, Piazza della Signoria, Firenze, Italy

XIII IUFRO-RBR group 9 september 2011 Paneveggio Natural Park Forest, Trento, Italy

XIII IUFRO-RBR group 9 semptember 2011 Val Venegia- Passo Rolle, Trento, Italy

XIII IUFRO-RBR group 8 september 2011 S. Martino di Castrozza, Trento, Italy

Session 1

Genomics and Plant-Pathogen Interactions

The *Heterobasidion irregulare* genome project

Å. Olson[1], A. Aerts[8], F.O. Asiegbu[2], L. Belbahri[18], O. Bouzid[20], A. Broberg[21], B. Canbäck[1], P.M. Coutinho[4], D. Cullen[17], K. Dalman[1], G. Deflorio[14], L.T.A. van Diepen[15], C. Dunand[12], S. Duplessis[3], M. Durling[1], P. Gonthier[22], J. Grimwood[9], C.G. Fossdal[5], D. Hansson[21], B. Henrissat[4], A.M. Hietala[5], K. Himmelstrand[1], D. Hoffmeister[11], N. Högberg[1], T.Y. James[15], M. Karlsson[1], A. Kohler[3], U. Kües[7], Y.H. Lee[13], Y.C. Lin[6], M. Lind[1], E. Lindquist[8], V. Lombard[4], S. Lucas[8], K. Lundén[1], E. Morin[3], C. Murat[3], J. Park[13], T. Raffaello[2], P. Rouzé[6], A. Salamov[8], J. Schmutz[9], H. Solheim[5], J. Ståhlberg[16], H. Vélëz[1], R.P. de Vries[19,20], A. Wiebenga[19], S. Woodward[14], I. Yakovlev[5], M. Garbelotto[10*], F. Martin[3*], I.V. Grigoriev[8*], J. Stenlid[1]

*These authors contributed to this work as senior authors

[1]*Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, Ullsväg 26, 750 05 Uppsala, Sweden.*
[2]*Department of Forest Ecology, PO Box 27 Latokartanonkaari 7, 00014 University of Helsinki, Finland.*
[3]*UMR INRA-UHP "Interactions Arbres/Micro-Organismes" IFR 110 "Genomique, Ecophysiologie et Ecologie Fonctionnelles" INRA-Nancy 54280 Champenoux, France.*
[4]*AFMB UMR 6098 CNRS/UI/UII, Case 932, 163 Avenue de Luminy 13288 Marseille cedex 9, France.*
[5]*Norwegian Forest and Landscape Institute, Høgskoleveien 8, NO-1432 Ås, Norway.*
[6]*VIB Department of Plant Systems Biology, Ghent University, Bioinformatics and Evolutionary Genomics, Technologiepark 927, B-9052 Gent, Belgium.*
[7]*Institute for Forest Botany, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany.*
[8]*US DOE Joint Genome Institute, Walnut Creek, CA 94598, USA.*
[9]*HudsonAlpha Institute for Biotechnology, 601 Genome Way Huntsville, AL 35806, USA.*
[10]*University of California, 338 Hilgard Hall Berkeley CA 94720 USA.*
[11]*Pharmaceutical Biology, Friedrich-Schiller-Universität Jena, Winzerlaer str. 2, 07745 Jena, Germany.*
[12]*University Paul Sabatier (Toulouse III), UMR5546- CNRS, Laboratory of Cell Surfaces and Plant Signalisation 24, Chemin de Borde-Rouge, BP 42617, Auzeville 31326 Castanet-Tolosan, France.*
[13]*Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea.*
[14]*University of Aberdeen, Institute of Biological and Environmental Sciences, Department of Plant and Soil Science, Cruickshank Building, St. Machar Drive, Aberdeen, AB24 3UU, Scotland UK.*
[15]*Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA.*
[16]*Department of Molecular Biology, Swedish University of Agricultural Sciences, Box 590, Husargatan 3, 751 24 Uppsala, Sweden.*

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

[17]*Forest Products Laboratory, Madison, WI 53726, USA.*
[18]*Laboratory of Soil Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland.*
[19]*CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.*
[20]*Microbiology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.*
[21]*Department of Chemistry, Swedish University of Agricultural Sciences, Box 7015, 750 05 Uppsala, Sweden.*
[22]*Department of Exploitation and Protection of Agricultural and Forest Resources (Di. Va. P. R. A.) – Plant Pathology, University of Torino, Via L. da Vinci 44, I-10095 Grugliasco, Italy.*
Corresponding author e-mail address: jan.stenlid@mykopat.slu.se

Abstract. We report on the annotated genome sequence and transcript profiling as well as quantitative trait loci mapping of one member of the *Heterobasidion annosum sensu lato* species complex; *H. irregulare.* Quantitative trait loci critical for pathogenicity and rich in transposable elements, orphan and secreted genes, were identified. A wide range of cellulose degrading enzymes is expressed during wood decay. In contrast, pathogenic interaction between *H. irregulare* and pine engages fewer carbohydrate active enzymes, but involves an increase in pectinolytic enzymes, transcription modules for oxidative stress, and secondary metabolite production. Our results show a trade-off in terms of constrained carbohydrate decomposition and membrane transport capacity during interaction with living host. The findings establish that saprotrophic wood decay and necrotrophic parasitism involve two distinct yet overlapping processes.

Introduction

Heterobasidion annosum sensu lato (*s.l.*) is a cosmopolitan fungal pathogen in conifer forests. In 1995 the economic losses were in the order of € 600 million annually to forest owners in Europe through tree mortality and wood decay (Woodward *et al.*, 1998). Although the economic consequences for North America forestry are less well documented, they are expected to be of similar magnitude. The species complex is comprised of three Eurasian (*H. annosum sensu stricto* (*s.s.*), *H. parviporum* and *H. abietinum*) and two North American (*H. occidentale* and *H. irregulare*) species, each with a different but overlapping host range (Niemelä and Korhonen, 1998; Otrosina and Garbelotto, 2010). Infections by *Heterobasidion* spp. are initiated in fresh wounds or newly created tree stump surfaces followed by spread via root to root infection through living bark and subsequent decay of and survival in the root and trunk of standing trees (Woodward *et al.*, 1998). This infection cycle relies on mechanisms for both saprotrophic wood decay and pathogenic interactions with a living host, allowing us to study potential trade-off between the two trophic strategies.

In this paper we describe the findings of a sequencing project that revealed the near complete genomic sequence of a strain of *H. irregulare* and provided an initial characterization of genome structure, gene content, and gene expression in various growth conditions.

Materials and Methods

Selection of *H. irregulare* strain and isolation of genomic DNA and RNA. The sequenced *H. irregulare* strain TC 32-1 (Chase, 1985) is well characterized and have been used in many studies.

Genome sequencing, assembly, and annotation. All sequencing reads for the whole genome shotgun sequencing were collected with standard Sanger sequencing protocols on ABI 3730XL capillary sequencing machines at the Department of Energy Joint Genome Institute in Walnut Creek, California, USA. Three different sized libraries were used as templates for the plasmid subclone sequencing process and both ends were sequenced. 214,143 reads from the 2.7 kb sized library, 192,768 reads from the 6.0 kb sized library, and 63,168 reads from a 39.1 kb fosmid library were sequenced. A total of 406,752 reads were assembled using a modified version of Arachne v.20071016 (Jaffe, 2003). This produced 53 scaffold sequences, with L50 of 2.2 Mb (the length of the scaffold that separates the top half (N50) of the assembled genome from the rest), 19 scaffolds larger than 100 kb, and total scaffold size of 33.9 Mb. Each scaffold was screened against bacterial proteins, organelle sequences and GenBank and removed if found to be a contaminant. The final draft whole genome shotgun assembly contained scaffolds that cover 33.1 Mb of the genome with a contig L50 of 127.0 kb and a scaffold L50 of 2.2 Mb.

Genome improvement. To fill large gaps, resolve larger repeats or to resolve chromosome duplications and extend into chromosome telomere regions shotgun sequencing and finishing of BAC fosmid clones were used. During the course of the improvement project, 5,376 BAC ends were sequenced to add additional contiguity. Finally, the sequences were compared to markers on the available genetic map (Lind *et al.*, 2007) and two map joins were made based on map evidence.

cDNA library construction and sequencing. *H. irregulare* TC 32-1 poly A+ RNA was isolated from total RNA for two RNA samples; RNA1 - cells grown in Liquid Hagem-medium (Stenlid, 1985) and RNA2 - cells grown in Liquid high nitrogen MMN-medium (Marx, 1969) using the Absolutely mRNA Purification kit and manufacturer's instructions (Stratagene, La Jolla, CA, USA). cDNA synthesis and cloning was a modified procedure based on the "SuperScript plasmid system with Gateway technology for cDNA synthesis and cloning" (Invitrogen, Carsbad, CA, USA). 1-2 µg of poly A+ RNA, reverse transcriptase SuperScript II (Invitrogen) and oligo dT-NotI primer (5' GACTAGTTCTAGATCGCGAGCGGCCGCCCT15VN 3') were used to synthesize first strand cDNA.

EST sequence processing and assembly. A total of 40,807 were processed through the JGI EST pipeline (ESTs were generated in pairs, a 5' and 3' end read from each cDNA clone). EST sequences were compared to the Genbank nucleotide database in order to identify contaminants; non-desirable ESTs such as those matching rRNA sequences were removed. Clustering and assembly of all 33,539 ESTs resulted in 10,126 consensus sequences and 1,503 singlets.

Whole-genome exon oligoarray. The *Heterobasidion irregulare* custom-exon expression array (4 × 72K) manufactured by Roche NimbleGen Systems Limited (Madison, WI, USA) (http://www.nimblegen.com/products/exp/index.html) contained five independent, nonidentical, 60-*mer* probes per gene model coding sequence. For 12,199 of the 12,299 annotated protein-coding gene models probes could be designed. For 2,032 probes, technical duplicates were included on the array. Total RNA was extracted using CTAB/phenol/chloroform and LiCl precipitation. The RNA was DNase I treated and cleaned with Qiagen RNA cleanup Kit. Arrays were performed from *H. irregulare* mycelium grown in liquid MMN medium (three biological replicates), from cambial zone of necrotic bark of pines inoculated with *H. irregulare* (21 dpi; three biological replicates), from fruiting bodies collected in California (four biological replicates) as well as from *H. irregulare* grown on wood shavings from pine (four biological replicates), grown in liquid medium amended with lignin (Kraft Pine lignin B 471003-500G; SIGMA-Aldrich) and growth in liquid medium amended with cellulose from Spruce (22182-KG Fluka; SIGMA-Aldrich). Cultures were harvested after 3 weeks of incubation 22°C in darkness. Total RNA preparations were amplified using the SMART PCR cDNA Synthesis Kit (Clontech) according to the manufacturer's instructions.

Genome annotation. The *H. irregulare* genome was annotated using the JGI annotation pipeline, which takes multiple inputs (scaffolds, ESTs, and known genes) and runs several analytical tools for gene prediction and annotation, and deposits the results in the JGI Genome Portal (http://www.jgi.doe.gov/Heterobasidion) for further analysis and manual curation. Measures of model quality include proportions of the models complete with start and stop codons (86% of models), consistent with ESTs (48% of models) and supported by similarity with proteins from the NCBI NR database (70% of models). About 90% of the models showed expression in at least one of the conditions (growth in liquid MMN medium, cambial zone of necrotic bark of pines inoculated with *H. irregulare*, fruiting bodies, *H. irregulare* growth on wood shavings from pine, growth in liquid medium amended with lignin or cellulose) analyzed in the NimbleGen array.

Results and Discussion

Using a whole genome shotgun approach, the 33.6 MB genome of *H. irregulare* was sequenced to 8.5 × coverage. Genome improvement, finishing and gap closure resulted in 33,649,967 bp with an estimated error rate of less than 1 error in 100,000 base pairs. The genome is represented in 15 scaffolds ranging in size from 3,591,957 to 8,087 bp. Six of the scaffolds represent complete chromosomes with sequence spanning from telomere to telomere. Seven other scaffolds have an identified telomere only at one end (Fig. 1).

The published linkage map (Lindm *et al.*, 2007) was anchored to the sequenced genome using Simple Sequence Repeats (SSR) markers designed from the genome sequence and evenly distributed across the scaffolds (Fig. 1). Segregation analysis of 179 sequence and SSR markers supported a genome organized into 14 chromosomes which is consistent with pulsed-gel electrophoresis data (Anderson *et al.*, 1993). The linkage map was used to locate quantitative trait loci (QTL) for pathogenicity, growth rate and fungal interactions (Lind *et al.*, 2005; Lind *et al.*, 2010; Olson *et al.*, 2006) onto the genome sequence, allowing identification of the genes in the targeted regions.

The mitochondrial genome (mt-genome), shown to influence *H. irregulare* virulence (Olson *et al.*, 2001), spans 114,193 bp and is one of the largest sequenced in fungi. In addition to genes coding for proteins of the oxidative phosphorylation system, we found 14 intron-containing genes, two genes and two pseudogenes probably derived from a mitochondrial plasmid and six non-conserved hypothetical genes. A total of 11,464 gene models have been predicted in *H. irregulare* with half of them shared across Basidiomycotina. The transcription factor distribution comparable with other fungal taxa and the signal transduction pathway are conserved. The largest gene families include transporters and signaling domains (MFS, p450, WD40, protein kinases). Sequenced ESTs and microarray analysis supported 90% of the predicted genes.

Heterobasidion spp. can produce at least 10 different secondary metabolites which are produced both in axenic cultures and during interaction with plants and other fungi (Sonnenbichler *et al.*, 1989). Genome analysis using a secondary metabolite unique regions finder (SMURF) web-tool (http://www.jcvi.org/smurf/index.php) and manual curation identified genes for three polyketide synthases (PKS), 13 nonribosomal peptide synthetase like (NRP-like) enzymes, 3 terpene cyclases and several tailoring enzymes, including one dimethylallyltryptophan synthase predicted to be involved in secondary metabolite production.

The *H. irregulare* genome shows a great potential for both saprotrophic and biotrophic lifestyles. It encodes a wide arsenal of enzymes required to digest cellulose, hemicellulose and pectin, making it almost as well equipped regarding plant cell wall degradation as obligate saprotrophs such as *S. commune* and *C. cinerea*. In contrast to *P. chrysosporum, H. irregulare* possesses two GH29

fucosidase genes that might act on living/fresh material and two GH5 that may have a role in softwood-specific glucomannan-degradation. Furthermore, *H. irregulare* growth on sucrose correlated well with the presence of an invertase (GH32) gene, a feature shared with many phytopathogens (Parrent *et al.*, 2009). Sucrose is one of the major sugars found in fresh pine stump surface (Asiegbu *et al.*, 1993) and the capacity to utilize it during the initial phase of colonization might provide *H. irregulare* with a selective advantage compared to saprotrophs lacking invertase activity, which could help explain why *H. annosum* spp. are so competitive in industrially managed forests.

Oxidative enzymes implicated in ligninolysis by white rot fungi include lignin peroxidase, manganese peroxidase, glyoxal oxidase and laccase (Hatakka, 1994). With its eight Mn-peroxidases and lack of lignin peroxidases *H. irregulare* has a lower peroxidase potential than *P. chrysosporum* but a higher number of phenol oxidases and laccases, 18 and 5, respectively. To generate H_2O_2, *H. irregulare* possesses 17 quinone oxidoreductases, 5 glyoxal oxidases, 34 glucose-methanol-choline oxidoreductases and four Mn-superoxide dismutases.

Heterobasidion annosum (*s.l.*) uses two ecological strategies; parasitism and saprotrophic wood decay. During the life cycle it infects and lives within standing conifer trees but it also continues colonizing and degrading the dead tissue. Our analyses of gene expression during these two trophic stages reveal a trade-off in terms of restricted energy acquisition. Fewer genes encoding carbohydrate active enzymes and transporters are expressed during pathogenic growth than during saprotrophic wood decay, indicating that the fungus is not using its full capacity for energy acquisition during necrotrophic growth, i.e. its full arsenal of wood degrading enzymes. Instead, the living tissue triggered an expanded metabolic repertoire involving genes associated with e.g., toxin production, protection against plant defenses, handling low oxygen pressure, and other abiotic stresses. We conclude that there is a trade-off present between maximal nutritional gain and access to a different ecological niche that the fungus has to balance. Gene expression during saprotrophic growth on wood correlated the most with expression during growth on cellulose and lignin, but just intermediately so with expression during growth in cambial zone of pine (Tab. 1). Presumably, *H. irregulare* detects wood as a source of cellulose-derived energy, whereas living tissue only partly function in this manner. The genes induced specifically during infectious growth enable *H. irregulare* to access energy sources, such as carbon bound in macro molecules of living organisms, unavailable to other organisms with which it would otherwise compete.

Re-mapping of two different virulence measurements on two hosts, pine and spruce, revealed three major regions on two chromosomes to be involved, harboring 178, 142 and 299 gene models, respectively (Fig. 1). These regions are characterized by a high number of transposable elements and orphan genes with no homologues genes reported from other species. These orphan models constitute a

resource for the exploration of novel enzymatic functions and biological mechanisms. Together the enrichment in orphan genes and repetitive elements indicates that these are highly dynamic regions with high evolutionary rate. The characteristics, with high number of orphan genes and many repetitive elements, in these regions are comparable with the effector regions identified in *Phytophthora infestans* (Haans *et al.*, 2009).

Transposable elements are not equally distributed within and among the chromosomes. The major TE´s observed are younger than 12 Mya and the decrease detected probably reflects element deterioration leading to loss of ability to detect older elements. Since TE proliferation within the pathogenicity QTLs is clearly younger than speciation (Dalman *et al.*, 2010), we hypothesize that transposon activity may have contributed to shaping the species specific characteristics of *H. irregulare*. Transposable elements have been implicated to co-locate with important factors for pathogenicity also in other pathosystems, eg. *Phytophthora infestans* (Cuomo *et al.*, 2007; Haans *et al.*, 2009).

Transcriptome analyses combined with QTL approach proved to be a powerful approach to reduce the number of candidate virulence genes of the QTL regions. Gene models who are present in QTL regions for virulence and significantly up-regulated during pathogenic interaction with pine are strong candidates. The most highly up-regulated ones were a high affinity sugar transporter (70 fold) present in QTL 2 on scaffold 12. In this QTL, a gene model with no sequence homology was found with 4 fold higher expressed in mycelia grown in cambium compared to liquid culture. In QTL 1 on scaffold 1their was one gene model with no sequence homology which was significantly lower expressed in mycelia grown on pine compared to in liquid media and a putative flavin containing monooxygenase, with 9.4 fold higher expressed during infection. This type of monooxygenase is needed for one of the biosynthetic steps required for the synthesis of phytotoxin fomannosin making it a very strong pathogenicity candidate. Two overlapping secondary metabolite clusters harboring altogether 43 gene models were located in the QTL region on scaffold 12. The clusters include three NRPS, several oxidative enzymes and transport proteins. Since the QTLs are based on a mapping population derived from a cross between *H. irregulare* and *H. occidentale*, these genes are the main candidates to explain the difference in virulence between the species. As host specificity probably plays an important role in speciation, these genes could constitute a crucial step towards understanding the separation of *H. annosum* (*s.l.*) into separate species.

Analysis of culture filtrates revealed presence of fomannosin and fomannoxin while genome analysis identified terpene cyclase and DMATS, possible involved in the respective synthesis of these known phytotoxins. In addition genes predicted to be involved in production of other secondary metabolites were identified. The presence of genes for putative PKS, NRPS-like enzymes and halogenase, however, implies that the biosynthetic capacity of *H. irregulare* has not been fully explored,

as to date, no polyketides, non-ribosomal peptides or halogenated compounds have been identified.

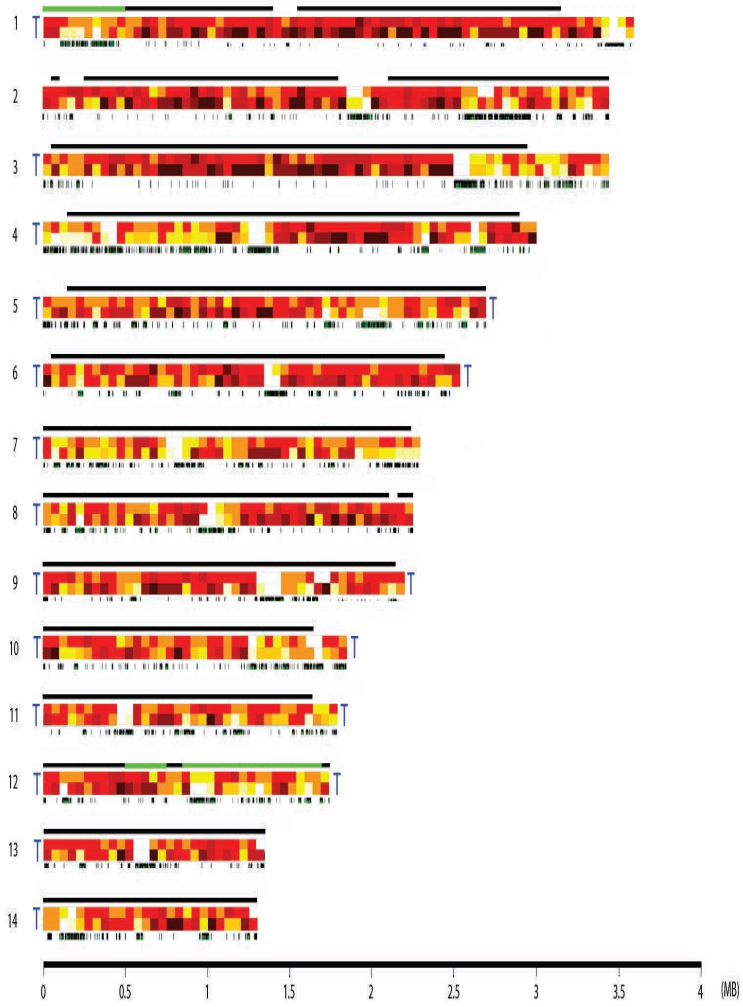


Figure 1. The 14 postulated chromosomes of *Heterobasidion irregulare*. The upper black bar of each chromosome denotes linkage map coverage, with pathogeniticy QTLs marked in green. The wide yellow-to-brown bar describes gene density (upper half) and gene model quality (lower half) for every 50 kB segment of the sequence. Gene density is calculated in number of gene models, ranging from over 27 (brown) to under 10 (white). Gene model quality is calculated based on microarray experiments using five probes for each gene model. The color indicates the percentage of models where all five probes hybridized, ranging from 100% (brown) to below 10% (white). The lowest bar of each chromosome indicates transposon regions, as per masked by RepeatMasker. Blue Ts stands for an identified telomere region in the corresponding chromosome end.

Table 1. Correlation of global gene expression under different growth conditions.

Condition	Lignin	Cellulose	Wood	Fruit body	Cambial zone
Lignin	1	0.6934[a]	0.7256	0.2965	0.5328
Cellulose		1	0.7365	0.2244	0.4880
Wood			1	0.3699	0.6158
Fruit body				1	0.2489
Cambial zone					1

[a] r^2; N=3,590

Acknowledgements

The work conducted by the U.S. Department of Energy Joint Genome Institute and was supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. Financial support from the Swedish Foundation for Strategic Research is gratefully acknowledged.

References

Anderson M., Kasuga T., Mitchelson K., 1993. A partial physical karyotype of *Heterobasidion annosum*. In: Johansson M., Stenlid J., (eds.). Proceedings of the eighth International Conference on Root and Butt Rots, Wik, Sweden and Haikko, Finland, pp. 303-313.

Asiegbu F.O., 2000. Adhesion and development of the root rot fungus (*Heterobsidion annosum*) on conifer tissue: effects of spore and host surface constituents. *FEMS Microbiology Ecology* 33: 101-110.

Chase T.E., 1985. Genetics of sexuality and speciation in the fungal forest pathogen *Heterobasidion annosum*. PhD thesis, University of Vermont, Burlington, VT, USA.

Cuomo C.A., Güldener U., Xu J.R., Trail F., Turgeon B.G., Di Pietro A., Walton J.D., Ma L.J., Baker S.E., Rep M. *et al.*, 2007. The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science* 317: 1400-1402.

Dalman K., Olson Å., Stenlid J., 2010. Evolutionary history of the conifer root rot fungus *Heterobasididion annsosum sensu lato*. *Molecular Ecology* 19: 4979-4993.

Haas B., Kamoun S., Zody M.C., Jiang R.H.Y., Handsaker R.E., Cano L.M., Grabherr M., Kodira C.D., Raffaele S., Torto-Alalibo T. *et al.*, 2009. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature* 461: 393-398.

Hatakka A., 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS Microbiology Reviews* 13: 125-135.

Jaffe D.B., Butler J., Gnerre S., Mauceli E., Lindblad-Toh K., Mesirov J.P., Zody M.C., Lander E.S., 2003. Whole-genome sequence assembly for mammalian genomes: Arachne 2. *Genome Research* 13: 91-6.

Lind M., Dalman K., Stenlid J., Karlsson B., Olson Å., 2007. Identification of quantitative trait loci affecting virulence in the basidiomycete *Heterobasidion annosum s.l. Current Geneicst* 52: 35-44.

Lind M., Olson Å., Stenlid J., 2005. An AFLP-marker based genetic linkage map of *Heterobasidion annosum* locating intersterility genes. *Fungal Genetics and Biology* 42: 519-527.

Lind M., Stenlid J., Olson Å., 2007. Genetics and QTL mapping of somatic incompatibility and intraspecific interactions in the basidiomycete *Heterobasidion annosum* s.l. *Fungal Genetics and Biology* 44: 1242-1251.

Marx D.H., 1969. The influence of ectomycorrhizal fungi on the resistance of pine roots to pathogenic infections. *Phytopathology* 59: 153-163.

Niemelä T., Korhonen K., 1998. Taxonomy of the Genus *Heterobasidion*. In: Woodward S., Stenlid J., Karjalainen R., Hüttermann A., (eds.). *Heterobasidion annosum* Biology, Ecology, Impact and Control. Wallingford, UK, CAB International, pp. 1-25.

Olson Å., 2006. Genetic linkage between growth rate and the intersterility genes S and P in the basidiomycete *Heterobasidion annosum sensu lato*. *Mycological Research* 110: 979-984.

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

Otrosina W.J., Garbelotto M., 2010. *Heterobasidion occidentale* sp. nov. and *Heterobasidion irregulare* nom. nov.: A disposition of North American *Heterobasidion* biological species. *Fungal Bioogyl* 114: 16-25.

Parrent J.L., James T.Y., Vasaitis R., Taylor A.F.S., 2009. Friend or foe? Evolutionary history of glycoside hydrolase family 32 genes encoding for sucrolytic activity in fungi and its implications for plant-fungal symbioses. *BMC Evolutionary Biology* 9: 1-16.

Sonnenbichler J., Bliestle I.M., Peipp H., Holdenrieder O., 1989. Secondary fungal metabolites and their biological activity, I. isolation of antibiotic compounds from cultures of *Heterobasidion annosum* synthesized in the presence of antagonistic fungi or host plant cells. *Biological Chemistry* 370: 1295-1303.

Stenlid J., 1985. Population structure of *Heterobasidion annosum* as determined by somatic incompatibility, sexual incompatibility, and isoenzyme patterns. *Canadian Journal of Botany* 63: 2268-2273.

Woodward S., Stenlid J., Karjalainen R., Huttermann A., 1998. *Heterobasidion annosum* Biology, Ecology, Impact and Control. Wallingford, UK, CAB International.

Molecular studies of the *Heterobasidion annosum s.l.* Norway spruce pathosystem: local and systemic host defense responses

C.G. Fossdal, N. Yaqoob, P. Krokene, A.M. Hietala, I. Yakovlev, H. Solheim

Norwegian Forest and Landscape Institute, Hogskoleveien 8, N-1432 Aas, Norway. Corresponding author e-mail address: foc@skogoglandskap.no

Abstract. The root-rot causing fungus *Heterobasidion annosum sensu lato* may enter Norway spruce through the roots and or trough wounds. It is acting as a necrotroph when in contact with living host tissue such as living bark and sapwood. Despite the high incidence of damage caused by this devastating fungus, the living tissues of Norway spruce trees have defences against this pathogen. We have studied the host responses to infection and methyl jasmonate (MJ) in Norway spruce at the transcriptional level spatiotemporally in both bark and sapwood. We compared gene expression in Norway spruce bark and sapwood in response to the pathogen *Heterobasidion parviporum*, wounding and methyl jasmonate (MeJ). The pathogen induced systemic up-regulation of defence related genes in both bark and sapwood, whereas responses to MeJ were most noticeable in the bark. Genes involved in lignin biosynthesis were up-regulated locally in the bark, and MeJ induced a stronger and more lasting response than the in response to inoculation with the pathogen. These results demonstrate local and systemic host responses to pathogen infection in both the bark and sapwood, and reveal similarities in the local defense responses in spruce to pathogen and MeJ.

Introduction

Trees face special challenges in defence due to their often very long lifespan and the fact that most of their biomass is allocated to wood that also must be protected from pathogen attack and decay (Hietala *et al.,* 2004; Deflorio *et al.,* 2011). The level of host resistance is determined by how efficiently the plant can coordinate its defences and how quickly the responses can be launched (Hietala *et al.,* 2004; Bonello *et al.,* 2006). Many plant hormones, such as abscisic acid, salicylic acid, jasmonic acid and ethylene, are involved in plant defence that lead to reinforcement of cell walls and production of hydrolytic enzymes. The constitutive and inducible defence systems in the bark is relatively well studied (Franceschi *et al.*, 2000; Hietala *et al.*, 2004; Fossdal *et al.*, 2006; Deflorio *et al.*, 2011). In response to infection Norway spruce elicits defence responses in the living bark and sapwood, including changes in size and phenolic content of the polyphenolic parenchyma cells (Franceschi *et al.,* 2000; Krokene *et al.*, 2003) and formation of a wound periderm (Franceschi *et al*., 2000; Nagy *et al*., 2000). There are both local and systemic defense responses in conifers and there are also indications of primed (induced or acquired) resistance (Krokene *et al.,* 2003; Bonello *et al.,* 2006; Fossdal *et al.,* 2007). The anatomical, physiological and chemical aspects of Norway spruce defences in the bark have been examined in recent studies (Erbilgin

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

et al., 2006; Zhao *et al.,* 2010). Less is known about defences in the wood, but *H. annosum* spreads further in wood than in bark of Sitka spruce seedlings, indicating that resistance responses are weaker in the wood (Bodles *et al.,* 2006). Gene expression profiling in the bark has been done to understand the resistance mechanisms in Norway spruce and Sitka spruce to *H. parviporum*, but much less is known about the local and systemic expression of putative defence genes in sapwood after *H. parviporum* infection.

For a more thorough understanding of the regulation of defence-related genes in Norway spruce is important to understand the mechanisms of tree resistance against *H. parviporum* and if and how MeJ can be used to prime tree resistance. The systemic expression of peroxidases and chitinases also suggests the presence of systemic signalling in Norway spruce (Fossdal *et al.,* 2001; Hietala *et al.,* 2004; Nagy *et al.*, 2004) but it is not established if the systemic signal from the local site of infection to more distal parts travel primarily in the bark or in the wood or in both.

Results and Discussion

In conifers and other plants induced defence responses to invading organisms are associated with cell wall modification through lignification and suberization. We found the lignin related peroxidases to be more highly expressed after inoculation, supporting the suggestion peroxidases participate in the host defence in many tissue types. The quick and strong expression of peroxidases systemically agrees with previous studies in very young Norway spruce seedlings and tissue cultures, where strong upregulation is observed shortly after inoculation (Hietala *et al.,* 2004; Nagy *et al.,* 2004; Fossdal *et al.,* 2006; Fossdal *et al.,* 2007). Rapid induction of peroxidases may be a potential future molecular markers to identify resistant genotypes of Norway spruce.

Interaction or cross-talk between the ethylene and MeJ signal transduction pathways is important in the defence responses. We studied two genes ACO and ACS in the ethylene pathway, but our data do not allow us to draw any firm conclusions about the role of the ethylene response of Norway spruce to pathogen infection and MeJ treatment. However we did see indications of down regulation of ethylene related transcripts.

Defense related genes are upregulated in both bark and woood of mature Sitka spruce trees (Deflorio *et al.,* 2011). Interestingly, data from Sitka spruce seedlings suggest that resistance responses to *Heterobasidion* infection are much weaker in the wood than in the bark (Bodles *et al.,* 2006). However, our results indicate that several defense genes are local and systemic defence upregulated in the sapwood of Norway spruce saplings, and some transcripts were even upregulated more strongly in the wood than in the bark. This agrees with the observation that 2-year-old Sitka spruce clones with high resistance to *H. annosum* are able to limit the growth of this pathogen not only in the bark but also in the wood (Bodles *et al.,* 2006).

Among the nine genes examined in this study, Peroxidases and a class IV chitinase were highly expressed in both bark and sapwood after pathogen inoculation and appeared to play the most important role in defence response both locally and systemically. Genes involved in lignin biosynthesis were more responsive to MeJ treatment than to inoculation with *H. parviporum*. In general, MeJ treatment induced somewhat higher transcript levels than fungal inoculation in the bark. However, fungal inoculation induced higher levels of *the* Class IV chitinase, and one Peroxidase, as well as systemic responses in the sapwood for the same gene products. In addition to increasing our knowledge of local and systemic defence responses in Norway spruce stem tissues to MeJ and pathogen infection this work may contribute to resistance breeding by providing breeders with possible tools to determine tree resistance at an early age by quantifying gene expression patterns in the bark and sapwood.

References

Adomas A., Heller G., Li G., Olson A., Chu T.M., Osborne J., Craig D., van Zyl L., Wolfinger R., Sederoff R., Dean R.A., Stenlid J., Finlay R., Asiegbu F.O., 2007. Transcript profiling of a conifer pathosystem: response of *Pinus sylvestris* root tissues to pathogen (*Heterobasidion annosum*) invasion. *Tree Physiology* 27: 1441-58.

Asiegbu F.O., Adomas A., Stenlid J., 2005. Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. s.l. *Molecular Plant Pathology* 6: 395-409.

Bodles W.J.A., Fossdal C.G., Woodward S., 2006. Multiplex real-time PCR detection of pathogen colonization in the bark and wood of *Picea sitchensis* clones differing in resistance to *Heterobasidion annosum*. *Tree Physiology* 26: 775-82.

Bonello P., Gordon T.R., Herms D.A., Wood D.L., Erbilgin N., 2006. Nature and ecological implications of pathogen-induced systemic resistance in conifers: a novel hypothesis. *Physiological and Molecular Plant Pathology* 68: 95-104.

Deflorio G., Horgan G., Woodward S., Fossdal C.G., 2011. Gene expression profiles, phenolics and lignin of Sitka spruce bark and sapwood before and after wounding and inoculation with *Heterobasidion annosum*. *Physiological and Molecular Plant Pathology* 75: 180-187.

Erbilgin N., Krokene P., Christiansen E., Zeneli G., Gershenzon J., 2006. Exogenous application of methyl jasmonate elicits defenses in Norway spruce (*Picea abies*) and reduces host colonization by the bark beetle *Ips typographus*. *Oecologia* 148: 426-36.

Fossdal C.G., Hietala A.M., Kvaalen H., Solheim H., 2006. Changes in host chitinase isoforms in relation to wounding and colonization by *Heterobasidion annosum*: early and strong defense response in 33-year-old resistant Norway spruce clone. *Tree Physiology* 26: 169-77.

Fossdal C.G., Nagy N.E., Johnsen O., Dalen L.S., 2007. Local and systemic stress responses in Norway spruce: similarities in gene expression between a compatible pathogen. *Physiological and Molecular Plant Pathology* 70: 161-173.

Fossdal C.G., Sharma P., Lonneborg A., 2001. Isolation of the first putative peroxidase cDNA from a conifer and the local and systemic accumulation of related proteins upon pathogen infection. *Plant Molecular Biology* 47: 423-35.

Franceschi V.R., Krokene P., Christiansen E., Krekling T., 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist* 167: 353-75.

Franceschi V.R., Krokene P., Krekling T., Christiansen E., 2000. Phloem parenchyma cells are involved in local and distant defense responses to fungal inoculation or bark-beetle attack in Norway spruce (*Pinaceae*). *American Journal of Botany* 87: 314-26.

Hietala A.M., Kvaalen H., Schmidt A., Johnk N., Solheim H., Fossdal C.G., 2004. Temporal and spatial profiles of chitinase expression by Norway spruce in response to bark colonization by *Heterobasidion annosum*. *Applied and Environmental Microbiology* 70: 3948-3953.

Hudgins J.W., Franceschi V.R., 2004. Methyl jasmonate-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. *Plant Physiology* 135: 2134-49.

Hudgins J.W., Ralph S.G., Franceschi V.R., Bohlmann J., 2006. Ethylene in induced conifer defense: cDNA cloning, protein expression, and cellular and subcellular localization of 1-aminocyclopropane-1-carboxylate oxidase in resin duct and phenolic parenchyma cells. *Planta* 224: 865-77.

Koutaniemi S., Warinowski T., Kärkönen A., Alatalo E., Fossdal C.G., Saranpää P., Lasko T., Fagerstedt K.V., Simola L.K., Paulin L., Rudd S., Teeri T.H., 2007. Expression profiling of the lignin biosynthetic pathway in Norway spruce using EST sequencing and real-time RT-PCR. *Plant Molecular Biology* 65: 311-28.

Krokene P., Nagy N.E., Solheim H., 2008. Methyl jasmonate and oxalic acid treatment of Norway spruce: anatomically based defense responses and increased resistance against fungal infection. *Tree Physiology* 28: 29-35.

Krokene P., Solheim H., Krekling T., Christiansen E., 2003. Inducible anatomical defense responses in Norway spruce stems and their possible role in induced resistance. *Tree Physiology* 23: 191-7.

Martin D., Tholl D., Gershenzon J., Bohlmann J., 2002. Methyl jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. *Plant Physiology* 129: 1003-18.

Nagy N.E., Fossdal C.G., Dalen L.S., Lonneborg A., Heldal I., Johnsen O., 2004. Effects of *Rhizoctonia* infection and drought on peroxidase and chitinase activity in Norway spruce (*Picea abies*). *Physiologia Plantarum* 120: 465-73.

Nagy N.E., Franceschi V.R., Solheim H., Krekling T., Christiansen E., 2000. Wound-induced traumatic resin duct development in stems of Norway spruce (*Pinaceae*): anatomy and cytochemical traits. *American Journal of Botany* 87: 302-13.

Ralph S.G., Hudgins J.W., Jancsik S., Franceschi V.R., Bohlmann J., 2007. Aminocyclopropane carboxylic acid synthase is a regulated step in ethylene-dependent induced conifer defense. Full-length cDNA cloning of a multigene family, differential constitutive, and wound- and insect-induced expression, and cellular and subcellular localization in spruce and Douglas fir. *Plant Physiology* 143: 410-24.

Zhao T., Krokene P., Bjorklund N., Langstrom B., Solheim H., Christiansen E., 2010. The influence of *Ceratocystis polonica* inoculation and methyl jasmonate application on terpene chemistry of Norway spruce, *Picea abies*. *Phytochemistry* 71: 1332-41.

Gene expression and metabolism of phenolic compounds in Sitka spruce clones inoculated with *Heterobasidion annosum*

G. Deflorio[1], G. Horgan[2], S. Woodward[1], C.G. Fossdal[3]

[1]*University of Aberdeen, Institute of Biological and Environmental Sciences, Department of Plant and Soil Science, Cruickshank Building, Aberdeen AB24 3UU, Scotland, UK.*
[2]*Biomathematics and Statistics Scotland, Aberdeen AB21 9SB, Scotland, UK.*
[3]*Norwegian Forest and Landscape Institute, Høgskoleveien 8, 1432 Ås, Norway.*
Corresponding author e-mail address: giulianade@gmail.com

Abstract. Expression of phenylpropanoid pathway genes (PAL, CCR1, HCT1, and CAD), peroxidase (PaPX3) and class IV chitinase (PaCHI4) were examined in bark and sapwood of Sitka spruce (*Picea sitchensis*) before and 3 days after wounding and artificial inoculation with *Heterobasidion annosum s.s.* All genes examined were up-regulated in bark at the site of inoculation. At 10 mm from the inoculation point in bark, however, only CAD was upregulated. All genes were down-regulated in sapwood, except for PaPX3 and PaCHI4; PAL, CCR1, HCT1 and CAD expression was lower around the inoculation site than in the distal zone. Compared to wounding only, inoculation with H. annosum triggered different CAD, PaPX3, and PaCHI4 levels in bark but not in sapwood. Concentrations of cell wall bound phenolic compounds (2 unknowns, coniferin, astringin, taxifolin, piceid, and isorhapontin) changed in bark after wounding and inoculation compared to healthy samples, whereas no change was found in sapwood. These results indicate that responses to wounding and pathogen inoculation are stronger in bark than sapwood of Sitka spruce.

Introduction

Expression of phenylpropanoid pathway genes (PAL, CCR1, HCT1, and CAD), peroxidase (PaPX3) and class IV chitinase (PaCHI4) were examined in bark and sapwood of Sitka spruce (*Picea sitchensis*) before and 3 days after wounding and artificial inoculation with *Heterobasidion annosum s.s.* All genes examined were up-regulated in bark at the site of inoculation. At 10 mm from the inoculation point in bark, however, only CAD was upregulated. All genes were down-regulated in sapwood, except for PaPX3 and PaCHI4; PAL, CCR1, HCT1 and CAD expression was lower around the inoculation site than in the distal zone. Compared to wounding only, inoculation with *H. annosum* triggered different CAD, PaPX3, and PaCHI4 levels in bark but not in sapwood.

Concentrations of cell wall bound phenolic compounds (2 unknowns, coniferin, astringin, taxifolin, piceid, and isorhapontin) changed in bark after wounding and inoculation compared to healthy samples, whereas no change was found in sapwood. These results indicate that responses to wounding and pathogen inoculation are stronger in bark than sapwood of Sitka spruce.

Although the biology of *Heterobasidion* and strategies for control and management are well documented, research on host resistance is in its infancy. To obtain disease-resistant plants, it is essential to enhance understanding of the

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

defence mechanisms that have evolved to withstand invasion by pathogens. Lignin deposition is important in host resistance (Hahlbrock and Scheel, 1989). Enzymes catalyzing the phenylpropanoid pathway have been quantified in *Picea abies* bark after inoculation with *Heterobasidion* (Karlsson *et al.,* 2007; Koutaniemi *et al.,* 2007) and expression profiles of a range of defence-related genes documented (Fossdal *et al.,* 2007). Little is known, however, of defence enzyme expression in Sitka spruce.

This work reports expression profiles of several phenylpropanoid pathway genes, a chitinase and a peroxidase in bark and sapwood of Sitka spruce clones with differing levels of susceptibility to *H. annosum*. Quantities of free and bound phenolic compounds were also compared in bark and sapwood.

Materials and Methods

Sitka spruce clones, treatments and sampling are detailed elsewhere (Deflorio *et al.,* 2011). Bark and sapwood were collected 0 and 3 days after wounding and/or inoculation and stored at -80°C until required. Sample areas were cut the site (A) and distal to the site of inoculation, approx. 1 cm away from the lesion boundary (B). Following grinding in liquid N_2 and RNA was extracted and purified (RNAcqueous Kit; Ribopure Kit (Ambion). cDNA was prepared from total RNA by reverse transcription. Primers specific for spruce genes were used (Hietala *et al.,* 2004; Nagy *et al.,* 2004) and amplification of cDNA carried out with the SYBR-Green PCR Master kit (Perkin-Elmer) on the ABI Prism 7700 Sequence Detection System (Applied Biosystems). Data acquisition and analysis was through Sequence Detection System software, version 1.4 (Applied Biosystems).

Soluble phenolic compounds were extracted from bark powder in 80% MeOH in water and analyzed by HPLC. Tissue residue was hydrolyzed in 1M NaOH at room temperature for approx. 3 hours, conc. H_3PO_4 (150 µl) added and the sample filtered prior to HPLC. HPLC was carried out using an Agilent 1100 system supplied using photodiodearray detection. Compounds were separated on an Eclipse XDB-C8 column (4.6 × 150 mm, 5 µm) at 30°C, flow rate 0.8 ml min^{-1} with a gradient of 0.05% TFA in water and (B) 0.05% TFA in acetonitrile.

Log transformed gene expression values and phenolic data were subject to ANOVA.

Results and Discussion

In bark tissues, wounding lead to up-regulation of all target transcripts by 3 days after treatment (Fig. 1a). Particularly large increases in in PaPAL and PaCHI4 expression occurred. Inoculation with *H. annosum* lead to down-regulation of CAD, PX3 and CHI4, compared with wounding alone. Proximity to the point of wounding had a highly significant effect on expression of target genes, except CAD.

In contrast, constitutive expression of transcripts was higher in sapwood than in bark, except for PaPX3 and PaCHI4 (Fig. 1b); in sapwood tissues, PAL, CCR1, HCT1 and CAD expression was higher distal to the wound than proximal 3 days after treatment.

Very high constitutive concentrations of astringin and isorhapontin were found in bark tissues, although piceid was present in relatively low concentrations (Tab. 1). Following wounding and inoculation concentrations of cell wall-bound isorhapontin and astringin increased two- and 24-fold, respectively. The quantities of these same compounds in sapwood were very low prior to wounding and decreased to trace/undetectable following wounding and inoculation.

The findings of this study indicate a stronger and earlier response to wounding and fungal pathogen inoculation in bark than sapwood of Sitka spruce in terms of (1) gene expression profiles of all monitored genes which were up-regulated in bark but down-regulated in sapwood, with the exception of class IV chitinase PaCHI4 and peroxidase PaPX3, compared to constitutive levels; (2) extractives (soluble and cell wall bound phenolic compounds) were present in greater amounts in bark than sapwood, and concentrations of all cell wall bound phenolic compounds increased greatly after treatment, reiterating the clear differences in defence response between these two tissues.

References

Deflorio G., Horgan G., Woodward S., Fossdal C.G., 2011. Gene expression profiles, phenolics and lignin of Sitka spruce bark and sapwood. *Physiological and Molecular Plant Pathology* 75: 180-187.

Fossdal C.G., Nagy N.E., Johnsen Ø., Dalen L.S., 2007. Local and systemic stress responses in Norway spruce: similarities in gene expression between a compatible pathogen interaction and drought stress. *Physiological and Molecular Plant Pathology* 70: 161-173.

Hahlbrock K. and Scheel D., 1989. Physiology and molecular biology of phenylpropanoid metabolism. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 347-369.

Hietala A.M., Kvaalen H., Schmidt A., Jøhnk N., Solheim H., Fossdal C.G., 2004. Temporal and spatial profiles of chitinase expression by Norway Spruce in response to bark colonization by *Heterobasidion annosum*. *Applied and Environmental Microbiology* 70: 3948-3953.

Karlsson M., Hietala A., Kvaalen H., Solheim H., Olson Å., Stenlid J., Fossdal C.G., 2007. Quantification of host and pathogen DNA and RNA transcripts in the interaction of Norway spruce with *Heterobasidion parviporum*. *Physiological and Molecular Plant Pathology* 70: 99-109.

Koutaniemi S., Warinowski T., Kärkönen A., Alatalo E., Fossdal C.G., Saranpää P., Laakso T., Fagerstedt K.V., Simola L.K., Paulin L., Rudd S., Teeri T.H., 2007. Expression profiling of the lignin biosynthetic pathway in Norway spruce using EST sequencing and real-time RT-PCR. *Plant Molecular Biology* 65: 311-328.

Nagy N.E., Fossdal C.G., Dalen L.S., Lönneborg A., Heldal I.M., Johnsen Ø., 2004. Effects of *Rhizoctonia* infection and drought on peroxidase and chitinase activity in Norway spruce (*Picea abies*). *Physiologia Plantarum* 120: 465-473.

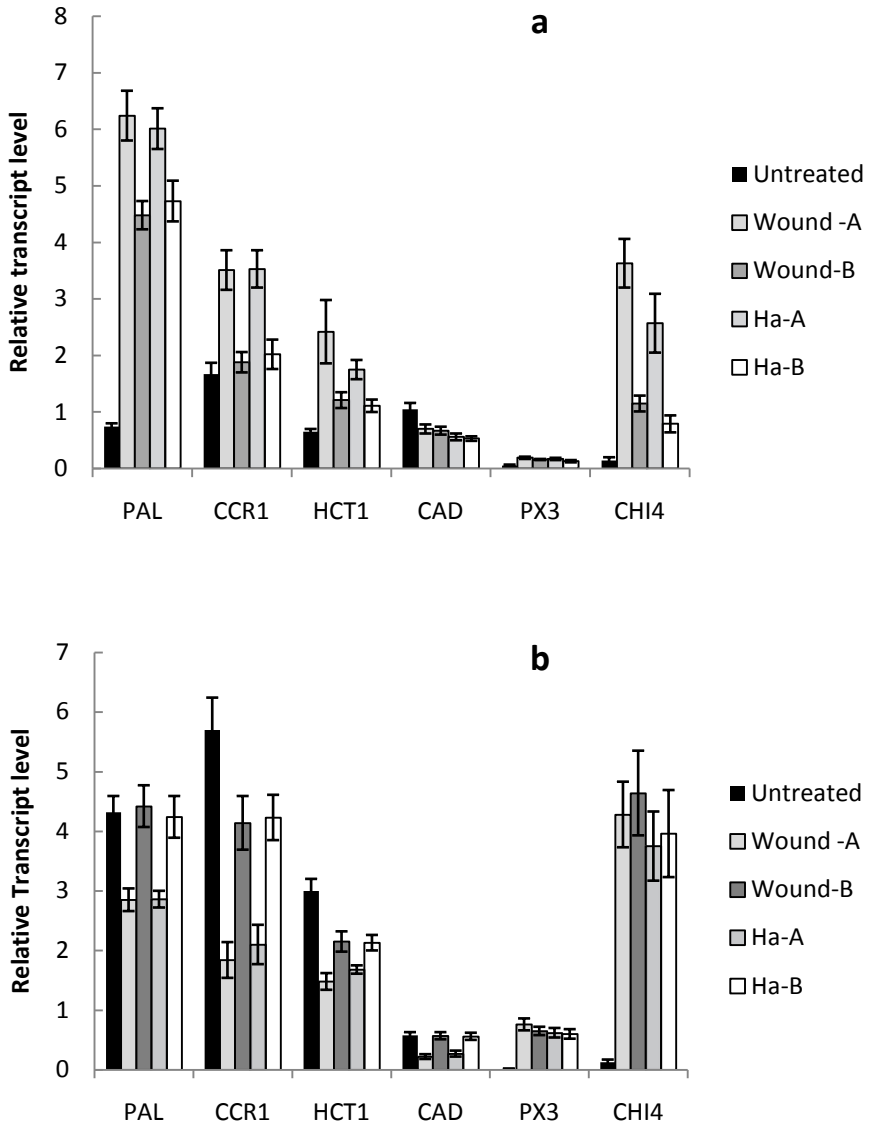


Figure 1. Relative expression of PA, CCR1, HCT1, CAD, PX3 and CHI4 in Sitka spruce bark (a) and sapwood (b), 3 days after wounding and inoculation with *Heterobasidion annosum*.

Table 1. Concentrations of free (area 2 ml mg^{-1} FW ± SE) and cell wall bound phenolic compounds (area mg^{-1} FW ± SE) extracted from bark and sapwood of Sitka spruce in healthy samples, collected at day 0, and 3 days after wounding and inoculation with *Heterobasidion annosum*, 1 cm away from inoculation site (n=8).

	Tissue	**Free phenolics**		**Cell wall bound phenolics**	
		Healthy	W + Ha-B	Healthy	W + Ha-B
Unknown1	Bark	n/d [a]	n/d	n/d	n/d
Unknown2	Bark	n/d	n/d	3.9 ± 0.7	6.4 ± 0.8
Unknown3	Bark	n/d	n/d	0.0 ± 0.0	8.8 ± 2.0
Coniferin	Bark	n/d	n/d	4.3 ± 1.3	7.2 ± 2.1
Astringin	Bark	1,471.3 ± 151.4	1,404.6 ± 72.2	4.1 ± 4.1	95.3 ± 20.7
Taxifolin	Bark	29.5 ± 3.5	44.3 ± 4.0	1.6 ± 0.5	2.5 ± 0.5
Piceid	Bark	74.3 ± 12.5	78.7 ± 11.9	0.5 ± 0.5	13.2 ± 3.3
Isorhapontin	Bark	1,116.1 ± 192.9	1,246.0 ± 63.5	123.0 ± 20.3	235.0 ± 15.7
Unknown1	Wood	46.0 ± 9.1	40.6 ± 6.3	n/d [a]	n/d
Unknown2	Wood	n/d	n/d	12.6 ± 3.1	12.1 ± 2.9
Unknown3	Wood	n/d	n/d	n/d	n/d
Coniferin	Wood	n/d	n/d	0.2 ± 0.1	0.2 ± 0.1
Astringin	Wood	19.3 ± 11.2	5.4 ± 3.0	n/d	n/d
Taxifolin	Wood	0.4 ± 0.2	0.0 ± 0.0	n/d	0.1 ± 0.1
Piceid	Wood	0.8 ± 0.6	0.3 ± 0.2	n/d	n/d
Isorhapontin	Wood	14.7 ± 7.4	6.6 ± 4.2	n/d	3.5 ± 3.5

[a] n/d = not detected.

'Clone' is not shown because concentrations were not significantly different for the majority of response variables.

Resistance responses of *Picea abies* to *Heterobasidion parviporum* in Southern Finland

S.E. Keriö[1], M. Niemi[1], M. Haapanen[2], F.O. Asiegbu[1]

[1]*Department of Forest Sciences, P.O. Box 27 (Latokartanonkaari 7), FI-00014 University of Helsinki, Finland.*
[2]*Finnish Forest Research Institute (Metla), P.O. BOX 18 (Jokiniemenkuja 1), FI-01301 Vantaa, Finland.*
Corresponding author e-mail address: susanna.kerio@helsinki.fi

Abstract. The resistance of fifteen Norway spruce (*Picea abies*) clones to a root and butt rot pathogen *Heterobasidion parviporum* infection was investigated in field conditions. The stems and roots of 40-year old Norway spruce trees were inoculated using spruce wood dowels which were either sterile (control) or pre-colonized by *H. parviporum*. Two inoculations were made to each tree, and each treatment was replicated at least twice within each clone. The inoculations were performed in late June, and the extension of necrotic reactions in phloem and xylem regions was recorded after four months. The necrotic responses due to infection were significantly stronger than those due to wounding. The average length of necrosis was 46 mm and 36 mm in infected roots and stems compared to 17 mm and 19 mm in wounded roots and stems, respectively. The average length of necrotic reactions to infection was 41 mm in phloem compared to 38 mm in xylem. A clone of Russian origin showed notably weak responses both to wounding (17 mm) and infection (21 mm) both in roots and in stem. In comparison, another clone from Central Finland responded strongly to infection both in roots (71 mm) and in stem (53 mm). Moreover, a clone selected from provenience hybrid (Southern Finland × Germany) exhibited significantly stronger responses in roots (106 mm) than in stems (31 mm). This study provides a starting point for further studies on the inherent factors contributing to the resistance of Norway spruce against *H. parviporum*.

Introduction

Heterobasidion parviporum causes severe economic losses in the Scandinavian countries. *H. parviporum* causes severe root and heart rot on *Picea abies* (Norway spruce), which is the host species of this fungus. *H. parviporum* infects fresh stump surfaces and lesions of trees with basidiospores, and spreads from stumps and infected trees to neighboring trees via root contacts. Trees with weakened roots may eventually fall down during storms (Stenlid and Redfern, 1998). Because the fungus is a necrotrophic pathogen, it is able to parasitize the living as well as feed on dead tissues of the host. There is yet no information on conifer species with absolute resistance against this pathogen. However, it has been shown that there are differences in the resistance of Norway spruce to this fungus between spruce clones (Von Weissenberg, 1975; Swedjemark and Stenlid 1997; Swedjemark and Karlsson 2004) but also within full-sib families (Arnerup *et al.,* 2010) and that the differences are heritable.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Materials and Methods

We studied the resistance responses of fifteen *P. abies* clones to *H. parviporum*. The ramet clones were planted in 1977 on former agricultural land in Southern Finland (lat. 60°30', long. 24°42', 100 m a.s.l.). Initially 1,240 plants were planted into 2 × 2 meter squares at 1 × 1 meter intervals, and the experiment had been thinned in 1988 and 1997. The selected clones were either of Southern Finnish (V48, V304, V315, V494, V3012, V3027, and V3031), Central Finnish (V374 and V375), Czech (V323), or Russian (V330) origin, or Finnish × German provenience hybrids (V477, V481, V483, and V488). The clones from Central Finland had the same tree either as their mother or father. Altogether 66 trees from the 15 different clones were inoculated. In a single tree, the stem and roots were either wounded (wounding), or wounded and infected (infection) with *H. parviporum*. Two inoculations were made both to stem and roots within one tree, and at least two separate trees were subjected to similar treatment within the clones. Inoculation method similar to Swedjemark and Stenlid (Swedjemark and Karlsson, 2004) was used. Circular wounds that reached the surface of the sapwood were produced with a hole puncher. Autoclaved spruce wood dowels with 10 mm diameter were used as inoculum. The dowels were placed for 3 weeks on 2% malt extract agar plates, which were either sterile or pre-colonized by *H. parviporum*. Wounded trees were inoculated with the sterile spruce dowels, and infected trees were inoculated with the dowels colonized by *H. parviporum*, and the inoculations were covered with Parafilm®. After 4 months, the extension of necrotic reactions was recorded from phloem and xylem surface. Non-parametric tests were used to compare the responses. For pair-wise comparisons between treatments, plant organs, and tissues, we used Wilcoxon signed rank test, and for between-clone comparison we used Kruskal-Wallis test. Trees with clear decay were excluded from the analysis. Five clones with too few valid cases were excluded from the analysis, and the analyses were based on the results from 10 clones. The necrosis measurements from each tree were aggregated so that up to four values, namely phloem and xylem responses both in roots and stems, remained for each tree. Statistical analyses were completed with PASW 18 Statistical package.

Results and Discussion

The overall necrotic responses due to infection were significantly stronger than those due to wounding both in phloem and xylem in roots and stems (Z significant at $P< 0.000$). The average length of necrosis in phloem was 51 mm and 37 mm in infected roots and stems compared to 16 mm and 20 mm in wounded roots and stems, respectively (Tab. 1).

Despite the clear overall difference between infected and wounded trees, the within-clone differences in the extension of necrosis between the two treatments were only nearly statistically significant for eight clones, and clearly insignificant

for two clones (V320, V483) (Z insignificant at P= 0.18 and P= 0.66, respectively). Root and stem responses differed significantly both in infected and wounded trees (Z significant at P= 0.01 and P= 0.04, respectively), but there were no statistically significant differences between roots and stems within clones. In infected stems, the average length of necrotic reactions was 37 mm in phloem compared to 28 mm in xylem, which was a statistically significant difference (Z significant at P= 0.002). In infected roots or in wounded trees, there were no significant differences between the phloem and xylem responses. Clone V330 showed notably weak average responses both to wounding (17 mm) and infection (21 mm) both in roots and in stem. In comparison, clone V375 responded strongly to infection both in roots (71 mm) and in stem (53 mm). Moreover, a provenance hybrid V477 exhibited significantly stronger responses in roots (106 mm) than in stems (31 mm). Due to the high within-clone variation and small sample size, the differences between the clones were not statistically detectable. This highlights the difficulty to breed for resistance against *Heterobasidion parviporum* only based on inoculation experiments.

Table 1. Extension of necrotic lesions in phloem and xylem in stems and roots. Results are means of two or three (V48 and V477: three infected trees) separate trees.

	Necrosis in stems (mm)								**Necrosis in roots (mm)**							
	Infection				Wounding				Infection				Wounding			
	Phloem		Xylem		Phloem		Xylem		Phloem		Xylem		Phloem		Xylem	
Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
V48	33	8.1	27	8.1	16	1.3	17	0.3	46	7.9	37	5	15	1.3	15	0.6
V315	22	5.8	22	1	15	2.8	19	4	21	15	31	6.8	14	0.8	18	0.5
V330	20	2	18	3.3	19	4.3	19	4.3	22	4	33	8.8	15	0.7	15	0
V374	39	7	27	2.3	22	2.5	20	0.8	64	12.5	41	2.5	23	4.5	19	2.5
V375	66	16	34	2.3	18	1	15	2	75	4	65*	-	15	1.5	16	2
V477	34	15.1	28	5.8	18	2.5	22	6.3	126	44.8	92*	-	20	3.5	16*	-
V481	35	4	31	1	18	4.3	19	1.5	28	10	34	14	15	1	15	0.7
V483	26	0.8	26	0.5	32	13	24	3.5	34	2.3	33	1	16	1	17	0.5
V494	65	29	43	16	24	7.5	28	12	35	1.8	30	1.8	17	1.3	18	1.3
V3027	34	3	29	2.8	17	0.3	17	2.3	56	14.3	50	13	18	1.5	18	2.5
Average	37	4.2	28	1.9	20	1.6	20	1.4	51	7	41	4	16	0.7	17	0.5

*Result represents only one tree.

References

Arnerup J., Swedjemark G., Elfstrand M., Karlsson B., Stenlid J., 2010. Variation in growth of *Heterobasidion parviporum* in a full-sib family of *Picea abies*. *Scandinavian Journal of Forest Research* 25: 106-110.

Stenlid J., Redfern D.B., 1998. Spread within the tree and stand. In: Woodward S., Stenlid J., Karjalainen R., Hüttermann A. (eds.). *Heterobasidion annosum* Biology, Ecology, Impact and Control. CAB International, Wallingford, Oxon OX10 8DE, UK, pp. 125-141.

Swedjemark G., Karlsson B., 2004. Genotypic Variation in susceptibility following artificial *Heterobasidion annosum* Inoculation of *Picea abies* clones in a 17-year-old field test. *Scandinavian Journal of Forest Research* 19: 103-111.

Swedjemark G., Stenlid J., 1997. Between-tree and between-isolate variation for growth of S-group *Heterobasidion annosum* in sapwood of *Picea abies* cuttings. *Canadian Journal of Forest Research* 27: 711-715.

von Weissenberg K., 1975. Variation in relative resistance to spread of Fomes annosus in four clones of *Picea abies*. *European Journal of Forest Pathology* 5: 112-117.

Molecular characterization of the expression and regulation of Scots pine (*Pinus sylvestris* L.) antimicrobial proteins (AMPs)

E. Jaber[1], S. Sooriyaarachchi[2], A. Sua´rez Covarrubias[3], W. Ubhayasekera[2], S.L. Mowbray[2], F.O. Asiegbu[1]

[1]*Department of Forest Sciences, University of Helsinki, Box 27, FI-00014 Helsinki, Finland.*
[2]*Department of Molecular Biology, Swedish University of Agricultural Sciences, Box 590, Biomedical Center, SE-751 24, Uppsala, Sweden.*
[3]*Department of Cell and Molecular Biology, Uppsala University, Box 596, Biomedical Center, SE-751 24, Uppsala, Sweden.*
Corresponding author e-mail address: jaber@mappi.helsinki.fi

Abstract. Scots pine (*Pinus sylvestris*) secretes a number of small, disulfide-rich proteins (Sp-AMPs) in response to challenges with fungal pathogens such as *Heterobasidion annosum*. We examined the expression patterns of these genes, as well as their structure and function. Northern blots and quantitative real time PCR showed increased levels of expression that are sustained during the interactions of host trees with pathogen, but not non-pathogens, consistent with a function in conifer tree defenses. Furthermore, the genes were up-regulated after treatment with salicylic acid and ethylene. Also, addition of exogenous glucan induced higher levels of Sp-AMP. The cDNA encoding one of the Sp-AMP proteins was cloned and expressed in *Pichia pastoris*. The purified protein exhibited antifungal activity against *H. annosum* and shown to bind soluble and insoluble b-(1,3)-glucans, specifically and with high affinity. Homology modeling and sequence comparisons suggest that Sp-AMP have a carbohydrate-binding site that can accommodate approximately four sugar units. We conclude that these proteins belong to a novel family of antimicrobial proteins (PR-19) that are likely to act by binding the glucans. In parallel to the above study, we further investigated the regulation of another defence related Pinus sylvestris defensin 1 (PsDef1) (1) gene. The results were discussed with reference to the impact of the fungal cell wall components in regulating the activity of AMP.

Scots pine (*Pinus sylvestris*) secretes a number of small, disulfide-rich proteins (Sp-AMPs) (Asiegbu *et al.,* 2003) in response to challenges with fungal pathogens such as *Heterobasidion annosum*. We examined the expression patterns of these genes, as well as their structure and function. *Sp-AMPs* expression level was investigated using northern blots and quantitative real time PCR during challenge with either a pathogenic (*Heterobasidion annosum*), mutualistic (*Lactarius rufus)* or saprotrophic (*Stereum sanguinolentum)* fungi. Results showed increased levels of expression that are sustained during the interactions of host trees with pathogen, but not non-pathogens (Fig. 1), consistent with a function in conifer tree defenses.

Furthermore, the genes were up-regulated after treatment with salicylic acid and ethylene, suggesting the involvement of both hormones in Sp-AMP regulation during biotic and abiotic stress. Also, addition of exogenous glucan induced higher levels of *Sp-AMP*. The cDNA encoding one of the Sp-AMP proteins was cloned

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

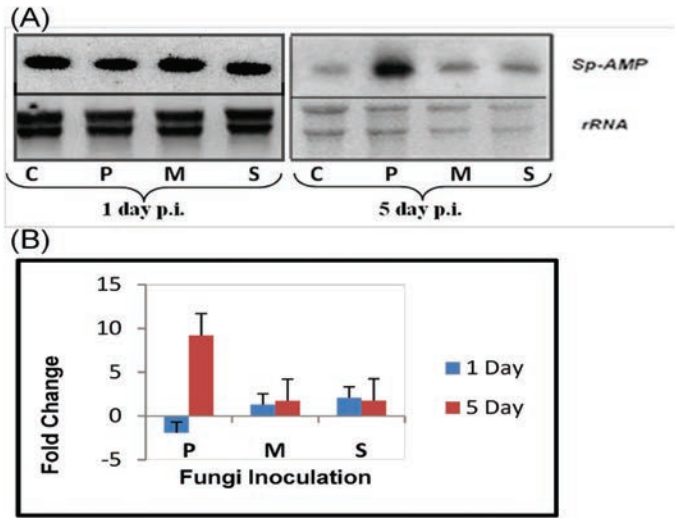


Figure 1. Effects of different functional groups of fungi on transcript abundance of the *Sp-AMP* genes. **(A)** In a Northern blot analysis, total RNA was extracted from roots inoculated with fungi that are pathogenic (*H. annosum*, P), beneficial/mutualistic (*L. rufus*, M) or saprotrophic (*S. sanquinolentum*, S), 1 and 5 days post-inoculation. The control seedlings (C) were inoculated with sterile water. **(B)** qRT-PCR was used to measure transcript abundance of *Sp-AMP* genes.). Data represent fold change of transcript abundance of *Sp-AMP* expressed in inoculated seedlings compared to that of the control seedlings inoculated with sterile water.

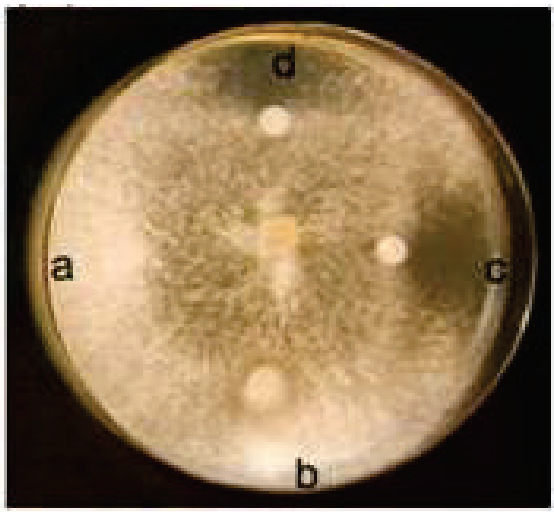


Figure 2. Sp-AMP3 inhibits *H. annosum* growth. The effects of various samples on *H. annosum* growth at 5 days, as follows: a. 20 mM HEPES, b. concentrated medium from the growth phase (before induction) of the transformed *Pichia* strain including Sp-AMP3 without His-tag, c. purified Sp-AMP3 without His-tag, d. purified Sp-AMP3 with His-tag.

and expressed in *Pichia pastoris*. Although the yield was very low, sufficient quantities were obtained from a *Pichia* expression system. The purified protein had antifungal activity against *H. annosum*, and caused an inhibition zone (Fig. 2). Binding studies revealed that it binds to soluble and insoluble beta-(1,3)-glucans, major components of the fungal cell wall, specifically and with high affinity. Homology modelling and sequence comparisons suggest that Sp-AMP have a carbohydrate-binding site that can accommodate approximately four sugar units. We conclude that these proteins represent a novel family of antimicrobial proteins, which we have named (PR-19), that are likely to act by binding the glucans that are a major component of fungal cell walls (Sooriyaarachchi *et al.,* 2001). In parallel to the above study, we further investigated the regulation of another defence related *Pinus sylvestris* defensin 1 (*PsDef1*) gene (Kovaleva *et al.,* 2009). *PsDef1* is significantly increased in Scots pine seedlings during germination and in their response to pathogenic infection with *Heterobasidion annosum* (Kovaleva *et al.,* 2011). We investigated the impact of the fungal cell wall components in regulating the activity of *PsDef1*. *PsDef1* expression was provoked by the treatments of exogenous chitin, chitosan or glucans only at prolonged time of the treatment (5 d.p.i.). Our results suggest that the fungal cell wall components have effect on the pine induction of the dual function *PsDef1* transcripts. Also, we conducted a complimentary study to investigate the possible mediatory role of salicylic acid, jasmonic acid and ethylene as signalling pathways in *PsDef1* regulation; our results indicate that *PsDef1* was up-regulated after treatment with ethylene precursor, ACC, suggesting the involvement of ethylene in *PsDef1* regulation.

References

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

Kovaleva V., Kiyamova R., Cramer R., Krynytskyy H., Gout I., Filonenko V., Gout R., 2009. Purification and molecular cloning of antimicrobial peptides from Scots pine seedlings. *Peptides* 30: 2136-2143.

Kovaleva V., Krynytskyy H., Gout I., Gout R., 2011. Recombinant expression, affinity purification and functional characterization of Scots pine defensin 1. *Applied Microbiology and Biotechnology*. 89: 1093-1101.

Sooriyaarachchi S., Jaber E., Covarrubias A.S., Ubhayasekera W., Asiegbu F.O., Mowbray S.L., 2001. Expression and b-glucan binding properties of Scots pine (*Pinus sylvestris* L.) antimicrobial protein (Sp-AMP). *Plant Molecular Biology* 77: 33-45.

Use of *in vitro* microcosm to validate the transcription pattern of two cysteine peptidases of *Heterobasidion annosum*

H. Chen and F.O. Asiegbu

Department of Forest Sciences, University of Helsinki, P.O. box 27, FI-00014, Helsinki, Finland.
Corresponding author e-mail address: fred.asiegbu@helsinki.fi

Abstract. *Heterobasidion annosum*, the root and butt rot pathogen of conifer trees can live as a saprotroph on dead wood and as a necrotroph on a living tree. The question is what kinds of genes play important roles during the transitional shift from one stage to the other? In this study, we investigated the regulatory pattern of two cysteine peptidase (bleomycin hydrolase and separin) under both growth stages. Peptidases are multifunctional enzymes involved in almost every aspect of physiology and development, also in signalling pathways and in the response to biotic and abiotic stresses. To investigate if they are involved in saprotrophic or necrotrophic growth stage, an in vitro microcosm was developed that mimics the natural infection biology of H. annosum. The pathogen pre-grown on dead Scots pine seedlings (saprotrophic stage) was allowed to spread to roots of neighboring living seedlings (pre-penetration necrotrophic stage). Quantitative real-time PCR results showed bleomycin hydrolase had higher expression during saprotrophic stage while separin had higher expression at pre-penetration necrotrophic stage, and both genes were down-regulated under all of the four tested salt stresses ($CaCl_2$, $MgCl_2$, NaCl and KCl). And the divalent salts had stronger negative influence on the gene expression than the monovalent salts. Our results suggest that bleomycin hydrolase and separin could have an important biological role during saprotrophic stage and pre-penetration necrotrophic stage, respectively. But both genes might not be involved in the tolerance to salt stress.

The root and butt rot pathogen, *Heterobasidion annosum*, can live as a saprotroph on dead wood and as a necrotroph on a living tree (Asiegbu *et al.,* 2005). The primary question is what kind of genes play important roles during the transitional shift from one stage to the other in this tree-pathosystem and how it can be assayed. In the plant-pathogen interaction, the degradation of exogenous proteins is essential for both plant resistance and pathogen survival. Intriguingly, cysteine peptidases are employed by both plants and their phytopathogen invaders at molecular battlefields (Shindo and van der Hoorn, 2008).
In plants, cysteine peptidases are used for protection against pests and pathogen attack. For example, papain was found to have negative influence on larval development (El Moussaoui *et al.,* 2001) and Pip1 and Rcr3 were found transcriptionally up-regulated and accumulated in the apoplast during pathogen challenge (Kruger *et al.,* 2002; Tian *et al.,* 2007).

In phytopathogenic bacteria, 21 cysteine peptidase effectors have been identified and classified into 4 families, YopJ family, XopD family, YopT family and AvrRpt2 family. Among these bacterial cysteine peptidase effectors, the

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

mechanism of AvrXv4, XopD, AvrPphB and AvrRpt2 in plant cells has been elucidated (Hotson and Mudgett, 2004).

The recent discovery of the cysteine peptidase effectors shows that the proteolysis of host substrates is an important strategy employed by their invaders to alter plant physiology. So do fungal pathogens also use cysteine peptidases as weapons too to invade and colonize the plants? Few cysteine peptidases have been studied in forest tree-pathogen interactions. In this study, we developed an *in vitro* microcosm which mimics the spread of the pathogen from decayed woody stump to adjacent living tree tissues, and used Real-time PCR to investigate the regulatory pattern of two cysteine peptidases under saprotrophic and pre-necrotrophic stages. The representatives of the saprotrophic and pre-necrotrophic stages were further validated by testing the expression of the genes on mature dead wood tissues as well as on a non-suberized living Scots pine seedlings with real-time PCR.

The *in vitro* microcosm (Fig. 1) was an apparent attempt to mimic the spread of the pathogen from decayed woody stump to adjacent living tree tissues. The results revealed that *H. annosum* was able to grow and utilize the dried dead seedlings as a carbon source. The fungal hyphae that grew out of the dead seedlings were also able to adhere to nearby living Scots pine seedlings. Closer microscopic examination revealed that the fungus was also able to develop infection structures to penetrate the tissues and caused necrosis on the roots of the seedlings.

The gene sequences of cysteine peptidase I and cysteine peptidase II encoded a protein of 500 and 653 amino acids, respectively. And the phylogenetic analysis showed that cysteine peptidase I could be assigned to cysteine peptidase clan CA, family C1, sub-family C1B (bleomycin hydrolase) and cysteine peptidase II to clan CD, C50 family (separase/separin).

Quantitative real-time PCR results showed that cysteine peptidase I had higher expression during saprotrophic stage, while cysteine peptidase II had higher expression at pre-necrotrophic stage (Fig. 2). The result of the expression during saprotrophic growth in microcosm was further confirmed during growth of the fungus on dead wood tissues which had similar expression pattern as on dead seedlings. Since it was very difficult to isolate the hyphae which grew inside the seedlings, as an alternative, we collected the hyphae around the living seedlings. So it only represented the pre-necrotrophic stage during contact of the fungus with the host. Both genes were also detected inside root tissues during necrotrophic growth, although it was impossible to quantify the expression level due to the different fungal biomass within the predominant plant tissue. Our results suggest that the microcosm we developed could successfully mimic the saprotrophic and pre-necrotrophic growth stage of *H. annosum*, and cysteine peptidase I and cysteine peptidase II might play an important biological role during saprotrophic stage and pre-necrotrophic stage, respectively.

References

Asiegbu F.O., Adomas A., Stenlid J., 2005. Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. *s.l. Molecular Plant Pathology* 6: 395-409.

El Moussaoui A., Nijs M., Paul C., Wintjens R., Vincentelli J., Azarkan M., Looze Y., 2001. Revisiting the enzymes stored in the laticifers of *Carica papaya* in the context of their possible participation in the plant defence mechanism. *Cellular and Molecular Life Sciences* 58: 556-570.

Hotson A., Mudgett M.B., 2004. Cysteine proteases in phytopathogenic bacteria: identification of plant targets and activation of innate immunity. *Current Opinion in Plant Biology* 7: 384-390.

Kruger J., Thomas C.M., Golstein C., Dixon M.S., Smoker M., Tang S.K., Mulder L., Jones J.D.G., 2002. A tomato cysteine protease required for Cf-2-dependent disease resistance and suppression of autonecrosis. *Science* 296: 744-747.

Shindo T., van der Hoorn R.A.L., 2008. Papain-like cysteine proteases: key players at molecular battlefields employed by both plants and their invaders. *Molecular Plant Pathology* 9: 119-125.

Tian M.Y., Win J., Song J., van der Hoorn R.A., van der Knaap E., Kamoun S., 2007. A *Phytophthora infestans* cystatin-like protein targets a novel tomato papain-like apoplastic protease. *Plant Physiology* 143: 364-377.

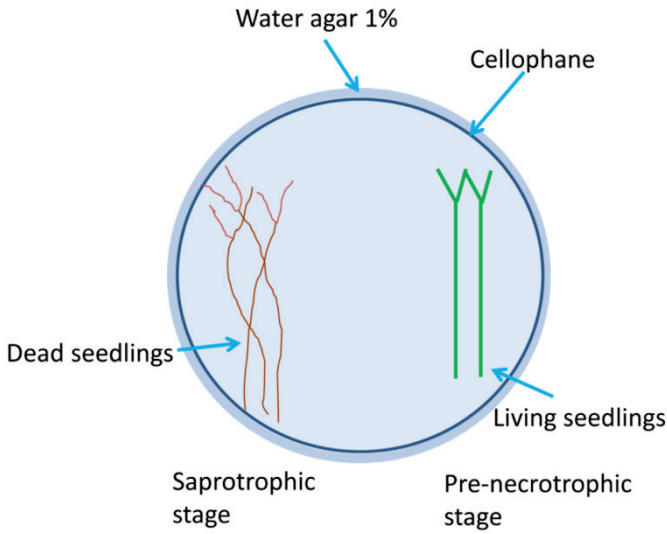


Figure 1. A schematic representation of the infection microcosm. The dead Scots pine seedlings (brown curve lines) colonized by *H. annosum* were transferred from malt extract agar medium to 1% water agar covered by cellophane. The mycelia grew by using the nutrient from the dead seedlings and the living seedlings (green lines) were transferred to the other side of the petri plate and contacted the mycelia directly. After 3, 7 and 15 days post inoculation, mycelia together with dead seedlings were harvested as saprotrophic stage, mycelia 1 cm surrounded living seedlings as necrotrophic stage and mycelia between dead seedlings and living seedlings as free-living stage.

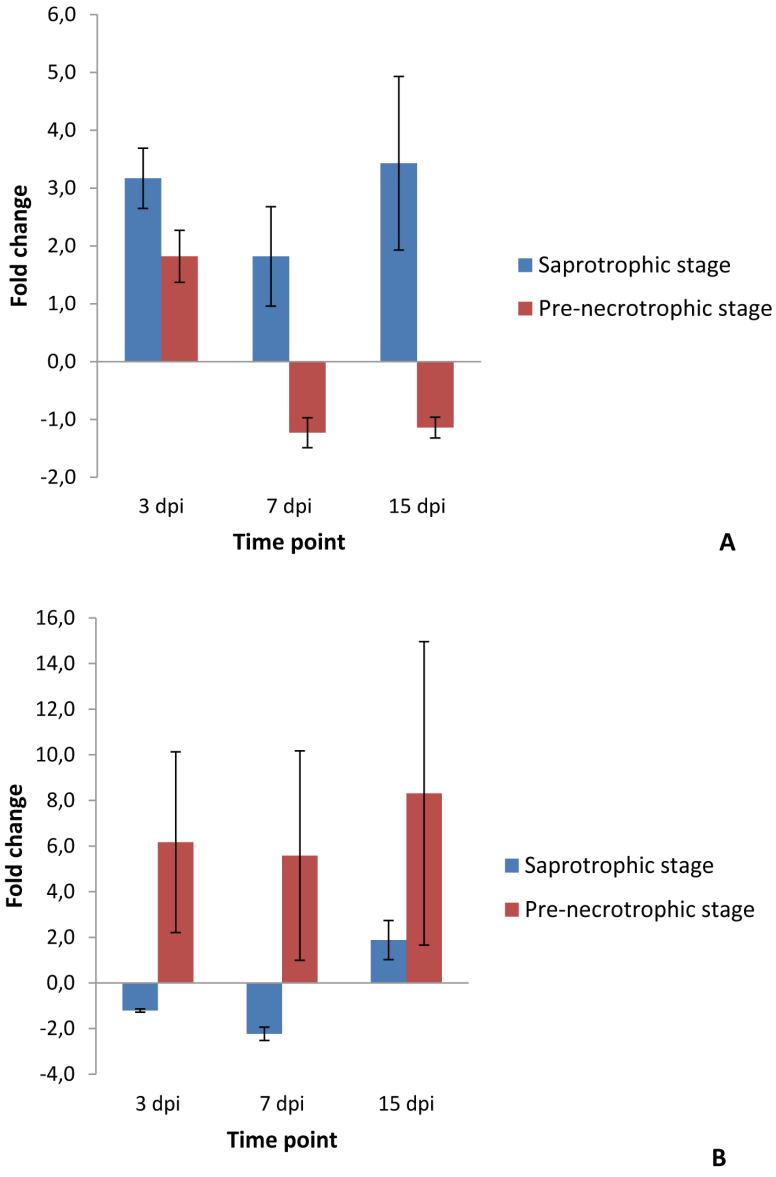


Figure 2. The transcription levels of cysteine peptidase I (A) and cysteine peptidase II (B) relative to GAPDH mRNA level among free-living, saprotrophic and pre-necrotrophic stages were measured by real-time PCR. Both expression levels of saprotrophic stage and pre-necrotrophic stage are compared to that of free-living stage. Positive number means the expression level is up-regulated and negative number means the expression level is down-regulated compared to the free-living stage. Three time points were tested: 3, 7, and 15 days post inoculation. Each point represented the mean + SEM from three independent experiments.

Molecular Analysis of Hydrophobins (*Pgh1* and *Pgh2*) from the biological control fungus, *Phlebiopsis gigantea*

A. Mgbeahuruike[1], H. Chen[1], W. Ubhayasekera[2], F.O. Asiegbu[1]

[1]*Department of Forest Sciences, University of Helsinki, Box 27, FI-00014, Helsinki, Finland.*
[2]*Department of Molecular Biology, Swedish University of Agricultural Sciences Uppsala, Sweden.*
Corresponding author e-mail address: Anthony.Mgbeahuruike@helsinki.fi

Abstract. Root and butt rot disease of conifers caused by *Heterobasion annosum s.l.* has been widely controlled with *Phlebiopsis gigantea*. We investigated the regulation of two hydrophobin genes (*Pgh1* and *Pgh2*) in strong and weak antagonistic isolates of *Phlebiopsis gigantea* under different substrate conditions. Transcript abundance of *Pgh1* was higher in single cultures of strong performing isolates than in the weak performing isolates at the early and late stages of the fungal growth ($P = 0.05$). High fold change of *Pgh1* and *Pgh2* was observed in the strong performing isolates than in weak performing group on sawdust media at the early stage of the antagonism. High transcript abundance of the two genes was also observed during growth in submerged and aerial condition ($P < 0.0001$ and $P = 0.0029$ for *Pgh1* and *Pgh2*, respectively). Both genes were up-regulated during emergence of aerial hyphae from submerged conditions. No correlation between antagonistic ability and sequence characteristics of either gene was found but a significant correlation was found between some strong performing isolates and the expression of *Pgh1*. Regulatory patterns of both *Pgh1* and *Pgh2* suggest a role during formation of aerial hyphae, and their potential roles in the biological control of *H. annosum* by *P. gigantea.*

Phlebiopsis gigantea has been widely used as the biocontrol fungus against the root and butt rot disease of conifers caused by *Heterobasion annosum* (Asiegbu *et al.,* 2005). Previous studies showed up-regulation of two hydrophobins *Pgh1* at the zone of interaction between the pathogen (*Heterobasidion annosum*) and the biocontrol fungus, *Phlebiopsis gigantea* (Adomas *et al.,* 2006). Hydrophobins are small secreted proteins with eight conserved cysteine residues and are involved in different roles in fungal life cycle (Kubicek *et al.,* 2008). We investigated the regulation of two hydrophobin genes (*Pgh1* and *Pgh2*) in high and low antagonistic isolates of the biological control agent *Phlebiopsis gigantea* (Mgbeahuurike *et al.,* 2011) under different treatment conditions. The evolutionary forces driving hydrophobin gene diversification in a subset of ecologically important fungi, the biocontrol agent (*Phlebiopsis gigantea*) and the conifer pathogen (*Heterobasidion annosum)* was also investigated. We also examined their evolutionary history by reviewing the distribution and copy numbers of hydrophobins in these two fungi as well as other fungal taxa. The phylogeny of hydrophobins was inferred from a subset of the selected fungal species. We examined a possible correlation between copy number of hydrophobin genes, overall genome size and their ecological strategy. Transcript abundance of thirteen hydrophobin genes from *H. annosum*

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

during saprotrophic growth on pine wood as well as on secondary metabolites from *P. gigantea* was further evaluated using micro-array. The protein structure of hydrophobins from a selected set of fungi was also modeled. The result revealed significant differences in the expression levels of all thirteen *H. annosum* hydrophobin genes which suggests possible differences in their regulatory patterns. Transcript abundance of *Pgh1* was higher in single cultures of high performing isolates than in the low performing isolates at the early and late stages of the fungal growth (P=0.05).

Higher fold transcript changes of *Pgh1* and *Pgh2* were observed in the high performing isolates at the early stage of the antagonistic interaction on modified Norkrans sawdust agar medium compared to the low performing isolates. Additionally, significant expansion of hydrophobin genes in basidiomycetes, (P=0.002) was documented while contraction was observed among the ascomycetes examined. Increased hydrophobin copy number appear to have significant effect on ecological strategy, with the non-pathogenic fungi having larger copies of hydrophobins than the pathogenic ones (necrotrophs; P=0.04). However, there was no significant relationship between hydrophobin copy number and genome size. Different folding patterns of the proteins were observed which may suggest differences in their ecological habits. Initial tests conducted show that *P. gigantea* and *H. annosum* hydrophobins may be under positive selection. The possible implication of these findings on their ecological habit will be discussed.

References

Adomas A., Eklund M., Johansson M., Asiegbu F.O., 2006. Identification and analysis of differentially expressed cDNAs during nonself-competitive interaction between *Phlebiopsis gigantea* and *Heterobasidion parviporum*. *FEMS Microbiology Ecology* 57: 26-39.

Asiegbu F.O., Adomas A., Stenlid J., 2005. Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. *s.l. Molecular Plant Pathology* 6: 395-409.

Kubicek C.P., Baker S., Gamauf C., Kenerley C.M., Druzhinina I.S., 2008. Purifying selection and birth-and-death evolution in the class II hydrophobin gene families of the ascomycete *Trichoderma/Hypocrea*. *BMC Evolutionary Biology* 8: 4.

Mgbeahuruike A.C., Hui S., Petra F., Risto K., Daniel J., Karlsson M., Asigbu F.O., 2011. Screening of *Phlebiopsis gigantea* isolates for traits associated with biocontrol of the conifer pathogen *Heterobasidion annosum*. *Biological Control* 57: 118-129.

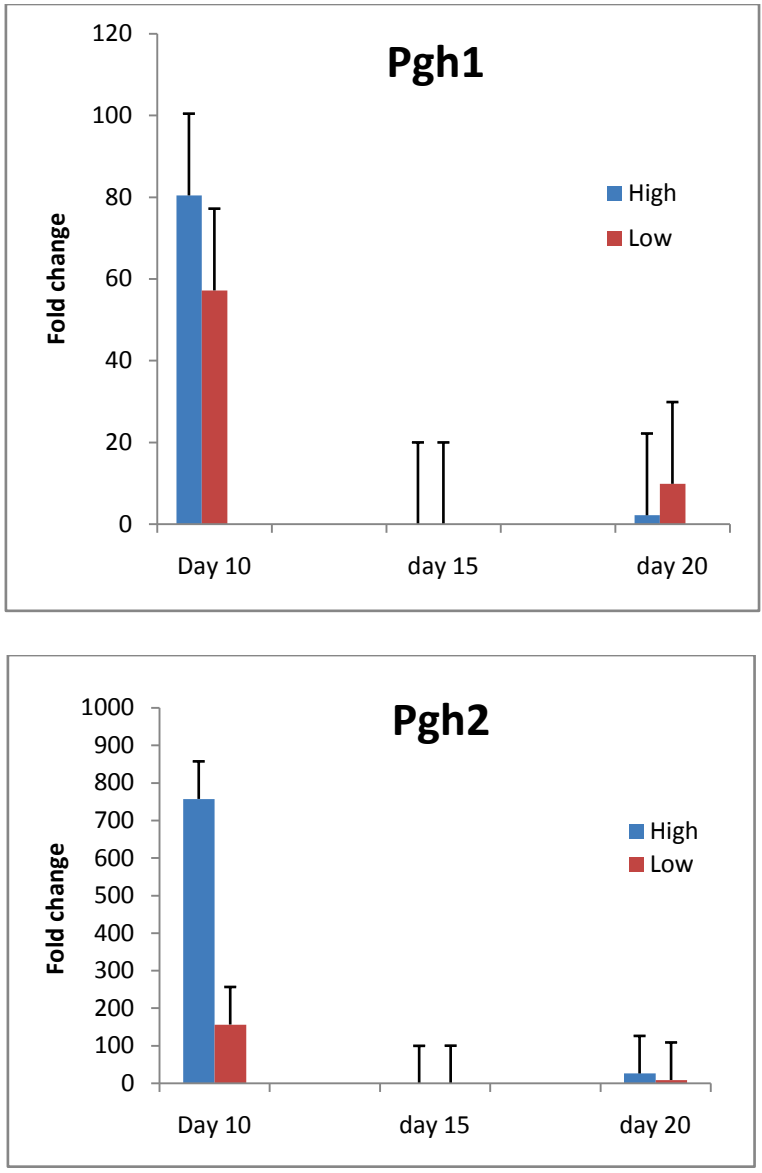


Figure 1. The expression profile of *Pgh1* and *Pgh2* in during antagonistic interaction of *H. annosum* and *P. gigantea*. *P. gigantea* was inoculated 70 mm from *H. annosum* on the same M/N sawdust media overlaid with cellophane membrane and cultures were harvested at 10, 15 and 20 d.p.i. Real Time RT-PCR was used to determine the expression patterns of the gene between the high performing isolates (blue bars) and the low performing isolates (red bars).

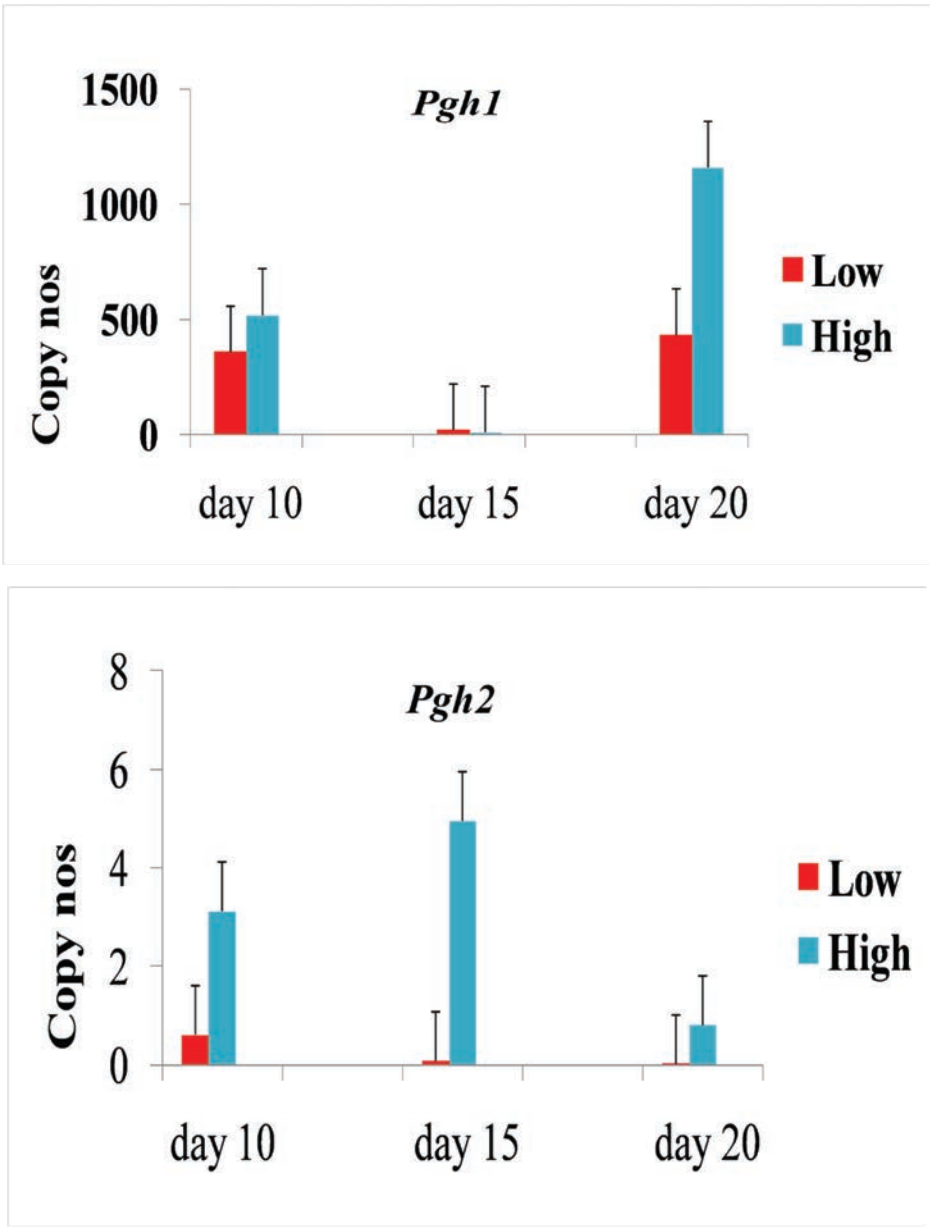


Figure 2. The expression profile of *Pgh1* and *Pgh2* during aerial hyphae formation in *P. gigantea*. *P. gigantea* was inoculated on M/N sawdust media overlaid with cellophane membrane and cultures were harvested at 10, 15 and 20 d.p.i. Real Time RT-PCR was used to determine the expression patterns of the gene between the high performing isolates (blue bars) and the low performing isolates (red bars).

Comparing pathogenicity and virulence of *Armillaria sinapina* and *Armillaria ostoyae* and host response to invasion on three conifer species

M. Cleary[1], B. van der Kamp[2], D. Morrison[3]

[1]*Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.*
[2]*University of British Columbia, Vancouver, BC, Canada.*
[3]*Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC, Canada.*
Corresponding author e-mail address: michelle.cleary@telia.com

Abstract. The relative pathogenicity and virulence of *Armillaria sinapina* on Douglas-fir, western hemlock and western redcedar was examined by i) comparing the frequency of fungal invasion on roots of 20-30 year trees following inoculation with *A. sinapina* and *A. ostoyae*, and ii) characterizing the anatomical changes in phloem (lignified impervious tissue and necrophylactic periderm) and cambial tissue (compartmentalization) following invasion by *A. sinapina*. Infection frequency did not differ between *A. sinapina* and *A. ostoyae* on the three hosts species. Inoculum potential was a key determinant of pathogenicity and virulence of *A. sinapina*. A negative correlation between lesion length and distance from inoculum source was observed. Root samples were challenged to a much greater degree with *A. ostoyae* than *A. sinapina* as evidenced by the larger proportion of roots showing no visible host response, larger zones of impervious tissue developed and the high frequency of breaching of both impervious tissue and necrophylactic periderm barriers. The intensity of host responses was greater in conifers infected with *A. ostoyae* than *A. sinapina,* and promoted further expansion of certain host responses in western redcedar involving induced rhytidome and traumatic resin duct formation in the phloem.

Six species of *Armillaria* are found in British Columbia, Canada, though most of these have only a limited occurrence and are not a concern to forest managers. *Armillaria ostoyae* (Romagn.) Herinkand *A. Sinapina* Bérubé & Dessureault have widespread occurrence in coastal and interior forests. The two species co-exist in the same forested stand, generally occupy the same ecological niche and can commonly be found on the same host. Traditionally *A. sinapina* is considered to be only weakly pathogenic and believed to exist mainly as a saprophyte. However, Morrison (Morrison, 2004) suggested that *A. sinapina* was capable of killing trees. Dettman and van der Kamp (Dettman and van der Kamp, 2001) also found *A. sinapina* killing more mature coniferous hosts and suggested that the species may be more pathogenic than previously thought.

There is a paucity of information concerning mechanisms involved in the infection biology of *A. sinapina* on conifers, and whether induced host reactions differ from those following invasion by *A. ostoyae*. The objective of this study was to assess the relative pathogenicity and virulence of *A. sinapina* on Douglas-fir [*P. menziesii* var. *glauca* (Beissn.) Franco], western hemlock [*Tsuga heterophylla*

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

(Raf.) Sarg.] and western red cedar (*Thuja plicata* Donn ex D.Don.) trees by comparing (i) the frequency of infection and lesion characteristics on roots following inoculation with *A. sinapina* and (ii) the anatomical changes in phloem and cambial tissue following infection by *A. sinapina* to that reported by Cleary *et al.* (Cleary *et al.,* 2011b) for *A. ostoyae* on the same host species.

In the field, roots of 20-30 year old trees were excavated and a single root was inoculated with *A. sinapina* previously grown on woody segments in the lab. The block and roots were reburied and harvested after 4 months and 1 year. Parallel investigations of host response to abiotic wounding and infection by *A. ostoyae* (Cleary *et al.,* 2011a,b) were also performed on separate trees within the same stand.Tissue samples werecryofixed and stored in liquid nitrogen. In the lab, frozen samples were sectioned in a cryostat and examined on a fluorescence microscope equipped with a freezing stage set at -35°C. The anatomical changes in cells leading to the formation of necrophylactic periderm (NP) formation in the bark and barrier zone formation associated compartmentalization of infected woody tissue were then characterized.

Inoculum blocks produced a profuse network of rhizomorphs sometimes extending up to 30 cm from the inoculum block.A negative correlation between lesion length and distance from inoculum source was observed. Infection frequency between *A. sinapina* and *A. ostoyae* did not differ on the three host species.However, large differences in the frequency of the types of host reactions occurred among species (Tab. 1).

Table 1. The frequency (%) of Douglas-fir, western hemlock and western redcedar root samples infected with *A. sinapina*and *A. ostoyae*after 4-5 months and 1 year showing no visible host response, initiation and breaching of impervious tissue (IT) and necrophylactic periderm (NP), respectively, androots compartmentalized and callused.

Host species		**Host Reaction Type**					
	Armillaria species	No host response	IT initiated	IT breached	NP formed	NP breached	Compart-mentalized& callused
Douglas-fir	*A. sinapina*	22	78	7	78	7	100
	A. ostoyae	60	40	50	31	57	10
Western hemlock	*A. sinapina*	20	80	5	72	22	45
	A. ostoyae	41	59	34	53	42	11
Western redcedar	*A. sinapina*	8	92	0	92	8	80
	A. ostoyae	2	98	0	98	17	92

Significantly more Douglas-fir and western hemlock roots infected with *A. ostoyae* showed no host response compared to those infected with *A. sinapina*. Nearly twice the percentage of Douglas-fir, and to a lesser extent, western hemlock infected with *A. sinapina* developed impervious tissue (IT) compared to those

infected with *A. ostoyae*. Breaching of IT and NP by *A. sinapina* occurred in fewer root samples compared to those infected with *A. ostoyae.* There were large differences in the frequency of compartmentalization and callusing between Douglas-fir roots and western hemlock roots infected with *A. ostoyae* and those infected with *A. sinapina*. No difference was found in the frequency of host reactions in western redcedar roots infected with either *Armillaria* species.

Of the three conifers studied, western hemlock was the least effective at containing *A. ostoyae,* and to a lesser extent *A. sinapina*. Resistant responses in western redcedar were consistently high when tested with both species of *Armillaria*. Root samples were challenged to a much greater degree with *A. ostoyae* than *A. sinapina* as evidenced by the larger proportion of roots showing no visible host response, larger zones of IT developed and the high frequency of breaching of both IT and NP barriers. Where damage from *A. sinapina* ensued, it was always associated with high inoculum potential.Thus, inoculum potential was a key determinant of pathogenicity and virulence of *A. sinapina*. Furthermore, the intensity at which certain host responses are formed in roots infected with *A. ostoyae* suggests a relationship between the degree of virulence of the *Armillaria* species and the intensity of the host response to infection.

A. ostoyae and *A. sinapina* are endemic throughout the southern interior of British Columbia. Climate models for British Columbia predict that large geographic areas of the southern interior will be under greater drought stress. These conditions could benefit *A. sinapina* which appears as more of an opportunistic species that flourishes under environmental stress. Behavioural changes of less virulent species of *Armillaria* under such stresses like climate change warrants further investigation.

References

Cleary M.R., van der Kamp B.J, Morrison D.J., 2011a. Effects of wounding and fungal infection with *Armillaria ostoyae* in three conifer species. I. Host response to abiotic wounding in non-infected roots. *Forest Pathology* [*in press*].

Cleary M.R., van der Kamp B.J., Morrison D.J., 2011b. Effects of wounding and fungal infection with *Armillaria ostoyae* in three conifer species. II. Host response to the pathogen. *Forest Pathology*. [*in press*].

Dettman J.R., van der Kamp, B.J., 2001. The population structure of *Armillaria ostoyae* and *Armillaria sinapina* in the central interior of British Columbia. *Canadian Journal of Botany*. 79: 600-611.

Morrison D.J., 2004. Rhizomorph growth habit, saprophytic ability and virulence of 15 *Armillaria* species. *Forest Pathology* 34: 15-26.

454 sequencing of transcriptomes for virulent and non-virulent *Armillaria ostoyae* strains and identification of their secretomes

G. Sipos[1,3], W. Qi[2], M. Künzli[2], M. Okoniewski[2], D. Rigling[1]

[1]*WSL, Swiss Federal Research Institute, Phytopathology Group, Birmensdorf, Switzerland.*
[2]*Functional Genomics Center, University of Zürich, Zürich, Switzerland.*
[3]*Institute of Silviculture and Forest Protection, University of West Hungary, Sopron, Hungary.*
Corresponding author e-mail address: gyoergy.sipos@wsl.ch

Abstract. Well growing mycelia were isolated from previously described virulent and non-virulent Armillaria ostoyae strains. While virulent mycelial fans were harvested from both autoclaved and fresh stems the non-virulent mycelia could be recovered only from autoclaved stems. Three full-length cDNA libraries were prepared and after normalization they were submitted to 454 sequencing. 1,34 million processed reads were available for transcriptome assembly and we identified 50,837 contigs (assembled reads) which fell into 19,658 isogroups. Traversal of contigs in the isogroups formed 45,286 individual transcripts from the libraries. About 60% of the isogroups had one transcript and 32% contained two to four transcripts. The potential coding sequences from the transcripts were identified with ESTScan. Score matrices, for codon preferences, were created using all mRNA sequence data from Basidiomycota and the unigene dataset of Filobasidiella neoformans. The assembled sequences were compared to the NCBI none redundant protein database and to the predicted protein dataset of Heterobasidion annosum. Altogether nearly 1,200 secretory proteins were identified based on direct screening for N-terminal signal peptides and looking for homologies to known secreted fungal proteins. Analysis of the differential expression profiles of the predicted genes, between virulent versus non-virulent strains, through exon-based microarrays is in preparation.

Armillaria ostoyae is considered as a primary pathogen within the *Armillaria* genus. Well growing mycelia were isolated from previously described virulent and non-virulent *Armillaria ostoyae* strains (Prospero *et al.,* 2004). We designed an in vitro system where segments of autoclaved and fresh spruce tree stems were infected in sterile jars. While virulent mycelial fans were harvested from the cambium of both autoclaved and fresh stems the non-virulent mycelia could be recovered only from autoclaved stems. We obtained stable total RNA extracts after grinding the fungal mycelia in liquid nitrogen and using Qiagen`s RNeasy kit. Three full-length cDNA libraries were prepared (Evrogen Mint kit) and after subsequent normalization (Evrogen`s Trimmer kit) they were submitted to 454 sequencing (Roche`s GS FLX). 1.34 million processed reads were available for the transcriptome assembly where 81.46% were assembled, 7.18% were partially assembled and 7.18% (95,306) were left as singletons. 50,837 contigs (assembled reads) were identified which fell into 19,658 isogroups. Traversal of contigs in the isogroups formed 45,286 isotigs reflecting the total number of individual transcripts from the libraries. Isotigs from the same isogroup can be inferred as splice-variants. About 60% of the isogroups had one transcript and 32% contained

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

two to four transcripts. The most dominant transcript length ranged from 400 to 1,200 nucleotides. The potential coding sequences from the transcripts were identified with ESTScan which requires codons preferences in the studied organism. Score matrices, for codon preferences, were created using all mRNA sequence data from *Basidiomycota* and the unigene dataset of *Filobasidiella neoformans*. The assembled sequences, using BLASTX with E value of 10-5 as the cutoff, were compared to the NCBI none redundant protein database and to the predicted protein dataset of *Heterobasidion annosum*. Based on the potential coding sequences, prediction of secretory proteins was performed using SignalP and SecretomeP. In addition, transcripts were compared to all secreted proteins downloaded from the Fungal Secretome KnowledgeBase (http://proteomics.ysu.edu/secretomes/fungi.php, 46,724 sequences from 53 fungal species). Altogether nearly 1200 secretory proteins were predicted based on direct screening for N-terminal signal peptides and looking for homologies to known secreted fungal proteins. Analysis of the differential expression profiles of the predicted genes, in terms of virulent versus non-virulent expression, using exon-based microarrays (Okoniewski *et al.,* 2007) is in preparation.

References

Okoniewski M.J., Yates T., Dibben S., Miller C.J., 2007. An annotation infrastructure for the analysis and interpretation of Affymetrix exon array data. *Genome Biology* 8: R79.

Prospero, S., Holdenrieder O., Rigling D., 2004. Comparison of the virulence of *Armillaria cepistipes* and *Armillaria ostoyae* on four Norway spruce provenances. *Forest Pathology* 34: 1-14.

A genome-wide association study identifies genomic regions for virulence in *Heterobasidion annosum s.s.*

K. Dalman, K. Himmelstrand, Å. Olson, M. Lind, M. Brandström-Durling, J. Stenlid

Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, P.O. Box 7026, S-75007 Uppsala, Sweden.
Corresponding author e-mail address: Kerstin.dalman@slu.se

Abstract. *Heterobasidion annosum* (Fr.) Bref. *sensu lato* (*s.l.*) is a necrotrophic pathogen that causes severe damage to coniferous forests in the Northern Hemisphere. A genome-wide association study analysing the virulence of *H. annosum sensu stricto* (*s.s.*) on spruce (*Picea abies*) and pine (*Pinus sylvestris*) using 23 homokaryotic haploid isolates was performed. The virulence of the isolates was measured as lesion length in the phloem and fungal growth within the sapwood following inoculations in the stem of 2-year-old pine and spruce seedlings. The fungal isolates were sequenced to between 2.6× and 12.6× coverage using the Illumina Genome Analyzer. This data set yielded 33,018 single nucleotide polymorphisms (SNPs), with a minor allelic frequency of at least two out of 23. These loci were present in all isolates. SNPs and mean values for each virulence trait were used for the association study. Twelve SNP markers distributed on seven contigs were found to be significantly associated with fungal virulence ($P< 0.0001$). These regions were characterized for linkage disequilibrium (LD) and gene contents. The LD blocks in these regions ranged between 1.2 and 31.2 kb when present. Nine genes encoding calcineurin, acetylglutamate kinase/synthase, cytochrome P450 monooxygenase, serine carboxypeptidase, quinone oxidoreductase (ToxD), two flavin-containing monooxygenases, exopolyphosphatase and a Swi5 transcription factor were identified as candidates for virulence.

The aim of this study was to conduct a genome-wide association (GWA) study, based on large scale single nucleotide polymorphism (SNP) identification, to identify virulence loci of *H. annosum sensu stricto* on *Picea abies* and *Pinus sylvestris*. The genomes of 23 *H. annosum s.s.* isolates were sequenced with an Illumina Genome Analyzer to a coverage between 2.6× and 12.6×. Virulence of *H. annosum s.s.* on *P. abies* and *P. sylvestris* was measured both as lesion length formed under the bark and fungal growth in the sapwood of the plants.

The general linear model implemented in TASSEL 2.1 (Bradbury *et al.,* 2007) revealed an association between 12 SNPs and virulence (Tab. 1). Six SNPs were found on contig 41,480; the remaining six SNPs were distributed on six different contigs. Four SNPs were associated with fungal growth in spruce (SFG) and the remaining eight with fungal growth in pine (PFG). The linkage disequilibrium (LD) heat maps show clear blocks of LD in the two contigs 4,128 and 41,480.

The LD block harbouring the SNP associated with SFG in contig 4,128 contained nine genes. They encode a serine protease, a transcriptional co-repressor, a quinone oxidoreductase (similar to ToxD, a host-selective toxin produced by *Pyrenophora tritici-repentis*), an inner centromere protein, a urea transporter, an

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

enzyme similar to a DNA-dependent-ATPase, a sorbitol dehydrogenase and two flavin-containing monooxygenases.

The LD block between 2.2 and 7.0 kb in contig 41,480 spanned a putative calcineurin and the end of an *N*-acetylglutamate kinase/synthase gene. Calcineurin is involved in calcium-dependent signal transduction pathways of many processes in eukaryotes. The protein confers a conserved function for virulence in several fungi, e.g. *Ustilago maydis* (Egan *et al.,* 2009). *N*-acetylglutamate is involved in the biosynthesis of arginine in prokaryotes, lower eukaryotes and plants (Kim *et al.,* 2007). An insertional mutation or deletion of genes for this protein lead to reduced virulence in *Gibberella zeae* (anamorph, *Fusarium graminearum*) (Kim *et al.,* 2007).

The marker associated with PFG in contig 9,600 is located to the transcription factor Swi5. The homolog of *Saccaromyces cerevisiae* SWI5 and ACE2 in *Candida albicans*, CaACE2, was shown to affect virulence (Kelly *et al.,* 2004).

In this study we show that GWA studies are useful for dissecting important complex traits of non-model organisms, such as fungi, that have small genomes and a haploid nature. We characterized seven genomic regions associated with fungal growth in the sapwood of spruce and pine and present candidate virulence genes.

Table 1. Significantly associated SNP markers (P_adj<1.00×10^{-4})

SNP Id	Contig	*P*_adj[a]	Trait[b]	Position in contig	Homolog in Hetan2[c]	Gene
2,541	4,128	1.00×10^{-4}	SFG	41,529	2:90,374	-
4,981	9,600	1.00×10^{-4}	PFG	30,323	1:562,288	SWI5 transcription factor
6,505	15,627	1.00×10^{-4}	SFG	792	13:53,143	-
7,164	16,590	1.00×10^{-4}	PFG	17,829	10:1,046,969	Exopolyphosphatase
31,888	41,480	1.00×10^{-4}	PFG	4,151	13:580,629	Putative calcineurin
31,890	41,480	1.00×10^{-4}	PFG	4,348	13:580,826	Putative calcineurin
31,891	41,480	1.00×10^{-4}	PFG	4,414	13:580,892	Putative calcineurin
31,898	41,480	1.00×10^{-4}	PFG	8,609	13:575,283	-
31,912	41,480	1.00×10^{-4}	PFG	11,461	13:569,318	Unknown
31,915	41,480	1.00×10^{-4}	PFG	12,127	13:569,985	Unknown
37,656	45,322	1.00×10^{-4}	SFG	6,635	3:163,145	Unknown
51,971	50,191	1.00×10^{-4}	SFG	17,454	5:438,018	-

[a]*P*-value adjusted after 10,000 permutations

[b]SFG, fungal growth in spruce sapwood, upstem and downstem combined; PFG, fungal growth in pine sapwood, upstem and downstem combined

[c]Scaffold: Position for the homolog position in *Heterobasidion annosum* v2.0, http://genome.jgi-psf.org/Hetan2/Hetan2.home.html

References

Bradbury P.J., Zhang Z., Kroon D.E., Casstevens T.M., Ramdoss Y., Buckler E.S., 2007. TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23: 2633-2635.

Egan J.D., García-Pedrajas M.D., Andrews D.L., Gold S.E., 2009. Calcineurin is an antagonist to PKA protein phosphorylation required for postmating filamentation and virulence, while PP2A is required for viability in Ustilago maydis. *Molecular Plant-Microbe Interactions* 22: 1293-1301.

Kelly M.T., MacCallum D.M., Clancy S.D., Odds F.C., Brown A.J.P., Butler G., 2004. The *Candida albicans CaACE2* gene affects morphogenesis, adherence and virulence. *Molecular Microbiology* 53: 969-983.

Kim J.E., Myong K., Shim W.B., Yun S.H., Lee Y.W., 2007. Functional characterization of acetylglutamate synthase and phosphoribosylamine-glycine ligase genes in *Gibberella zeae*. *Current Genetis* 51: 99-108.

Terpenes as markers for relative resistance of Sitka spruce clones to *Heterobasidion annosum*

V. Martini[1], S. Woodward[2], G. Deflorio[2], P. Capretti[1], M. Michelozzi[3]

[1]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università di Firenze, Firenze, Italy.*
[2]*University of Aberdeen, IBES, Plant and Soil Science, Aberdeen, UK.*
[3]*CNR-IGV, Via Madonna del Piano, 50019, Firenze, Italy.*
Corresponding author e-mail address: s.woodward@abdn.ac.uk

Abstract. Variations in *Picea sitchensis* resin terpene composition following inoculation with *Heterobasidion annosum* were determined in bark. Four clones, two relatively susceptible and two relatively resistant, of Sitka spruce growing at Scootmore, Moray, Scotland, were wounded and inoculated with the pathogen. There were significant differences in constitutive terpene profiles between different clones. Moreover, proportions of several monoterpenes varied in secondary resin produced in bark tissues surrounding the lesions. Total absolute quantities of monoterpenes were significantly higher in secondary than in primary resin in both resistant and susceptible clones. This effect was more evident in tissues following inoculation, compared with the pseudo-inoculated samples. These findings are discussed in the context of ecological interactions and demonstrate that terpenoid metabolism may provide a useful biochemical marker for resistance to *H. annosum* in selection and breeding programmes.

Introduction

Variations in *Picea sitchensis* resin terpene composition following inoculation with *Heterobasidion annosum* were determined in bark. Four clones, two relatively susceptible and two relatively resistant, were wounded and inoculated with *H. annosum*. There were differences in constitutive terpene profiles between clones. Proportions of monoterpenes varied in secondary resin in bark tissues around the lesions. Total quantities of monoterpenes were higher in secondary than in primary resin in both resistant and susceptible clones, an effect more evident following inoculation, compared with pseudo-inoculated samples.

Spruce produce many secondary metabolites, some of which may help to repel attack by pests and pathogens (Croteau *at al.,* 2000). Following damage, traumatic resin forms, with a different overall composition to the primary resin. Previous work suggested that terpene profiles of spruce may be used markers for relative susceptibility to *Heterobasidion* (Woodward *et al.,* 2007). The aim of the work reported here was to examine changes in terpene profiles of mature clones of *P. sitchensis* following inoculation with *H. annosum*.

Materials and Methods

Terpene composition was analyzed in bark of the four 20 year old Sitka spruce clones described elsewhere (Deflorio *et al.,* 2011), forming the shortest (20,198

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

and 20,206) and longest (20,179 and 20,204) lesions after inoculation with *H. annosum*. Bark was prepared and analysed as described previously (Woodward *et al.,* 2007).

Results and Discussion

Thirteen monoterpenes were found in bark of the clones. Relative proportions of (+)-α-pinene, myrcene, sabinene and terpinolene differed between clones ($P< 0.05$; Fig. 1). Proportions of myrcene and terpinolene were higher in clones 20,204, 20,198 and 20,206 than in 20,179. These results were confirmed when data were expressed as mg monoterpenes/g tissue.

Increased total terpene concentrations were found with both treatments in all clones, an effect more evident in infected tissues 43 days after inoculation in clones 20,179, 20,198 and 20,206 than in 20,204.

By day 43, wounded and inoculated tissues had higher terpene concentrations than wounded treatments in clones 20,179, 20,198, and 20,206. Position of sampling in relation to the point of wounding and inoculation had no effect on terpene concentrations.

Samples collected at day 3 days had higher amounts of (-)-α-pinene, myrcene, β-phellandrene and γ-terpinene in the wounded and wounded plus inoculated than in control tissues in clones 20,179, 20,204 and 20,206 ($P< 0.05$).

Concentrations of (+)-α-pinene increased following wounding and wounding plus inoculation in clones 20,198, 20,204 and 20,206; in clone 20,179, highest amounts were in wounded bark. Myrcene concentrations also significantly increased after wounding and wounding plus inoculation treatments in all four clones.

Further studies on the use of terpenes as markers in the selection of less susceptible Sitka spruce chemotypes may be warranted, using greater numbers of clones.

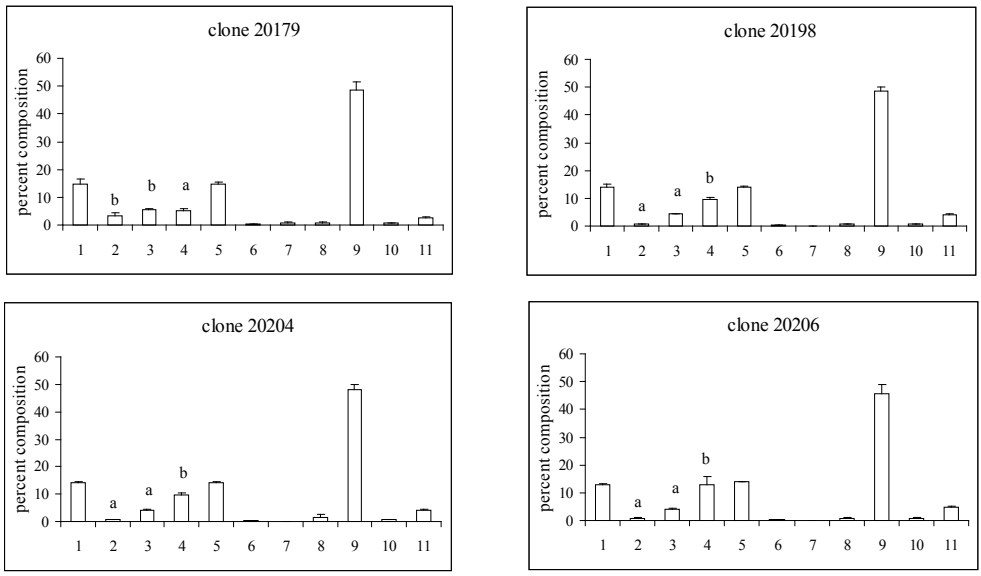


Figure 1. Proportions of constitutive terpenes in clones of Sitka spruce. 1, (–)-α-pinene; 2, (+)-α-pinene; 3, sabinene; 4, myrcene 5, (–)-β-pinene; 6, (+)-β-pinene; 7, (–)-limonene; 8, (+)-limonene; 9, β-phellandrene; 10, γ-terpinene; 11, terpinolene.

References

Croteau R., Kutchan T.M., Lewis N., 2000. Natural products (secondary metabolities). In: Buchanan B., Gruissem W., Jones R., (eds.). *Biochemistry and Molecular Biology of Plants, American Society of Plant Physiologists*. pp. 1250-1318.

Deflorio G., Horgan G., Woodward S., Fossdal C.G., 2011. Gene expression profiles, phenolics and lignin of Sitka spruce bark and sapwood before and af ter wounding and inoculation with *Heterobasidion annosum*. *Physiological and Molecular Plant Pathology* 75: 180-187.

Woodward S., Bianchi S., Bodles W.J.A., Beckett L., Michelozzi M., 2007. Physical and chemical responses of Sitka spruce (*Picea sitchensis*) clones to colonization by *Heterobasidion annosum* as potential markers for relative host susceptibility. *Tree Physiology* 27: 1701-1710.

^{1}H NMR fingerprinting detects defence response in Sitka spruce inoculated with *Heterobasidion annosum*

G. Deflorio[1], G. Horgan[2], S. Woodward[1], M. Jaspars[3]

[1]*University of Aberdeen, IBES, Plant and Soil Science, Aberdeen, UK.*
[2]*BioSS, Aberdeen, UK.*
[3]*University of Aberdeen, Chemistry, Aberdeen, UK.*
Corresponding author e-mail address: giulianade@gmail.com

Abstract. Using ^{1}H NMR, differences in metabolite profiles were observed in bark and sapwood tissues of Sitka spruce (*Picea sitchensis*) in different clones of Sitka spruce following inoculation with *Heterobasidion annosum*. Compared to samples collected before treatment, wounding and inoculation in bark lead to increasing numbers and quantities of aromatic compounds, whereas in sapwood lower amounts of all metabolites were observed in inoculated samples. Multivariate statistical analysis (ANOVA-PCA) showed highly significant effects of reference, position, and time (PC1), and significant effects of clone and fungus. Differences between clones apparent in sapwood were due to peaks in the aliphatic and carbohydrate regions. In bark carbohydrate peaks decreased with time, with a concomitant increase in aliphatic and aromatic peaks. In contrast, all peaks decreased in sapwood extracts, followed by an increase in carbohydrate and aliphatic peaks. Changes in carbohydrate concentrations were greater within the lesion compared to more distal sampling points on in both bark and sapwood.

Introduction

^{1}H NMR showed differences in metabolite profiles in bark and sapwood of different Sitka spruce clones after inoculation with *Heterobasidion annosum*. Wounding and inoculation in bark lead to increased quantities of aromatic compounds, whereas in sapwood all metabolites decreased in inoculated samples. Statistical analysis showed highly significant effects of reference, position, and time and significant effects of clone and fungus. Differences between clones seen in sapwood were due to peaks in the aliphatic and carbohydrate regions. In bark carbohydrate peaks decreased with time, whilst aliphatic and aromatic peaks increased. All peaks decreased in sapwood extracts followed by an increase in carbohydrate and aliphatic peaks.

Defences against fungal pathogens in spruce include cell wall lignification and suberisation in invaded bark tissues, preformed chemicals and antifungal proteins (Pearce, 1996; Franceschi *et al.,* 2005). ^{1}H nuclear magnetic resonance (^{1}H NMR) - based techniques have been applied in several areas of research (Holmes *et al.,* 2006), including stresses caused by fungi. NMR, however, has not been used to study metabolites in bark and sapwood of trees after challenge with pathogens.

In this work, metabolite fingerprints were determined in bark and sapwood of 20 year old Sitka spruce clones using ^{1}H NMR, after wounding and inoculation with *Heterobasidion annosum*.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Materials and Methods

Sample trees, inoculations and sampling methods used are described elsewhere (Deflorio *et al.,* 2011).

Samples were milled, the powder extracted twice in 1.5 ml H_2O/methanol (60:40) and combined extracts dried. The residue was dissolved in 0.1 M phosphate buffer, pH 7, with 1 mM TSP. NMR spectra were recorded in CD_3OD on a Varian INOVA spectrometer interfaced to a 9.4-T magnet (resonance frequency 400 MHz, 291 K, spin 20 Hz). Spectral processing used standard methods (Veselkov *et al.,* 2009). Data were analysed using principal component analysis (PCA), and split plot ANOVA. F-statistics were used to find peaks which differed between levels of the fixed effects. All statistical analyses were carried out with R v.2.8.

Results and Discussion

Carbohydrate and aromatic regions dominated ^{1}H NMR spectra in bark (Fig. 1a); in sapwood the aliphatic peaks dominated (Fig. 1b). The main effects of the treatments were:

- In bark, no clear separation occurred between resistant and susceptible clones in ANOVA-PCA.
- In sapwood, a resistant clone clustered apart from all others along PC2.
- Samples from wounded and wounded and inoculated treatments clustered in PCA; separation from healthy bark was not apparent. Sapwood showed a similar pattern.
- Over time, carbohydrate peaks decreased in bark, with increases in aliphatic and aromatic peaks.
- All peaks decreased in sapwood, followed by increases in carbohydrate and aliphatic peaks.
- Tissues sampled distal to wounds had more sugars than at the point of inoculation.

This work showed that metabolic processes differed in bark and sapwood of Sitka spruce. Identification of individual compounds in the spruce metabolome will enable full interpretation of roles of different metabolites as constitutive or induced defence compounds.

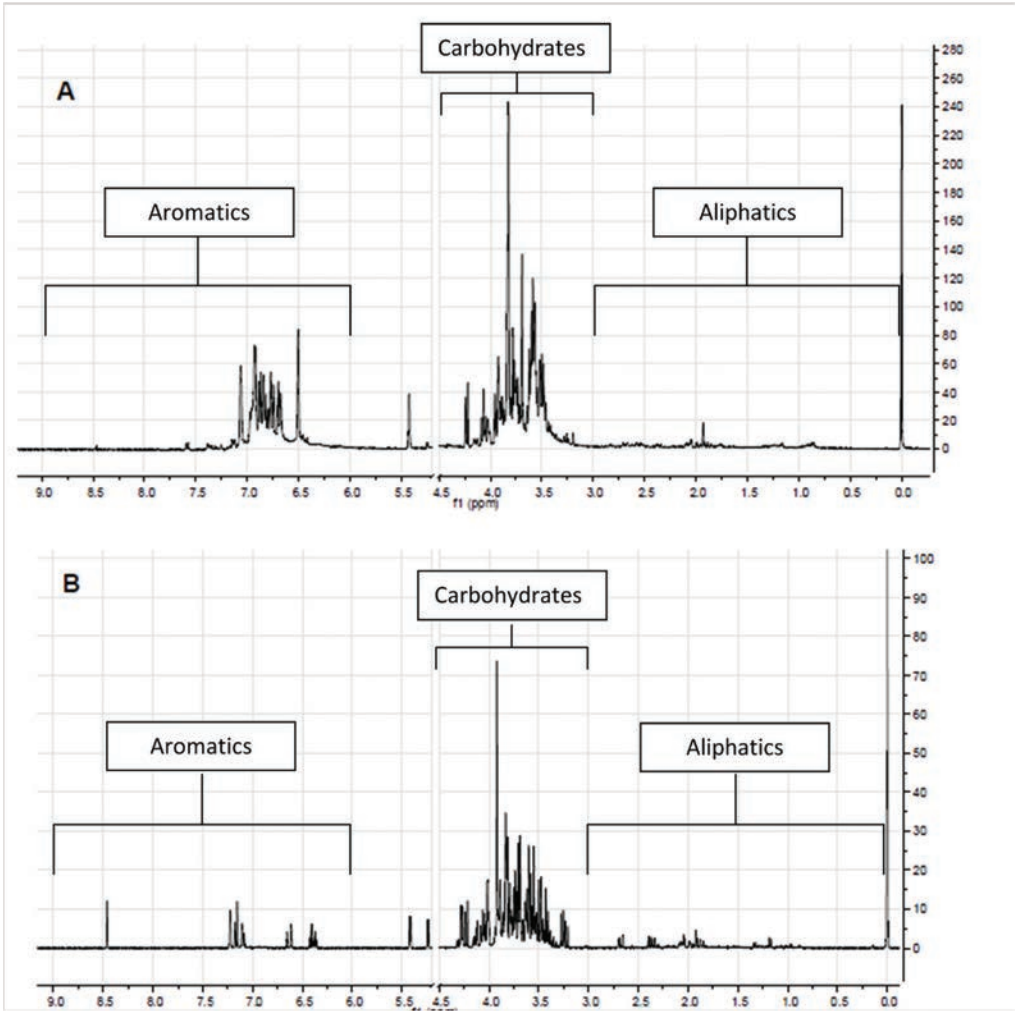


Figure 1. ^{1}H NMR fingerprints for (A) bark and (B) sapwood from healthy Sitka spruce.

References

Deflorio G., Horgan G., Woodward S., Fossdal C.G., 2011. Gene expression profiles, phenolics and lignin of Sitka spruce bark and sapwood before and after wounding and inoculation with *Heterobasidion annosum*. *Physiological Molecular Plant Pathology* 75: 180-187.

Franceschi V.R., Krokene P., Christiansen E., Krekling T., 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist* 167: 353-376.

Holmes E., Tang H., Wang Y., Seger C., 2006. The assessment of plant metabolite profiles by NMR-based methodologies. *Planta Medica* 72: 771-785.

Pearce R.B., 1996. Antimicrobial defences in the wood of living trees. *New Phytologist* 132: 203-233.

Veselkov K.A., Lindon J.C., Ebbels T.M.D., Crockford D., Volynkin V.V., Holmes E., Davies D.B., Nicholson J.K., 2009. Recursive segment-wise peak alignment of biological (1)h NMR spectra for improved metabolic biomarker recovery. *Analytical Chemistry* 81: 56-66.

Distribution of elements in the bark of Sitka spruce following wounding and inoculation with *Heterobasidion annosum*

M. Siebold[1], P. Leidich[2], M. Bertini[2], G. Deflorio[1], J. Feldmann[2], E. Krupp[2], E. Halmschlager[3], S. Woodward[1]

[1]*University of Aberdeen, IBES, Plant and Soil Science, Aberdeen, UK.*
[2]*University of Aberdeen, Chemistry, Aberdeen, UK.*
[3]*Universität für Bodenkultur Wien, Austria.*
Corresponding author e-mail address: s.woodward@abdn.ac.uk

Abstract. Element distribution in the bark of two 20 year old clones of *Picea sitchensis* showing greater or lesser susceptibility to extension growth of *Heterobasidion annosum* was studied using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Clones were wounded and artificially inoculated with *H. annosum* and bark sampled 0, 3, and 43 days later for analysis using a focused Nd:YAG laser. Intensities of ^{13}C, ^{25}Mg, ^{27}Al, ^{31}P, ^{32}S, ^{39}K, ^{48}Ca, ^{55}Mn, ^{57}Fe, ^{63}Cu and ^{64}Zn were measured by ICP-MS to determine elemental distribution across the bark samples. There was a clear accumulation of Mg, P and K at the boundary zone between the lesion and healthy tissue in the wounded and inoculated samples of both clones, more distinctively at 43 than at 3 days after wounding and inoculation. These accumulations suggest a major role of Mg, P and K in the non-specific response of Sitka spruce both to wounding and to artificial inoculation with *H. annosum*, possibly in roles as co-factors to enzymes and in energy utilisation.

Introduction

Element distribution in bark of two 20 year old clones of *Picea sitchensis* showing greater or lesser susceptibility to *Heterobasidion annosum* was studied using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Intensities of ^{13}C, ^{25}Mg, ^{27}Al, ^{31}P, ^{32}S, ^{39}K, ^{48}Ca, ^{55}Mn, ^{57}Fe, ^{63}Cu and ^{64}Zn were measured by ICP-MS to determine elemental distribution across the bark samples.

Clear accumulation occurred of Mg, P and K at the boundary zone between the lesion and healthy tissue in the wounded and inoculated samples of both clones, more distinctively at 43 than at 3 days after wounding and inoculation. These accumulations suggest a major role of Mg, P and K in the response of Sitka spruce to wounding and to artificial inoculation with *H. annosum*, possibly in roles as co-factors to enzymes and in energy utilisation.

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a highly sensitive technique widely utilised for multi-element analysis of solid samples (Perkins *et al.,* 1991). The objective of this study was to develop a LA-ICP-MS method suitable for the analysis of element distribution in Sitka spruce bark following wounding and inoculation with *H. annosum.*

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Materials and Methods

Sample trees, inoculations and sampling methods used are described elsewhere (Deflorio *et al.,* 2011). Bark samples were polished using a stainless steel microtome blade, mounted on glass slides and dried at 25°C for one week. Laser ablation (Nd:YAG, LSX 200+, CETAC, Omaha, US) was carried out using DIGI LAZ™ software. All isotopes listed in Tab. 1 were measured simultaneously for each scan. For each path, one pre-ablation was carried out before data-gathering in order to remove any surface contamination remaining from the sample preparation process.

Results and Discussion

LA-ICP-MS demonstrated that Mg, K and P accumulated at the boundary between necrotic and healthy tissues. No changes in the distributions of other elements analysed (Ca, Mn, Al, Fe, Cu, Zn, S, C). A 2-D scan proved that these accumulations were associated with ligno-suberized boundary zone and wound periderm formation (LSZ/WP; Fig. 1). The intensity of the response was more marked at 43 days after treatment than at 3 days. There were no clear differences in the accumulation of these elements between resistant and susceptible clones of Sitka spruce.

These results indicate that P, Mg and K are important for the host responses occurring following wounding, during the de-differentiation and redifferentiation process that occurs in the host cortex tissues in order for wound closure to occur. The elements are probably required for the enhanced enzymatic and energy activity in the LSZ and WP.

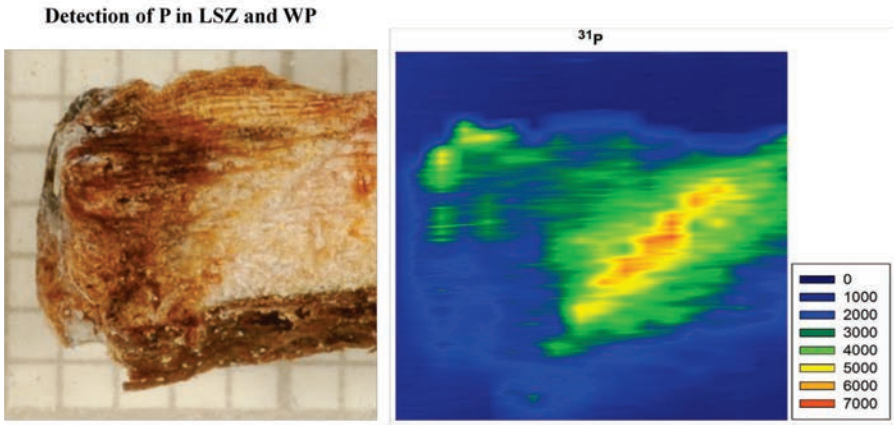


Figure 1. LA-ICP-MS detection of ^{31}P in bark of Sitka spruce following wounding. The legend indicates relative intensity of ^{31}P. Highest intensity is in the LSZ/WP.

References

Deflorio G., Horgan G., Woodward S., Fossdal C.G., 2011. Gene expression profiles, phenolics and lignin of Sitka spruce bark and sapwood before and after wounding and inoculation with *Heterobasidion annosum*. *Physiological Molecular Plant Pathology* 75: 180-187.

Perkins W.T., Fuge R., Pearce N.J.G., 1991. Quantitative analysis of trace elements in carbonates using laser ablation inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry* 6: 445-449.

Comparative genomic analysis of rot fungi: insights into the evolution of specialized functions

G. Emiliani[1], G. Sablok[2], N. La Porta[2]

[1]*Trees and Timber Institute, IVaLSA-CNR, S. Michele a/Adige (TN).*
[2]*Sustainable Agro-ecosystems and Bioresources Department, IASMA Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, (TN), Italy.*
Corresponding author e-mail address: nicola.laporta@fmac.it

Abstract. The availability of completely sequenced genomes offers the possibility to analyze molecular evolutionary processes like gene duplication and the functional fate of paralogous genes possibly leading to functional innovations. The aim of the present work are to apply *in silico* analysis approaches to three fungi showing different ecological role and functions in forest ecosystems; the pathogen *Heterobasidion annosum s.l.* is widely recognised as the agent of devastating root rot in conifer stands and plantations. *Postia placenta* and *Phanerochaete chrysosporium* on the contrary are the responsible of wood decay processes known, respectively, as brown rot (depolymerization of cellulose without significant lignin removal) and white rot (simultaneous degradation of lignin and cellulose). The comparison of duplicate genes content in the 3 genomes and the functional properties of paralogous gene families may explain the differential lifestyle and ecological niche occupied by fungi. Preliminary data shows a different amount of duplicated genes in the 3 genomes: in *H. annosum* 3,344 (27%) genes out 12,270 predicted gene models are duplicated while in *P. chrysosporium* 3,679 out of 10,480 (35%) and 3,439 out of 9,113 (37%) in *P. placenta*. The comparative analysis of orthologous genes among the three genomes showed that there are 4,916, 2,188 and 2,181orthologs between *H. annosum and P. chrysosporium, H. annosum* and *P. placenta, P. chrysosporium* and *P. placenta,* respectively. From a biological perspective the paralogous gene families are not randomly distributed among functional categories showing that gene duplication and loss is highly constrained by the functional properties and interacting partners of genes. Ad example, stress-related genes exhibit many duplications, whereas basal processes like growth-related genes show selection against such changes. The ongoing comparative analysis of paralogous gene families across the three 3 genomes will possibly enable to shed some light on the evolution of specialized ecological peculiar features of the rot and pathogen fungi.

Gene duplication has been recognized as a major force in shaping genomes and in the evolution of metabolic innovations through the functional divergence of paralogous genes (Wapinski *et al.,* 2007).

The availability of completely sequenced genomes offers the possibility to analyze evolutionary processes and to perform comparative genomic analyses to shed some light on metabolic divergence of organisms.

Therefore, the aim of the present work was to apply *in silico* analysis approaches to three fungi showing different ecological roles and functions in forest ecosystems; the pathogen *Heterobasidion annosum s.l.* is widely recognised as the agent of root rot in conifer stands and plantations. *Postia placenta* (Martinez *et al.,* 2009) and *Phanerochaete chrysosporium* (Martinez *et al.,* 2004), on the contrary, are the responsible of wood decay processes known, respectively, as brown rot (depolymerization of cellulose without significant lignin removal) and white rot (simultaneous degradation of lignin and cellulose).

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

The comparison of duplicate genes content in the 3 genomes and the functional properties of paralogous gene families may explain the differential lifestyle and ecological niches occupied by 3 fungi.

Preliminary data show a different amount of duplicated genes in the 3 genomes: in *H. annosum* 3,344 (27%) genes out 12,270 predicted gene models are duplicated while in *P. chrysosporium* 3,679 out of 10,480 (35%) and 3,439 out of 9,113 (37%) in *P. placenta*. The comparative analysis of orthologous genes (Fig. 1) among the three genomes showed that there are 4,916, 2,188 and 2,181orthologs between *H. annosum* and *P. chrysosporium, H. annosum* and *P. placenta, P. chrysosporium* and *P. placenta,* respectively. 2,040 orthologous genes are shared by 3 genomes building their "core genome".

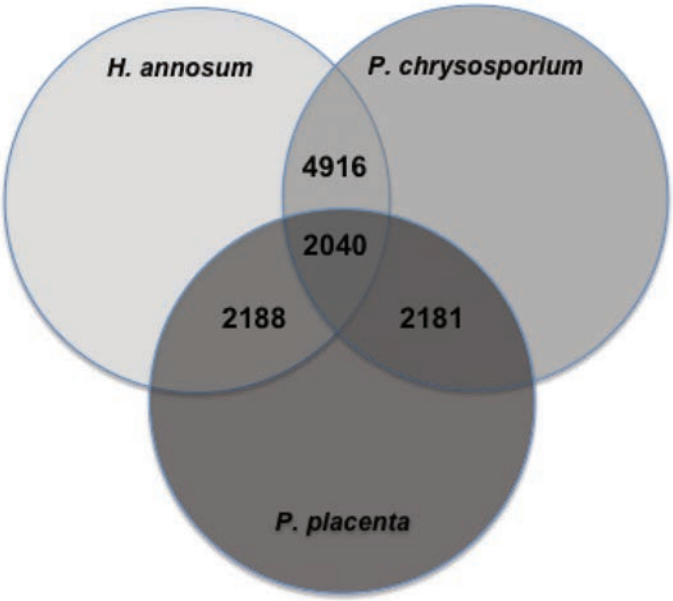


Figure 1. Venn diagram showing the number of orthologous genes among the 3 fungi

The paralogous gene families are not randomly distributed among functional categories, showing that gene duplication and loss is highly constrained by the functional properties and interacting partners of genes. Ad example, stress-related genes exhibit many duplications, whereas basal processes like growth-related genes show selection against such changes. The ongoing comparative analysis of paralogous gene families across the 3 genomes will shed some light on the evolution of specialized ecological features of the rot and pathogen fungi.

References

Martinez D., Challacombea J., Morgenstern I., Hibbett D., Schmoll M., Kubicek C.P., Ferreira P., Ruiz-Duenas F.J., Martinez A.T., Kersten P., Hammel K.E., Wymelenberg A.V., Gaskell J., Lindquist E., Sabat G., BonDurant S.S., Larrondo l.F., Canessa P., Vicuna R., Yadav J., Doddapaneni H., Subramanian V., Pisabarro A.G., Lavìn J.L., Oguiza J.A., Master E., Henrissat B., Pedro M. Coutinho P.M., Harris P., Magnuson J.K., Baker S.E., Bruno K., Kenealy W., Hoegger P.J., Kües U., Ramaiya P., Lucas S., Salamov A., Shapiro H., Tu H., Chee C.L., Misra M., Xie G., Tetero S., Yaver D., James T., Mokrejs M., Pospisekt M., Grigoriev I.V., Brettin T., Rokhsar D., Berka R., Cullen D., 2009. Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. *PNAS* 106: 1954-1959.

Martinez D., Larrondo L.F., Putnam N., Gelpke M.D.S., Huang K., Chapman J., Helfenbein K.G., Ramaiya P., Detter J.C., Larimer F., Coutinho P.M., Henrissat B., Berka R., Cullen D., Rokhsar D., 2004. Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. *Nature Biotechnology* 22: 695-700.

Wapinski I., Pfeffer A., Friedman N., Regev A., 2007. Natural history and evolutionary principles of gene duplication in fungi. *Nature* 449: 54-61.

Multivariate analysis revealed translational selection and mutational bias in *Heterobasidion irregulare*-destructive fungal pathogen of conifers in the Boreal hemisphere

G. Sablok[1], K.C. Nayak[2], E. Potenza[3], G. Emiliani[4], N. La Porta[1]

[1]*Sustainable Agro-ecosystems and Bioresources Department, IASMA Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, (TN), Italy.*
[2]*Bioinformatics Centre, Institute of Life Sciences, Department of Biotechnology, Nalco Square, Bhubaneswar, India.*
[3]*Genomics and Biology of Fruit Crops Department, IASMA Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, (TN), Italy.*
[4]*Trees and Timber Institute, National Research Council, IVaLSA-CNR S. Michele all'Adige (Trento), Italy.*
Corresponding author e-mail address: nicola.laporta@fmac.it

Abstract. Codon usage has a profound effect at the intra and inter- proteome level and even at the organismal level. *Heterobasidion annosum s.l.* is one of the most destructive fungal pathogen of conifers in the Boreal hemisphere. Recently the whole genome sequence of the *H. annosum* was released at DOE Joint Genome Institute (DOE-JGI) with 8.23 X coverage (Olson *et al.,* 2009) covering nuclear genome assembles in 39 scaffolds of total 33.7 Mbp estimated to cover 98.1% of the complete genome. We have carried out a genome wide codon usage analysis of this pathogenic fungus using mutivariate analysis and opular indices of codon usage. The results show that the G+C contents at three positions of codon are different GC1 (mean value of 58.37% ± 0.07); GC2 (mean value of 47.5% ± 0.09); GC3 (mean value of 60.9% ± 0.13) explain heterogeneity in the composition of the genes. For the identification of gene expression values we have systematically searched for the ribosomal proteins and have used them as a reference for calculating codon adaptation index (CAI). The heterogeneity in the genome was also relaeaved by plotting coordinates of *H. annosum* genes on axis 1 (COA/RSCU) which showed high negative correlation with GC_{3s} and GC content (r=-0.965, P<0.01 and r=-0.858, P<0.01), and significant high positive correlation with Nc (*r=0.689, P<0.01*) Furthermore, high significant negative correlation was observed between Nc and GC_{3s} (r=-0.674, P<0.01) which also complements the observation that C-ending codons are preferred over G-ending codons in highly expressed genes. It was also observed that CAI value and GC3s also had a significant correlation (r=0.789, P<0.01) which suggest that genes with higher expression level tend to use C or G at synonymous positions compared to genes with lower expression level. We further analyzed the correlation between the nucleotide bias and amino acid composition using the two other phylogentically closed fngal genomes.

Codon usage signature implies the variation in the usage of the codon at the wobble position. This variation in the usage has a profound effect at the intra- and inter-proteome level. It has widely demonstrated that except methionine (Met) and tryptophan (Trp), most of the amino acids are biased towards codon degeneracy (Hershberg and Petrov, 2009). *Heterobasidion irregulare s.l.* is one of the most destructive necrotrophic fungal pathogen of conifers in the Boreal hemisphere that produces a range of extracellular enzymes and a multitude of toxins. Most conifer

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

trees are susceptible to infection by this basidiomycete, causative agent of a root and but rot disease and widely regarded as the most economically important forest pathogen in temperate forests devastating conifer plantations and natural forests.

H. irregulare complex has a wide geographical distribution particularly in many parts of Europe, North America, China and Japan (Dai *et al.,* 1999; Dai *et al.,* 2003). Besides *H. irregulare*, the other species are known from East Asia, Australia and adjacent areas (Niemelä *et al.,* 1998). Currently, eight distinct taxonomic species have been described within the genus.

The virulence of this pathogen has been shown to be partly under mitochondrial control. Recent progress in the *H. irregulare* research include: possibilities for fruiting and classical genetics, publication of a genetic linkage map, expressed sequence tags (ESTs) compilation of more than 3,000 sequences are available, transformation system, knowledge of genetics of interspecific recognition, phylogeny of the species complex, pathogenicity factors in nucleus and mitochondria. The complete sequence of *H. irregulare* is the first plant pathogenic homobasidiomycete with comprehensive genome coverage; and this is challenging for investigations in many areas, including pathogenicity factors, interactions with host organisms, lignin degradation and bioremediation applications, and fungal biology and evolution. In this study, we used the available complete genome sequence of this organism and analysed its codon usage, aiming to understand the genetic organization of the *H. irregulare* genome.

The complete genome sequence and coding sequences of *H. irregular* was obtained from JGI (http://genome.jgi-psf.org). Sequences were initially checked for sequencing errors and finally 6,497 CDS sequences of *H.irregulare* having more than 100 codons were extracted directly to avoid sampling bias in calculations of codon usage (Wright, 1990). All the sequencing errors were removed using in-house developed perl/Bio-perl scripts and BioSeq modules have been used to prepare the final sequence dataset. All the popular indices of codon usage (Nc, RSCU) have been used to estimate the levels of biased usage of the codon. All the statistical analysis have been carried out using SPSS 17.0 (http://www.spss.com/) , Origin professional 8.0 (http://www.originlab.com/) and R (http://www.r-project.org/). All correlations are based on the Nonparametric Spearman's Rank Correlation analysis method.

Genome of *H. irregulare* is GC rich (52.0%), which predicts the biased usage of G- /or C- ending codons in the coding regions of this genome and the same was observed. Since there were wide variations in the GC bins of the genome, therefore to evaluate GC variation, G+C content was calculated at all the three codon positions. The results showed that the G+C contents at three positions of codon differ significantly, GC1 (58.37% ± 0.07); GC2 (47.5% ± 0.09); GC3 (60.9% ± 0.13), which explain occurrence of heterogeneity in the composition of the genes. To evaluate the compositional constraints, Nc plot was further analyzed. Nc plot revealed that a high number of the genes are lying below the expected curve and

poised towards GC3s, which clearly demonstrates that besides the compositional bias, there are several other factors also influencing the biased patterns of codon usage in this genome.

To further investigate the constraints on the codon usage biology we carried out correspondence analysis of the RSCU in a 59-dimensional hyperscape and the data was partitioned and analyzed across the first four major axes. We observed that the first major axis (axis1/COA; 20.4%) revealed the major inertia of 59-dimensional hyperspace and the subsequent axes/COA (2, 6.02%; 3, 4.7%; and 4, 3.5%) showed decreased variation subsequently.

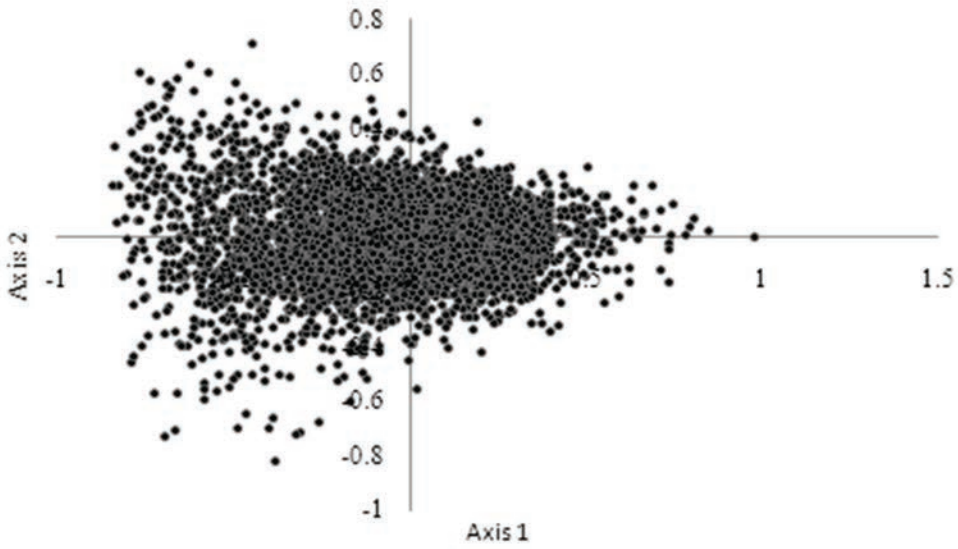


Figure 1. Plot for coordinates of first two major axes of correspondence analysis for *Heterobasidion irregulare*

To evaluate the levels of gene expression, codon adaptation index (CAI) was calculated; ribosomal proteins were systematically searched and used as reference sequences for calculating CAI (Gupta *et al.,* 2004). To evaluate the effect of gene expression level on synonymous codon usage bias spearman's rank correlation analysis was carried out between CAI and the coordinates of genes along the axis1/COA. A high negative correction between these two variables (r=-0.812, P<0.01) was observed whereas high significant positive correlation was found between CAI and GC_{3s}, GC (r=0.789, P<0.01; r=0.682, P<0.01). These results are in complete agreement with the finding that the genes with higher expression level exhibit a greater degree of codon usage bias towards GC ending codons and prefer to use codons with C or G at the synonymous position as compared to weakly

expressed genes. In summary, this study has shown that the codon usage variation among the genes of *H. irregulare* is influenced by mutational bias and translational selection. As more complete genomes of this phylogenetic taxa being sequenced, different factors shaping the pattern of codon usage might be found.

References

Dai Y.C., Korhonen K., 1999. *Heterobasidion annosum* group S identified in northeastern China. *European Journal of Forest Pathology* 29: 273-279.

Dai Y.C., Vainio E.J., Hantula J., Niemelä T., Korhonen K., 2003. Investigations on the *Heterobasidion annosum* complex in central and eastern Asia with the aid of mating tests and DNA fingerprinting. *Forest Pathology*. 33: 269-286.

Gupta S.K., Bhattacharyya T.K., Ghosh T.C., 2004. Synonymous codon usage in *Lactococcus lactis*: mutational bias versus translational selection. *Journal of Biomolecular Structure and Dynamics* 21: 527-536.

Hershberg R., Petrov D.A., 2009. General Rules for Optimal Codon Choice. *PLoS* Genet 5: e1000556.

Niemelä T., Korhonen K., 1998. Taxonomy of the genus Heterobasidion. In: Woodward S., Stenlid J., Karjalainen R., Hüttermann A. (eds.). *Heterobasidion annosum* Biology, Ecology, Impact and Control. CAB International, Wallingford, Oxon, OX10 8DE, UK, pp. 27-33.

Wright F., 1990. The 'effective number of codons' used in a gene. *Gene* 87: 23-29.

Tree-ring as proxies of stress caused by *Heterobasidion parviporum* at three different mature stands in Trentino

Y. Gori[1], F. Camin[2], P. Cherubini[3], N. La Porta[1]

[1]*IASMA Research and Innovation Centre, Fondazione Edmund Mach, Environment and Natural Resources Area, Italy.*
[2]*IASMA Research and Innovation Centre, Fondazione Edmund Mach, Stable Isotope and Traceability Area, Italy.*
[3]*WSL Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland.*
Corresponding author e-mail address: yuri.gori@iasma.it

Abstract. In this study we used a tree ring-proxy analysis to analyse the impact of the fungus *Heterobasidion parviporum* causal agent of root and butt rots of conifers. This fungal pathogen is causing the most economically serious damages in the conifer forests and many studies have been carried out to estimate the wood losses of this disease. However, a significant gap of knowledge is still present on the growth failure caused by this pathogen. The aim of this study was to test the potential of tree ring analysis to estimate the missing growth due to *H. parviporum* infection on Norway spruce. Three Norway spruce mature stands infected by *H. parviporum* were selected for sampling in the South-Eastern Alps: Baselga (BAS), Val Maggiore (VAL) and Cermis (CER) at different altitude respectively at 850-900, 1,300 and 1,950 m a.s.l. Health (HT) and infected trees (IT) were sampled. The main goals of the study were: (1) to clarify the role of climate conditions on infected trees by analyzing the climate-growth relationship at forest ecosystem level; (2) to forecast the development of this pathogen under a climate warming trend; (3) to estimate the indirect volume losses due to the prolonged presence of the fungus within the wood, on different managed forest in the Eastern Alps; (4) to test the hypothesis that tree-ring patterns may be used as an indicator of tree health, drought susceptibility and physiological change of infected trees. IT ring width was significantly lower than that of the control trees (HNS) with average basal area losses of 45% at Baselga and 49% at Val Maggiore, and only 30% at Cermis. (Fig. 2). HT growth rates were significantly reduced with time at each site. When crossdated samples were used to identify period of growth suppression as "abrupt growth reduction", were observed significant tree-ring growth divergence observed at Baselga, Val Maggiore and Cermis commencing in 1980, 1965 and 1945 respectively.

In this study we used a tree ring-proxy analysis to analyse the impact of the fungus *Heterobasidion parviporum* causal agent of root and butt rots of conifers. This fungal pathogen is causing the most economically serious damages in the conifer forests of the boreal hemisphere and is particularly dangerous in weakening their root system and to predispose them to windfall.

Specifically, the objectives were (1) to clarify the role of climate conditions on infected trees; (2) to forecast the development of this pathogen under a climate warming trend; (3) to estimate the indirect volume losses due to the prolonged presence of the fungus within the wood; (4) to test the hypothesis that tree-ring

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

patterns are a useful tool to indicate tree health, drought susceptibility and physiological change in infected trees.

For this purpose we evaluated the diseases at three different elevation site by measuring the tree-ring widths of Norway spruce [*Picea abies* (L.) Karst.] mature stands infected by *Heterobasidion parviporum*. In October 2009 10 healthy Norway spruce (control, 'CON') and 10 infected Norway spruce (infected, 'INF') were selected at three altitudinal different stands. The stands were:

- High elevation stand, 1,910 m a.s.l.
- Medium elevation stand, 1,320 m a.s.l.
- Low elevation stand, 870 m a.s.l.

Two cores, at 180°C from each other, were extracted with a 0.5 cm diameter Pressler increment borer from each one of the 60 trees, perpendicular to the slope direction to avoid possible compression wood and minimize stem eccentricity. Ring-width measurement were made to the nearest 0,01 mm on each core, using the Time Series Analysis Programme (TSAP) measurement equipment, coupled to a Leica MS5 stereoscope. Raw Tree-Ring-Widths (TRW) of each curves were plotted, cross-dated visually and then cross-dated statistically. Once measuring and crossdating was verified, original measurement time series were detrended to remove the decreasing trend and long-term noises caused by *H. parviporum*. To check suppressions (growth reduction) showed by INF, a running calculation of percent-growth change, for a 4 years periods, was applied to each raw ring-width and mean ring-width master chronologies. This "suppression index" was progressively shifted of 1 year, and calculated as (A1-A2)/A2 and , in which A1 equals average growth over the prior 4 years and A2 equaled average growth over the subsequent four years (Schweingruber *et al.,* 1990). In this way we also detected abrupt growth reduction (AGR) when the average ring width of the four rings was at least 40% smaller than the previous. Pointer years were used as additional ecological indicators capable to record the reactions of trees to environmental factor limiting radial growth (Schweingruber *et al.,* 1990).

The influence of climatic variables upon tree growth was investigated among the three regional tree-ring indexed chronologies. We used a correlation function analysis for the common period (1937-2009), comparing each CON and INF tree-ring index with the gridded monthly temperature and precipitation anomalies from the HISTALP dataset (Auer *et al.,* 2007) and the gridded scPDSI. Monthly analysis were performed using eight independent climate variables sequenced from January to August of the year of growth. The cumulative basal area chronologies was calculated for healthy and infected Norway spruce at each stand to assess the growth decline caused by fungal infection.

Our results from tree-ring and climate correlation patterns highlight a strongly different behavior of *P. abies* infected by *H. parviporum* at different environmental conditions. In our dendroecological study, Norway spruce affected by *H.*

parviporum reports a slow growth decline over many decades in natural spruce forest (at high and medium altitude), while at lower altitude *H. parviporum* acts as primary pathogen, since a final rapid decline and a cambial mortality was found in infected trees at this stand. From a climatic perspective, the results of this study show that *H. parviporum* drastically reduces the ability to withstand soil water-deficit al low altitude. Instead, at medium and high altitude, the ability to overcome drought stress is the same for both infected and uninfected trees. We found that altitude play a decisive role in plant-pathogen interaction and host resistance to disease. We hypothesize that, under a likely climate warming trend, *H. parviporum* could become more aggressive also at higher altitude.
From a management point of view, silvicultural control methods could include thinning to enhance water availability and presence of native species such as *Fagus sylvatica* or *Ostrya carpinifolia*, that are resistant to *Heterobasidion parviporum*. Since thinning operations could also increase the risk of stump infection, the urea treatment or other chemical and biological control, are therefore recommended.

In conclusion, our study demonstrate that tree-ring data are a useful tool to indicate a tree health, host resistance and physiological change in the pathogen-host system, but we understand that tree disease history could not be fully reconstructed since, to date, tree ring are not able to check the onset of infection.

References

Auer I., Böhm R., Jurkovic A., Lipa W., Orlik A., Potzmann R., Schöner W., Ungersböck M., Matulla C., Briffa K., Jones P., Efthymiadis D., Brunetti B., Nanni T., Maugeri M., Mercalli L., Mestre O., Moisselin J.M., Begert M., Müller-Westermeier G., Kveton V., Bochnicek O., Stastny P., Lapin M., Szalai S., Szentimrey T., Cegnar T., Dolinar M., Gajic-Capka M., Zaninovic K., Majstorovic Z., Nieplova E., 2007. HISTALP – Historical instrumental climatological surface time series of the greater alpine region. *International Journal of Climatology* 27: 17-46.

Schweingruber F.H., Eckstein D., Bachet S.F., Bräker O.U., 1990. Identification, presentation and interpretation of event years and pointer years in dendrochronology. *Dendrochronologia* 8: 9-38.

Session 2

Systematic, Taxonomy and Phylogeography

Evolutionary history of the conifer root rot fungus *Heterobasidion annosum sensu lato*

K. Dalman, Å. Olson, J. Stenlid

Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, P.O. Box 7026, S-75007 Uppsala, Sweden.
Corresponding author e-mail address: kerstin.dalman@slu.se

This work has been published in *Molecular Ecology* (2010) 19: 4979-4993.

Abstract. We investigated two hypotheses for the origin of the root rot fungus *Heterobasidion annosum* species complex: (i) that geology has been an important factor for the speciation (ii) that co-evolutionary processes with the hosts drove the divergence of the pathogen species. The *H. annosum* species complex consists of five species: three occur in Europe, *H. annosum s.s.*, *Heterobasidion parviporum* and *Heterobasidion abietinum*, and two in North America, *Heterobasidion irregulare* and *Heterobasidion occidentale*; all with different but partially overlapping host preferences. The evolution of the *H. annosum* species complex was studied using six partially sequenced genes, between 10 and 30 individuals of each species were analysed. Neighbour-joining trees were constructed for each gene, and a Bayesian tree was built for the combined data set. In addition, haplotype networks were constructed to illustrate the species relationships. For three of the genes, *H. parviporum* and *H. abietinum* share haplotypes supporting recent divergence and/or possible gene flow. We propose that the *H. annosum* species complex originated in Laurasia and that the *H. annosum s.s./H. irregulare* and *H. parviporum/H. abietinum/H. occidentale* ancestral species emerged between 45 and 60 Ma in the Palaearctic, well after the radiation of the host genera. Our data imply that *H. irregulare* and *H. occidentale* were colonizing North America via different routes. In conclusion, plate tectonics are likely to have been the main factor influencing *Heterobasidion* speciation and biogeography.

Over the past two decades, several studies have examined different aspects of the phylogenetic relationships of the different species within the *H. annosum* species complex (Kasuga *et al.,* 1993; Garbelotto *et al.,* 1998; Johannesson *et al.,* 2003; Linzer *et al.,* 2008). Different factors that may be important for the speciation of the *H. annosum* species complex have been put forward, including co-evolution with the host, modern forestry, geological factors and glacial periods. Although several hypotheses on evolutionary scenarios for the species complex have been put forward (Otrosina *et al.,* 1993; Linzer *et al.*, 2008), no formal estimates of divergence times have been published previously.

Phylogenetic trees were constructed for the five species in the *H. annosum* species complex: *H. annosum s.s.*, *H. irregulare*, *H. occidentale*, *H. parviporum* and *H. abietinum*. In addition, *H. insulare s.l.* and *H. araucariae* were used as outgroups. The single-locus Neighbour-Joining genealogies for *TF*, *EFA* and *G3P* each generated a phylogeny where each species grouped into separate clades. By contrast, the phylogenies for *GST1* and *ITS* revealed a mixed clade for *H. abietinum* and *H. parviporum* isolates. However, the combined Bayesian

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

phylogeny for the intronic regions of *GST1*, *TF*, *EFA* and *G3P* generated a tree where each species grouped into a well-supported clade (Fig. 1).

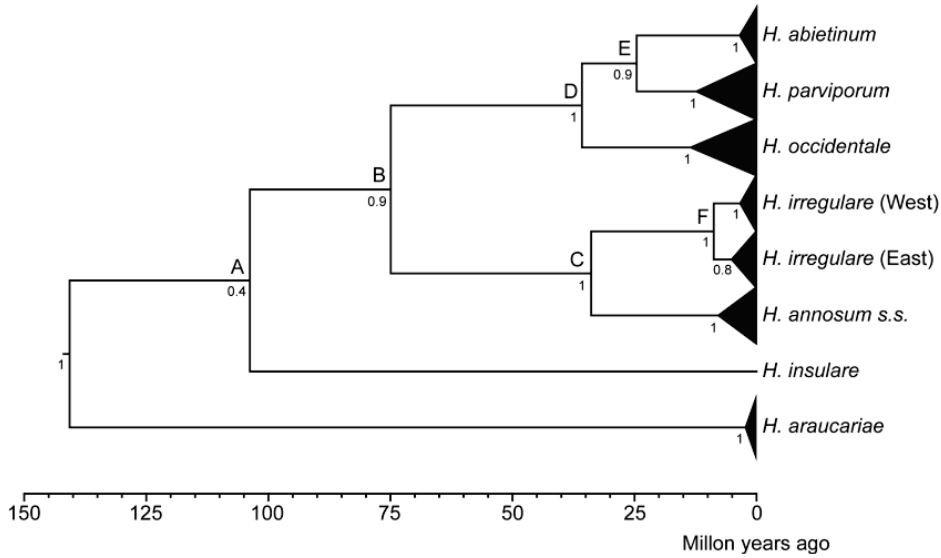


Figure 1. Bayesian phylogeny for the non-coding regions of *TF*, *GST1*, *G3P* and *EFA* estimated with BEAST using the HKY substitution model. Numbers correspond to the support for the nodes (posterior probabilities). Divergence times were estimated for the nodes A-F and are shown in Tab. 1. This tree was generated using the strict clock model and a mutation rate of 0.9×10^{-9} substitutions per site and year.

Tajima's relative rate test (Tajima, 1993) could not be rejected, assuming equal substitution rates among the different branches in the studied set of *Heterobasidion* spp. By using the given neutral substitution rates found, 0.9×10^{-9} and 16.7×10^{-9} substitutions per site per year, the limits for the divergence times could be estimated (Kasuga *et al.*, 2002). The Bayesian MCMC sampling generated posterior mean estimates of the time for the most recent common ancestor (TMRCA) and corresponding 95% highest posterior density intervals shown in Tab. 1 for the HKY model applied in BEAST.

The ancestor of the *Heterobasidion* genus emerged a maximum of 162 million years ago, which is in line with the appearance of gymnosperms (Chaw *et al.*, 1997). The current distribution of the *H. annosum* species complex is consistent with a geographic origin to be located in Laurasia. This hypothesis is supported by the phylogeny. Two groups diverged from the ancestor about 75 million - 85 million years ago: one group evolved into infecting pines (*H. annosum s.s.*/*H. irregulare*) and the other group did not (*H. parviporum*/*H. abietinum*/*H. occidentale*. This split took place well after the split of the host genera *Pinus* and *Picea*, which has been estimated to have happened between 150 million and 225 million years ago (Eckert and Hall, 2006)

The origin of the *H. parviporum/H. abietinum/H. occidentale* species was either in western North America or in eastern Asia. The ancestral *H. parviporum/H. abietinum/H. occidentale* colonized eastern Asia or North America around 30 million - 40 million years ago. The westward spread was probably stopped at the Turgai Sea, which existed 30 million - 160 million years ago between what today is Asia and Europe. If the ancestor of *H. occidentale* spread from Eurasia it was probably via the Trans-Bering Bridges, which were open for exchange between 100 million and 3.5 million years ago. The current distribution of *H. occidentale* is consistent with the appearance of this species in western North America. There was a dispersal barrier between eastern and western North America, about 35 million years ago (Sanmartin *et al.,* 2001), consisting of an arid region that was probably colonized by pines, which are nonhosts to this species. The current distribution of *H. parviporum* in Europe was probably a recolonization event together with its host after the last glaciation periods from refuges in southern Europe and the southern Ural mountain range. *H. abietinum* arose around 20 million years ago, became specialized to *Abies* species and has had a different biogeographical history to that of *H. parviporum*.

Table 1. Bayesian estimates of divergence times (in millions of years) for the most recent common ancestor (TMRCA). Values are posterior mean estimates of TMRCAs and 95% highest posterior density (HPD) intervals. A combined set of introns was used from the four genes *GST1*, *TF*, *EFA* and *G3P* consisting of 384 sites from 93 *Heterobasidion* spp. Isolates.

TMRCA	**Mutation rates per site per year**			
	0.9×10^{-9}		16.7×10^{-9}	
Clock model[a]	Mean	95% HPD	Mean	95% HPD
A.[b] *H. annosum s.l.*, *H. insulare*	103.8	No interval	7.6	4.8-10.6
B. *H. annosum s.l.*	75.0	54.3-95.5	4.0	2.9-5.1
C. Pine infecting species	33.9	20.5-47.5	1.8	1.1-2.6
D. Pine non-infecting species	35.8	23.5-48.2	1.9	1.3-2.6
E. European pine non-infecting species	24.5	14.6-34.5	1.3	0.8-1.9
F. *H. irregulare* (east and west)	8.8	3.2-14.3	0.5	0.2-0.8
Relaxed exponential clock model[a]				
A. *H. annosum s.l.*, *H. insulare*	162.4	58.1-303.7	8.6	2.9-17.0
B. *H. annosum s.l.*	85.2	34.5-142.8	4.5	1.9-8.0
C. Pine infecting species	41.3	13.4-71.3	2.2	0.6-4.0
D. Pine non-infecting species	42.1	17.8-73.3	2.2	0.9-3.9
E. European pine non-infecting species	27.7	9.8-42.5	-	-
F. *H. irregulare* (east and west)	13.2	2.7-21.0	0.6	0.1-1.2
Relaxed lognormal clock model[a]				
A. *H. annosum s.l.*, *H. insulare*	159.7	66.1-258.3	8.2	3.6-13.9
B. *H. annosum s.l.*	81.0	43.8-123.4	4.4	2.4-6.7
C. Pine infecting species	38.2	15.5-65.5	2.0	0.9-3.5
D. Pine non-infecting species	39.8	11.7-43.2	2.2	1.0-3.6
E. European pine non-infecting species	26.4	11.7-43.2	1.4	0.6-2.3
F. *H. irregulare* (east and west)	10.1	2.5-17.6	0.5	0.1-1.0

[a] The substitution model was HKY with the site heterogeneity model Gamma + Invariant sites using six gamma categories.

[b] The nodes are as indicated in Fig. 1.

The common ancestor of *H. annosum s.s.* and *H. irregulare* probably emerged in the western part of Eurasia about 60 million years ago and was further spread across the Trans-Atlantic bridge, which was a possible route of spread until about 35 million years ago. Further spread of *H. annosum s.s.* east in Eurasia was restricted by the Ural Mountains and the Turgai Strait (Sanmartin *et al.,* 2001) and by the *H. insulare* complex partly occupying its niche. This study proposes a different route of spread for *H. annosum s.s./H. irregulare* compared with those previously published (Otrosina *et al.,* 1993; Linzer *et al.,* 2008).

References

Chaw M.S., Zharkikh A., Sung H.-M., Lau T.-C., Li W.-H., 1997. Molecular phylogeny of extant gymnosperms and seed plant evolution: analysis of nuclear 18S rRNA sequences. *Molecular Biology and Evolution* 14: 56-68.

Eckert A.J., Hall B.D., 2006. Phylogeny, historical biogeography, and patterns of diversification for *Pinus* (*Pinaceae*): Phylogenetic tests of fossil-based hypotheses. *Molecular Phylogenetics and Evolution* 40: 166-182.

Garbelotto M., Otrosina W.J., Cobb F.W., Bruns T.D., 1998. The European S and F intersterility groups of *Heterobasidion annosum* may represent sympatric protospecies. *Canadian Journal of Botany* 76: 397-409.

Johannesson H., Stenlid J., 2003. Molecular markers reveal genetic isolation and phylogeography of the S and F intersterility groups of the wood-decay fungus *Heterobasidion annosum*. *Molecular Phylogenetics and Evolution* 29: 94-101.

Kasuga T., White T.J., Taylor J.W., 2002. Estimation of nucleotide substitution rates in eurotiomycete fungi. *Molecular Biology and Evolution* 19: 2318-2324.

Kasuga T., Woods C., Woodward S., Mitchelson K., 1993. *Heterobasidion annosum* 5.8s ribosomal DNA and internal transcribed spacer sequence: rapid identification of European intersterility groups by ribosomal DNA restriction polymorphism. *Current Genetics* 24: 433-436.

Linzer R.E., Otrosina W.J., Gonthier P., Bruhn J., Laflamme G., Bussieres G., Garbelotto M., 2008. Inferences on the phylogeography of the fungal pathogen *Heterobasidion annosum*, including evidence of the interspecific horizontal genetic transfer and of human-mediated, long-range dispersal. *Molecular Phylogenetics and Evolution* 46: 844-862.

Otrosina W.J., Chase T.E., Cobb F.W., Korhonen K., 1993. Population structure of *Heterobasidion annosum* from North America and Europe. *Canadian Journal of Botany* 71: 1064-1071.

Sanmartín I., Enghoff H., Ronquist F., 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biological Journal of the Linnean Society* 73: 345-390.

Tajima F., 1993. Simple methods for testing molecular clock hypothesis. *Genetics* 135: 599-607.

Species delimitation of *Armillaria cepistipes* and A. *gallica* in Central Europe

M. Tomšovský[1], V. Antonin[2], P. Sedlák[3], L. Jankovsky[3]

[1]*Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemedelska 1, 61300 Brno, Czech Republic.*
[2]*Moravian Museum, Department of Botany, Zelny trh 6, 659 37 Brno, Czech Republic.*
[3]*Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemedelska 1, 61300 Brno, Czech Republic.*
Corresponding author e-mail address: michal.tomsovsky@mendelu.cz

Abstract. *Armillaria cepistipes* and *A. gallica* (Basidiomycota, *Physalacriaceae*) are morphologically similar species, and they are often nearly indistinguishable using DNA methods targeting the ITS region of ribosomal DNA. The aim of our study was to examine morphological and ecological features of *A. cepistipes* and *A. gallica*, and to test other DNA-based methods to distinguish the two species. Our results revealed discriminative macro- and micromorphological features between these two species, especially the presence of a distinct central pileus ocella, the shape of the annulus, the character of the velar stipe remnants and the length of the terminal cells of the pileus scales. Ecologically, *A. gallica* generally prefers warmer areas in lowlands (oak and alluvial forests), while *A. cepistipes* is more common in hilly and lower montane beech forests in Central Europe. Nevertheless, certain locations between 300 and 500 m a.s.l. are known to sympatrically support both species. The sequences of the translation elongation factor 1-alpha showed high interspecific variability, and this gene is a more appropriate candidate for distinguishing *A. gallica* from *A. cepistipes*.

Armillaria cepistipes and *A. gallica* (Basidiomycota, *Physalacriaceae*) are morphologically similar species, which are nearly indistinguishable using DNA methods targeting the ITS region of ribosomal RNA genes. Therofore, *A. cepistipes* - *A. gallica* assemblage is the most difficult european *Armillaria* group to identify. Both species occur in the Northern hemisphere in similar temperate deciduous forest biomes. In Central Europe, the primary centres of *Armillaria cepistipes* distribution are beech stands at altitudes of 500-900 m. *Armillaria gallica* is distributed predominantly in lowlands (oak and alluvial stands), and it spreads to higher elevations along rivers and streams. The distribution of both species overlaps at altitudes of approx. 300-500 m. Indeed, sympatric occurrence of both species at elevations as high as 1,400 m in the Swiss Alps was reported (Prospero *et al.,* 2003). *A. cepistipes* was recorded on two conifers and four broadleaved hosts and *A. gallica* on 13 conifers and 37 broadleaved hosts from the area of South Moravia (Czech Republic) (Jankovský, 2003). Both species are less likely to kill trees than the more virulent fungus *A. ostoyae.* The aim of our study was to examine morphological features of *A. cepistipes* and *A. gallica*, to screen samples for their PCR-RFLP patterns, and to obtain DNA sequences of their translation elongation factor 1- alpha gene.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

PCR-RFLP method have been developed for rapid and cost-effective DNA-based identification of *Armillaria* samples (Lochman *et al.,* 2004). This method uses an *Armillaria*-specific primer pair targeting the ITS region of nuclear ribosomal DNA, and the nested-PCR assay is suitable for low-quality DNA obtained from rhizomorphs, soil samples and older herbarium specimens. However, some *A. cepistipes* - *A. gallica* specimens tested with this PCR-RFLP method have yielded ambiguous restriction patterns. Since multiple copies of nuclear ribosomal DNA (rDNA) are present in fungal genomes, some heterozygous specimens were detected. If a copy of the gene includes a base transition, the change may cause the elimination of a particular restriction site (Lochman *et al.,* 2004). These individuals showing an intermediate restriction pattern were studied in detail to determine the placement of the specimens. The individuals were hypothesized to be interspecific hybrids.

The partial sequence of gene coding translation elongation factor 1- alpha (tefa), which includes two partial exons and one complete exon, as well as two introns, was chosen as a promising tool in distinguishing *A. cepistipes* - *A. gallica* specimens. According to the tefa sequences, *A. cepistipes* and *A. gallica* are clearly distinguishable, while specimens with intermediate PCR-RFLP profiles undoubtly belong to *A. cepistipes*. The sequences of the translation elongation factor 1-alpha showed high interspecific variability, and this gene is an appropriate candidate for distinguishing *A. gallica* from *A. cepistipes*. The topology of the tefa-derived phylogram does not support close taxonomic relationships of the two species. Therefore the intraspecific hybridisation is improbable.

Our results revealed discriminative macro- and micromorphological features between *A. cepistipes* and *A. gallica*. In summary, *A. cepistipes* differs from *A. gallica* by:

I.) scales fibrillose to tomentose, which disappear very soon from the pileus surface except the centre (so-called central ocella). In *A. gallica*, the scales are long fibrillose, almost pyramidal at centre, disappearing toward the margin only.

II.) annulus breaking mostly irregularly (often regular, star-shape breaking in *A. gallica*).

III.) stipe surface generally covered only with sparse fibrillose velar remnants (rich fibrillose or fibrillose-tomentose velar remnants in *A. gallica*).

The results of the our research were published by Antonín et al. (Antonín *et al.,* 2009).

References

Antonín V., Tomsovsky M., Sedlak P., Majek T., Jankovsky L., 2009. Morphological and molecular characterization of the *Armillaria cepistipes* - *A. gallica* complex in the Czech Republic and Slovakia. *Mycologycal Progress* 8: 259-271.

Jankovský L., 2003. Distribution and ecology of *Armillaria* in Southern Moravia, Czech Republic. *Czech Mycology* 55: 173-186.

Lochman J., Sery O., Mikes V., 2004. The rapid identification of European *Armillaria* species from soil samples by nested PCR. *FEMS Microbiology Letters* 237: 105-110.

Prospero S., Rigling D., Holdenrieder O., 2003. Population structure of *Armillaria* species in managed Norway spruce stands in the Alps. *New Phytologyst* 158: 365-373.

Species distribution and host spectrum of *Heterobasidion annosum s.l.* in the Czech Republic

P. Sedlák and M. Tomšovský

Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic.
Corresponding author e-mail address: petrsedlak@klikni.cz

Abstract. The root-rot fungus *H. annosum* (Fr.) Bref. species complex consists of three species, separated by their host affinity. The distribution and host spectrum of this root pathogen has not been sufficiently studied in the Czech Republic till now. The work is focused on the stand and substrate specificity, frequency, distribution, and divergence of each species: *H. annosum*, *H. abietinum*, and *H. parviporum* in the country. The taxon-specific competitive-priming was used for rapid identification of species. The results were not able to clearly distinguish *H. parviporum* and *H. abietinum*. Therefore, new specific primers (HAF, HAR) based on the translation elongation factor 1-alfa gene were designed for identification of the two species. The phylogenetic relationship of the species complex was studied by analysing DNA sequences of three gene regions: glyceraldehyde 3-phosphate dehydrogenase, translation elongation factor 1-alfa, and mitochondrial ATP synthase subunit 6. In total, 71 fungal specimens originated from Czech Republic were examined. *H. annosum s.s.* has the largest spectrum of host trees (*Pinus, Picea, Fraxinus, Betula, Corylus, Alnus, Abies, Acer* and *Prunus*). *H. parviporum* was found only on spruce and *H. abietinum* was observed on *Abies, Picea*, and *Pinus*.

The root-rot fungus *H. annosum* (Fr.) Bref. species complex consists of three species, separated by their host affinity. The distribution and host spectrum of this root pathogen has not been sufficiently studied in the Czech Republic till now. The work is focused on the stand and substrate specificity, frequency, distribution, and divergence of each species: *H. annosum*, *H. abietinum*, and *H. parviporum* in the country.

Fungal material for this work was collected in the wild from the various localities from Czech Republic. For rapid identification of *Heterobasidion annosum s.l.* we used taxon-specific competitive-priming (TSCP-PCR) method modified according to (Gonthier *et al.,* 2003). According to ambiguous results of TSCP-PCR in discrimination of *H. abietinum* and *H. parviporum* new pair of specific primers HAF/HAR was designed. These primers were selected from a heterogenic part of translation elongation factor 1-alpha gene sequence and were designed for differentation of the *H. abietinum* and *H. parviporum*. The other PCRs were used for amplification three DNA fragments: glyceraldehyde 3-phosphate dehydrogenase (GPD1/GPDR) (Gonthier P. *et al.*, 2003), translation elongation factor 1-alfa (EFaR/EFaF) (Linzer *et al.,* 2008) and mitochondrial ATP synthase subunit 6 (ATP6 2/ ATP6 3) (Dai *et al.,* 2006). Samples were sequenced by Macrogen (Seoul, Korea). Sequences were aligned manually using freeware BioEdit. Phylogeny was generated by MEGA 5.05 using minimum evolution analysis with bootstrap method (1,000 replications).

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

All loci were successfully amplified, with a few exceptions: we were not able to amplify ATP6 gene and GPD of some samples, probably because of low-quality DNA of herbarium specimens. The most of specimen fell into a *Heterobasidon annosum s.s.* clade. *H. annosum* was collected on the widest range of hosts: *Abies alba, Acer spp, Alnus glutinosa, Betula pendula, Corylus avellana, Fraxinus excelsior, Picea abies, Pinus sylvestris, Prunus domestica,* and *Salix caprea. Heterobasidion parviporum* was collected only from spruce (*Picea abies*) and *Heterobasidion abietinum* was observed on *Abies alba* and *Picea abies.*

We document occurrence of these three European species in the area of Czech Republic. Distribution of *H. parviporum*, as species occuring on spruces, is less widespread as we expected. We recorded its occurence mainly in indigenous spruce stands. Therefore, in next work, we will focus on the localities with indigenous spruce and fir to obtain more specimens of *H. parviporum* and *H. abietinum*.

References

Dai Y.C., Wang Z., Binder M., Hibbett D.S., 2006. Phylogeny and a new species of *Sparassis* (Polyporales, Basidiomycota): evidence from mitochondrial *atp6*, nuclear rDNA and *rpb2* genes. *Mycologia* 98: 584-592.

Gonthier P., Garbelotto M., Nicolotti G., 2003. Swiss stone pine trees and spruce stumps represent an important habitat for *Heterobasidion* spp. in subalpine forests. *Forest Pathology* 33: 191-203.

Linzer R.E., Otrosina W.J., Gonthier P., Bruhn J., Laflamme G., Bussières G., Garbelotto M., 2008. Inferences on the phylogeography of the fungal pathogen *Heterobasidion annosum*, including evidence of interspecific horizontal genetic transfer and of human-mediated, long-range dispersal. *Molecular Phylogenetics and Evolution* 46: 844-862.

Session 3

Ecology

Climate change effects on soil functionality and soil-plants interactions: practical approaches

M.T. Ceccherini[1], N. Luchi[2], P. Capretti[3], G. Pietramellara[1]

[1]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università di Firenze, piazzale delle Cascine, 28 - 50144 Firenze, Italia.*
[2]*CNR-IPP, Via Madonna del Piano 10, 50019, Sesto Fiorentino, Firenze, Italia.*
[3]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università di Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italia.*
Corresponding author e-mail address: mariateresa.ceccherini@unifi.it

Abstract. The cause and consequences of climate change on heart ecosystem are still debated between scientific community. The soil response to climate change in terms of functionality is strongly studied in the last years due to its relevance to support life on earth ecosystem. The soil microbial community represent the main responsible of the soil functionality an there are several studies on its response in terms of resilience and resistance to climate change effects, especially on soil chemical and physical characteristics. The relation of the soil functionality and plant survival are very strictly and markedly influenced the plant metabolism and physiological status with relevant consequence on plant-pathogens interactions. The results of all of these studies will be fundamental to put forward new models that better predict the short and long term future consequences of climate change on soil functionality and their attended effects on the whole earth ecosystem.

Olive trees represent a large part of the Mediterranean landscape, thus playing an important role in cultural, ecological, environmental and social fields. In Tuscany, olive constitutes a resource favourable to economic activity. Unfortunately, the Verticillium wilt does not save this region and until now, no products are registered in Italy to fight against this wilt. In this study, we proposed a Real-time PCR approach to detect and quantify *Verticillium dahliae* in soil and in olive tree tissues. Reliable insight in this relation is needed by growers, related to the importance of the cultivation and thus, highly relevant for tree nursery growers (Luchi *et al.,* 2006). Specific and accurate quantitative measurements in host tissues are fundamental to detect pathogenic sequences soon after infection and prior to the appearance of wilt, providing a tool to accelerate the diagnosis (Atallah *et al.,* 2007).

We set up a molecular approach based on Real-time PCR that allows for a simpler quantification, in addition to an increased sensitivity compared with conventional PCR (Ceccherini *et al.,* 2003). Assays were conducted using the specific primer pair DB19/DB22, that defines amplicons of 539-bp in D isolates (Mercado-Blanco *et al.,* 2003), to detect and quantify a specific Defoliating *Verticillium dahliae* sequence in olive plants of the genotype *Leccino*, by monitoring the amount of pathogen DNA in a time-course after inoculation. The sensitivity of detection of *V. dahliae* was quite high; in non sterile soil inoculated with known decreasing amounts of the pathogen DNA, we were able to detect 11.4

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

fg of sequence per ng of total DNA; considering that 148 fg of DNA are equivalent to approximately five haploid genomes of *V. dahliae* (Collins *et al.,* 2003), the sensitivity of our Real-time assays allowed for the detection of as few as 0.38 haploid genomes of *V. dahliae* (Mahuku and Platt, 2002).

In 18 months old olives, maximal absolute amounts of the pathogen DNA were quantified in soil and roots, 1.71 and 0.763 ng ng^{-1} of total DNA, respectively, at the death (185 days after the infection). At the same time lesser amounts of pathogen DNA were quantified in collar and stem tissues and in soil without plant. Interestingly, at T5 the pathogen sequences were detected in soil (3.62×10^{-5}) and in the collar (8.00×10^{-6}) when the plants were apparently healthy (no wilt symptoms). Since we experienced from the investigation that after 185 days olives withered of a suddenly death, we could consider the amounts of pathogen sequences in soil and collar, as a "threshold of alert" for the wilt, in the conditions used for the experiment.

A general difficulty in predicting Verticillium wilt in trees is that disease symptoms are more qualitative (presence or absence of wilt) than quantitative and expression of symptoms seems best under circumstances that also favour plant growth (Goud, 2003). Therefore, a rapid and cost-effective method to extract DNA directly from soil and plant tissue samples, which can be utilized in PCR amplification to effectively detect specific soil pathogens, is desirable.

Sampling of rhizospheric soil and non symptomatic tissues from an olive, would be the best use for the molecular detection of *V. dahliae*, because of the benefits of early detection of latent infections in plant tissues in which the pathogen could be biologically active.

Acknowledgments

This research was supported by grants from the Fondazione Monte dei Paschi di Siena.

Thanks are due to Rafael M. Jiménez-Dìaz (Departamento de Protección de Cultivos, CSIC, Instituto de Agricultura Sostenible – IAS, Córdoba, Spain) for providing *V. dahliae* isolates V138I.

References

Atallah Z.K., Bae J., Jansky S.H., Rouse D.I., Stevenson W.R., 2007. Multiplex real-time quantitative PCR to detect and quantify *Verticillium dahliae* colonization in potato lines that differ in response to Verticillium Wilt. *Phytopathology* 97: 865-872.

Ceccherini M.T., Potè J., Kay E., Tran Van V., Maréchal J., Pietramellara G., Nannipieri P., Vogel T.M., Simonet P., 2003. Degradation and transformability of DNA from transgenic leaves. *Applied and Environmental Microbiology* 69: 673-678.

Collins A., Okoli C.A.N., Morton A., Parry D., Edwards S.G., Barbara D.J., 2003. Isolates of *Verticillium dahliae* pathogenic to crucifers are of at least three distinct molecular types. *Phytopathology* 93: 364-376.

Goud J.C., 2003. Verticillium wilt in trees. Detection, prediction and disease management. Ph.D. Thesis. Wageningen Universiteit, Wageningen, Netherlands.

Luchi N., Capretti P., Vettraino A.M., Vannini A., Pinzani P., Pazzagli M., 2006. Early detection of *Biscogniauxia nummularia* in symptomless European beech (*Fagus sylvatica* L.) by TaqMan™ real-time PCR. *Letters in Applied Microbiology* 43: 33-38.

Mahuku G.S., Platt H.W., 2002. Quantifying *Verticillium dahliae* in soils collected from potato fields using a competitive PCR assay. *American Journal of Potato Research* 79: 107-117.

Mercado-Blanco J., Collado-Romero M., Parrilla-Araujo S., Rodríguez-Jurado D., Jiménez-Díaz R.M., 2003. Quantitative monitoring of colonization of olive genotypes by *Verticillium dahliae* pathotypes with real-time polymerase chain reaction. *Physiological and Molecular of Plant Pathology* 63: 91-105.

Consequences of climate warming on the activity of *Heterobasidion parviporum* in Finland

M.M. Müller[1], R. Sievänen[1], E. Beuker[2], H. Meesenburg[3], N. La Porta[4], J. Ekojärvi[1], I. Pavlov[5], J. Hantula[1], K. Korhonen[1]

[1]*Finnish Forest Research Institute, Vantaa Research Unit, Finland.*
[2]*Finnish Forest Research Institute, Punkaharju Research Unit, Finland.*
[3]*Northwest German Forest Research Station, Germany.*
[4]*Sustainable Agro-ecosystems and Bioresources Department, IASMA Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, (TN), Italy.*
[5]*Siberian State Technological University, Department of Forest Cultures, Krasnoyarsk, Russia.*
Corresponding author e-mail address: Michael.mueller@metla.fi

Abstract. The respiration activity of *Heterobasidion parviporum* isolates at various temperatures from -4°C to 33°C was determined by measuring CO_2-production in air-tight glass vials with saw dust of Norway spruce as the only substrate. The activity of *H. parviporum* at different temperatures and temperature measurements in soil and air at three spruce forest sites (in Germany, southern and northern Finland) were used to estimate the dependence of the annual cumulative respiration activity of *H. parviporum* on the annual average air temperature. Soil temperatures at 20 cm depth were considered to represent average root temperatures and air temperatures at 2 m height were considered to represent stem temperatures. A linear regression was found between the annual average air temperature and the cumulative annual respiration activity of *H. parviporum*. According to this regression model, an increase in the annual average air temperature by 4°C would increase the activity of *H. parviporum* in spruce roots in southern and northern Finland by 67% and 109%, respectively. In stems the corresponding values are 48% and 68%. This increase in degradation activity would exceed considerably the predicted increase in forest growth when climate changes according to scenario A2 (IPCC), forecasting an increase of 4°C during this century. Instead, in northern Finland the increase of forest growth would equal or exceed the increase of activity by *H. parviporum*. The respiration rate at different temperatures did not differ significantly between isolates from distant localities in Europe (Italy, Denmark and Finland) with different climate. Neither did genetic studies involving length variation in five microsatellites and sequence analyses of three DNA-markers reveal differentiation among these populations, suggesting considerable gene flow between them. Hence, populations of *H. parviporum* will probably adapt well to possible changes in temperature caused by climate change.

According to the climate change scenario A2 made by the International Panel on Climate Change (IPCC) an increase of 6°C of the annual average temperature has been predicted for Finland during this century. It is likely that the climate change with increasing temperatures during all seasons will enhance the spread of *Heterobasidion parviporum*. There are several reasons for this. Firstly, a higher proportion of forest harvesting will take place at temperatures when the fungus produces spores, resulting in an increased risk of fresh stumps to become infected. Secondly, more harvesting will be carried out on non-frozen soil, resulting in increasing logging damage to tree roots. Thirdly, storm damages, exposing trees to new infections, will increase because the period of frozen soil (with well anchored

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

trees) becomes shorter. A warmer climate will also allow faster growth and higher decay activity of the fungus. However, it is not known how this increasing activity of the fungus relates to increasing forest growth. Neither is it known whether subpopulations of *H. parviporum* are adapted to local climate, and how well they are able to adapt to changing climate. We investigated these questions by collecting pure cultures from various climatic regions and measuring their respiration activity at different temperatures. In addition, the possibility of gene flow was investigated by comparing genetic variation among remote subpopulations of the fungus.

The respiration activity of *Heterobasidion parviporum* isolates at temperatures varying from -4°C to 33°C was determined by measuring the CO_2-production in air-tight glass vials with saw dust of Norway spruce as the only substrate. These results were used to calculate an annual cumulative amount of respiration activity, using soil and air temperature data obtained from three spruce forest sites representing different climatic conditions: North Finland, South Finland and Central Germany. Soil temperatures at 20 cm depth were considered to represent average root temperatures and air temperatures at 2 m height were considered to represent stem temperatures. A linear regression was found between the annual average air temperature and the cumulative annual respiration activity of *H. parviporum*. According to this regression model, an increase in the annual average air temperature by 6°C would increase the activity of *H. parviporum* in spruce roots in southern and northern Finland by 67% and 109%, respectively. Corresponding values in stems are 48% and 68%. This increase in degradation activity would exceed considerably the predicted increase in forest growth in southern Finland (Kellomäki *et al.,* 2008) if the climate changes according to the scenario A2 (IPCC), forecasting an increase of 6°C during this century. Instead, in northern Finland the increase of forest growth would equal or exceed the increase of activity by *H. parviporum*.

Adaptation of subpopulations to local climate was investigated with pure cultures of *H. parviporum* originating from Italy, Denmark, Finland and Central Siberia (Krasnojarsk and Irkutsk regions). We determined the response of respiration rate to a temperature change from 2°C to 6°C, from 6°C to 20°C and from 20°C to 25°C as follows: response = absolute value of the activity at X°C subtracted by the activity at Y°C and divided by their average. The temperature responses did not differ significantly between European isolates, but when Siberian isolates were included and the responses were compared with the average temperature of the coldest month in the region from where the isolates originated, a highly significant correlation was found between responses to temperature changes from 2° to 6°C and from 20° to 25°C with the average temperature of the coldest month in the region from where the isolates originated. This result suggests that some adaptation to local climate exists between subpopulations in regions with large differences in climate. However, local variation of activity between isolates was high compared to variation between subpopulations.

Genetic differences between the subpopulations of *H. parviporum* used for respiration measurements (111 isolates) were determined from variation of five microsatellite loci according to Johannesson and Stenlid (Johannesson and Stenlid, 2004). Among European subpopulations only 1% of the total genetic variation was due to between subpopulation variation while 99% was due to within subpopulation variation. The low variation between subpopulations suggests that gene flow is possible between European subpopulations. When the Siberian isolates were included, 5% of the total genetic variation was due to between subpopulation variation.

Hence, the activity of *H. parviporum* will probably increase at least as much as forest growth if the climate warming of 6°C takes place, as predicted for Finland during this century. Subpopulations of *H. parviporum* will probably adapt well to such change because of their low specialisation to local climate and high variation in individual responses to temperature changes. Gene flow between remote European subpopulations will allow genetic adaptation over long periods of time. Our results support the hypothesis that damages caused by *Heterobasidion* in spruce forests will probably increase considerably in future if control measures are not intensified.

References

Johannesson H., Stenlid J., 2004. Nuclear reassortment between vegetative mycelia in natural populations of the basidiomycete *Heterobasidion annosum*. *Fungal Genetics and Biology* 41: 563-570.

Kellomäki S., Peltola H., Nuutinen T., Korhonen K.T., Strandman H., 2008. Sensitivity of managed boreal forests in Finland to climate change, with implications for adaptive management. *Philosophical Transactions of the Royal Society B, Biological Sciences* 363: 2341-2351.

Heterobasidion irregulare in central Italy: where have we got to?

E. Motta, L. D'Amico, T. Annesi, M. Scirè

C.R.A. - Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy.
Corresponding author e-mail address: emma.motta@entecra.it

After the first record of *Heterobasidion irregulare* Garbelotto & Otrosina in Castelporziano Presidential Estate (Rome, Italy), several aspects of its biology and epidemiology were investigated in our country, in order to understand the threat it could create to natural vegetation and habitats.

The results gathered during a ten-year patchwork study are summarized as follows:

- *H. irregulare* is distributed in *Pinus pinea* forests of the western coast of central Italy, within an area approximately 100 km long (D'Amico *et al.,* 2007).

- In Mediterranean climate, *H. irregulare* has a continuous spore production, regardless the temperature level, in relation with rain periods only (D'Amico *et al.,* 2008).

- *H. irregulare* and *H. annosum* (Fr.) Bref. coexist in the National Park of Circeo (D'Amico *et al.,* 2007a; D'Amico *et al.,* 2007b) and in Rome public and private garden (Scirè *et al.,* 2009), but no more in the coastal pinewood of Castel Fusano, where *H. annosum* disappeared from in 2000 because of a fire (Motta *et al.,* 2008).

- At least two separate introduction of *H. irregulare* have taken place in Italy, as in Lazio Region the 1.5 kb mitochondrial intron only is present in the examinated isolates except in the Circeo National Park, where isolates with a 1.8 kb intron are present (D'Amico *et al.,* 2007b).

- In some conditions, *H. irregulare* is a faster colonizer than *H. annosum*, but this does not imply that it is more efficient in inducing decay (Scirè *et al.,* 20010; Scirè *et al.,* 2011).

- *H. irregulare* is able to attack *P. halepensis* in its natural distribution area, where this species is undamaged by *H. annosum* (Scirè *et al.,* 2008; Scirè *et al.,* 2010).

- The use of selected isolates of *Phlebiopsis gigantea* (Fr.) Jülich for controlling *H. annosum* is successful against *H. irregulare* too (Annesi et al., 2005; Motta *et al.,* 2009).

These data, seen as a whole, strongly suggest that a permanent survey on pines and other conifers in forest stands and plantations should be launched in Lazio and in the neighbouring Regions, also supplemented by monitoring *H. irregulare* spore dispersal, in order to prevent a further diffusion of this pathogen in Italy.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

References

Annesi T., Curcio G., D'Amico L., Motta E., 2005 Biological control of *Heterobasidion annosum* on *Pinus pinea* by *Phlebiopsis gigantea*. *Forest Pathology* 35: 127-134.

D'Amico L., Annesi T., Scirè M., Curcio G., Motta E., 2008. Importanza e caratteristiche di *Heterobasidion annosum* in alcune pinete del Lazio. *Micologia Italiana* 37: 42-47.

D'Amico L., Motta E., Annesi T., Scirè M., Luchi N., Hantula J., Korhonen K., Capretti P., 2007. The North American P group of *Heterobasidion annosum s.l.* is widely distributed in *Pinus pinea* forests of the western coast of central Italy. *Forest Pathology.* 37: 303-320.

D'Amico L., Scirè M., Annesi T., Motta E., 2007. *Heterobasidion annosum* in the National Park of Circeo. *Journal of Plant Pathology* 89: S37.

Motta E., Annesi T., D'Amico L., Curcio G., Sequino S., Scirè M., 2009. Uso sperimentale di un isolato autoctono di *Phlebiopsis gigantea*: efficacia ed ecosostenibilità. *Forest@* 6: 148-153.

Motta E., D'Amico L., Annesi T., Scirè M., Curcio G., L. Puddu A., 2008. Effect of fire on the infection centres of *Heterobasidion annosum* in a *Pinus pinea* wood. *Petria* 18: 27-35.

Scirè M., D'Amico L., Annesi T., Motta E., 2010. Some observations on two Mediterranean Pinus species facing the *Heterobasidion irregulare* introduction in Italy. *Petria* 20: 127-128.

Scirè M., D'Amico L., Motta E., Annesi T., 2008. North American P type of *Heterobasidion annosum* shows pathogenicity towards *Pinus halepensis* in Italy. *Forest Pathology* 38: 299-201.

Scirè M., D'Amico L., Motta E., Annesi T., 2009. Alcuni aspetti fitosanitari nella "foresta" della città di Roma. Atti del III Congresso Nazionale di Selvicoltura. Taormina (ME), 16-19 ottobre 2008. Accademia Italiana di Scienze Forestali, Firenze, pp. 1424-1428.

Scirè M., Motta E., D'Amico L., 2011. Behaviour of *Heterobasidion annosum* and *Heterobasidion irregulare* isolates from central Italy in inoculated *Pinus pinea* seedlings. *Mycological Progress* 10: 85-91.

Heterobasidion annosum in coniferous ecosystems of Northern Spain

N. Mesanza and E. Iturritxa

Production and Plant Protection Neiker Tecnalia. Granja Modelo de Arkaute. 46 Post. Vitoria Gasteiz, 01080 Spain.
Corresponding author e-mail address: eiturritxa@neiker.net

Abstract. *Heterobasidion annosum* (Fr.) Bref. *sensu lato* is an important pathogen that causes root rot in several conifer species. This fungus is widely distributed in the northern hemisphere from central Finland in the north to northern Africa and Central America in the south. In Europe three species have been identified: *Heterobasidion parviporum* Niemelä & Korhonen (or S type) primarily infecting *Picea abies*, *Heterobasidion abietinum* Niemelä & Korhonen (or F type) mainly causing damages in *Abies alba* and *Heterobasidion annosum sensu stricto* (or P type) prevalent on *Pinus* spp. his fungus has been rarely recorded from Spain. It was recorded in 1941, in Balsain (Segovia), center of Spain, in a stump of *Pinus sylvestris* and as a factor associated in the decline of *Abies alba* Mill. in the Pyrenees, Northern Spain, and it was identified in south of Spain where it causes root diseases in *A. pinsapo* Boiss. The objective of this study was to determine the population structure of *H. annosum s.l.* and host range in conifer plantations and native forest of the North of Spain. A total of 109 stands were surveyed and then, where the disease was found *H. annosum* basidiocarps were collected. In all cases pure cultures were obtained from basidiocarps using BDS (Benomyl dichloran streptomycin), a semi-selective medium, and were incubated at 20°C for two weeks. A total of 46 isolates were obtained and included in the molecular analysis. DNA from the pure cultures was extracted with the Quiagen DNA extraction Kit (Quiagen Inc., Chatsworth, CA), following the manufacturer's instruction. The internal transcribed spacer region (ITS) of the ribosomal (r) DNA was amplified using primers ITS1-F and ITS4. Sequences were aligned with those of other *Heterobasidion* spp. obtained from NCBI database by performing a nucleotide BLAST search. Gene tree was built using the Neighbor-Joining method and the evolutionary distances were computed using the Maximum Composite Likelihood method. The branch support of the gene tree was assessed by the posterior probabilities obtained from the 50% majority-rule-consensus tree calculated by bootstrapping (2000 replicates) with Mega4. *H. annosum* ss. was found in different host species: *Chamaecyparis lawsoniana, Pinus pinaster, Pinus sylvestris, Pinus radiata, Pseudotsuga menziesii, Picea abies and Pinus nigra.* Basidiomes were encountered in the 42.20% of the sampled places. All the isolates were identified as European *Heterobasidion annosum* (European P-type) based on ITS sequencing.

During the last 250 years, Spanish Atlantic forests have been in a state of uninterrupted decline and degradation. Prior to the beginning of 20th century, the native forest cover in de Basque Country, Northern Spain, composed mainly by *Fagus sylvatica* L., *Quercus robur* L., *Q. pyrenaica* Wild, *Fraxinus excelsior* L., *Alnus glutinosa* (L.) Gaertn, had already been dramatically reduced.

In the second half of the 20th century, the forested surface in Northern Spain began to increase in a process of reforestation and replacement of native forest, mostly by introduction of monocultures of exotic coniferous species.

The increase in charcoal and timbers demand due to their use in the production of iron in foundries and the farming and shipbuilding sectors, was the main cause of this deforestation (Martin de Agar, 1995; Martin Martín, 2001).

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Coniferous plantations started spreading in the 20th century, covering many of the hills that had been deforested.

In the 1940's, a slow abandonment of agricultural land began because agriculture was no longer economically profitable and younger generations were moving to cities that offered more attractive opportunities (Eusko Jaurlaritza, 2002) in this context introduction of exotic coniferous species seemed a good use of agricultural land, given by the low price of planting and the fast growth of some species. Meanwhile, native forest continued being deforested.

Nowadays, in the Basque Country *P. radiata* D. Don is the predominant conifer species (137,466 Ha) followed by:

- *P. sylvestris* (17,233Ha),
- *Pinus nigra* Arnold (13,560Ha),
- *Larix* spp. (8,137Ha),
- *P. pinaster* Aiton (7,262Ha),
- *Pseudotsuga menziessi* (Mib.) Franco (5,717Ha),
- *Chamaecyparis lawsoniana* (A. Murray) Parl (3,325Ha),
- *Picea abies* (482Ha)

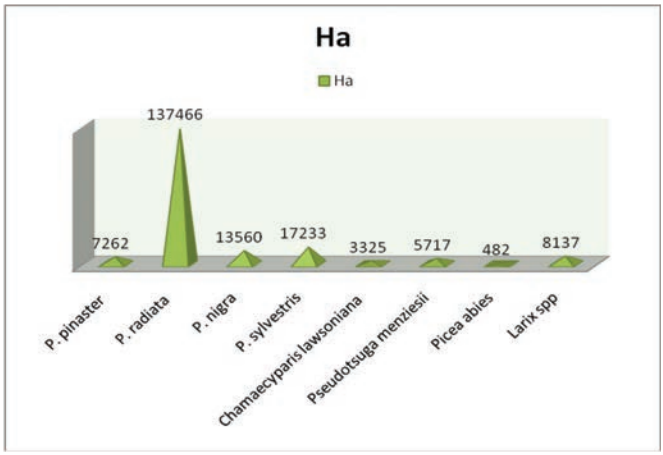


Figure 1. Abundance of the conifer species in Basque Contry. Data by European Forest Institute, 2005; IKT, 2005.

The pathogen *Heterobasidion annosum s.l.* has been occasionally reported in Spain. Segovia, on stumps of *P. sylvestris* (Martinez, 1943).

H. abietinum was found in *Abies pinsapo* Boiss stands of Sierra de las Nieves Natural Park, Málaga (Sanchez *et al.*, 2007) and in *Abies alba* Mill. stands of the Pyrenees (Oliva and Colinas 2007).

H. annosum s.s. had been previously reported in the Pyrenees on *Pinus nigra* (Oliva *et al.,* 2008).

This study is a first investigation of the state of the disease caused by *Heterobasidion* in the Spanish Atlantic forest.

The survey was carried out by stratified random sampling (Muller-Dombois and Ellemberg, 1974), paying attention to trees in pockets of mortality and to decayed trees.

In all cases cultures were obtained from basidiocarps using the semi-selective medium BDS (Benomyl Dichloran Streptomycin) (Worral, 1991) and were incubated at 20°C for two weeks. For each strain, conidia were isolated and single spore cultures were cultured.

The internal transcribed spacer region (ITS) of the ribosomal (r) DNA was amplified and sequenced using primers ITS1-F and ITS4 (Worral *et al.*, 2010). Sequences were aligned with those of other *Heterobasidion* spp. using ClustalW algorithm and then manually with Mega software version 4.0 (Tamura *et al.*, 2007). *H. insulare* and *H. araucariae* ITS sequences from GenBank were used as outgroups (Asiegbu *et al.*, 2004).

The phylogenetic tree was built using the Neighbor-Joining method (Saitou and Nei, 1987) and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004).

The mapping of spatial distribution of *Heterobasidion* population was conducted using ArcGis 9.2 Software. The nearest neighbour index (NNI) was applied in this study, as a measure of clustering (Mitchell 2009) to analyse the distribution pattern of the *Heterobasidion* population.

H. annosum was detected in 42% of the sampled stands, all of them with disease symptoms. Basidiocarps were occasionally found on windthrown trees and in the internal and external part of decayed trees, broken roots, root collars and stumps.

H. annosum showed a wide range of coniferous hosts which represent the most economically important group of plantations for the development of the North of Spain (Montero *et al.*, 2003; European Forest Institute, 2005; IKT, 2005).

To the best of our knowledge, this is the first report, in Spain, of *H. annosum s.s.* causing damages on *Chamaecyparis lawsoniana*, *Pinus pinaster*, *Pinus radiata*, *Pseudotsuga menziesii and Picea abies.* Being also detected in plantations of *P. nigra* and plantations and native forest of *P. sylvestris*.

No evidence of the presence of disease was found in coniferous species which are considered to be hosts such as *Sequoia sempervirens* (D. Don) Endl., *Sequoiadendron giganteum* (Lindl.) J. Buchholz, *Larix leptolepis* (Siebold & Zucc.) Gordon and *Larix decidua* Mill., could be connected with the fact that they only represent 5% of coniferous surface in the area (European Forest Institute, 2005; IKT, 2005).

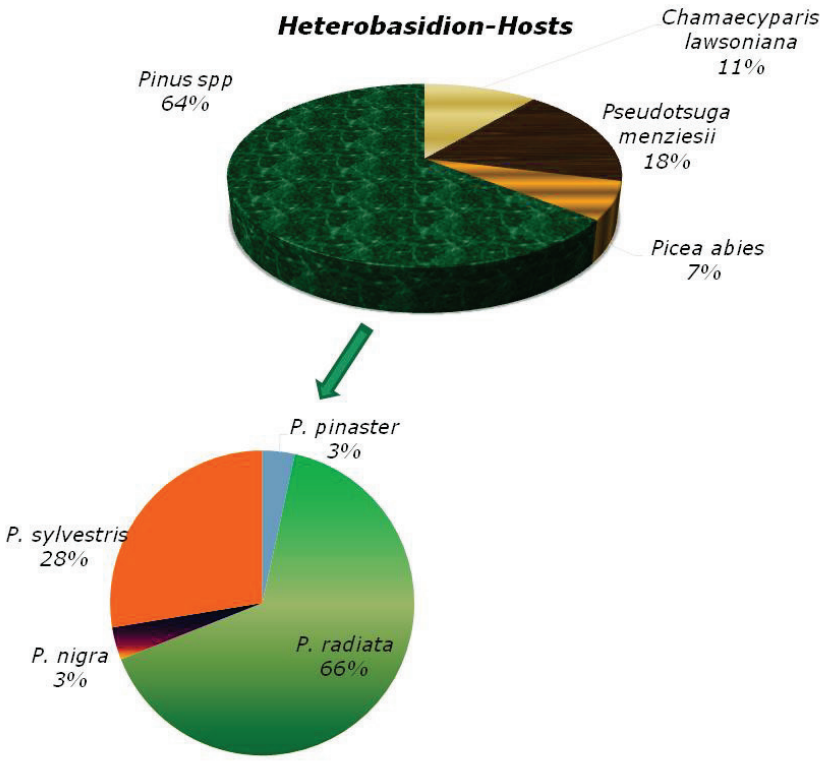


Figure 2. *Heterobasidion* conifer hosts

All the isolates were identified as European *Heterobasidion annosum* (European P-type) based on ITS sequencing (Worral *et al.*, 2010), and the most of their sequences were identical to *H. annosum* B298 GeneBank sequence (*Picea*, Finland) except isolates H63 (A→G transition at nucleotide 412), H67 (A→T transversion at nucleotide 188), H94 (G insertion at nucleotide102), and H77, H50, H90, H97(C→T transition at nucleotide 9). The bootstrap value for the branch connecting *H. annosum* isolates was 92%. Representative ITS sequences of this specie for each host were deposited in GenBank.

Spatial distribution of *H. annosum* in the Basque Country is not randomly. In this case, the average of nearest neighbor index, NNI, was equal to 0.44 with a Z score of -7.26 ($P< 0.01$). The null hypothesis is rejected (Mitchell, 2009) and the results suggest a statistically significant, high degree of clustering in the population of *H. annosum* from the Basque Country.

Host and spatial distribution of *Heterobasidion* cultures from the Basque Country and point density map are shown in Fig. 3

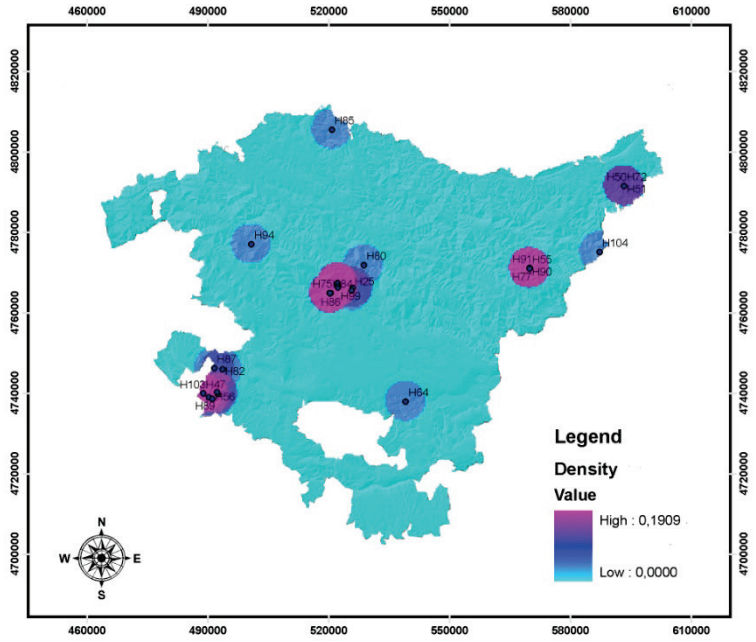


Figure 3. Point density map of host and spatial distribution of *Heterobasidion* cultures from the Basque Country

Heterobasidion has probably become more frequent due to the dominance of productive coniferous ecosystems subsequent to reforestation established in the 1940s (Eusko Jaurlaritza-Gobierno Vasco, 2002).

These plantations replaced many of the deciduous native species such as *Fagus sylvatica, Quercus robur, Q. pyrenaica*, *Fraxinus excelsior*, *Alnus glutinosa*, as well as some coniferous native forest of *P. sylvestris* and former pastureland (Ruiz Urrestarazu, 1992). *P. sylvestris* and *Fagus sylvatica* are potential hosts of this pathogen (Puddu *et al.,* 2003).

This situation has implications for management in the Atlantic forest ecosystems of Spain. In areas where *Fusarium circinatum* Niremberg & O'Donnell has previously been detected, stands of *Pinus* spp. are currently being substituted by other conifers as spruces, Lawson cypress, potential hosts of *Heterobasidion annosum*.

A revision needs to be carried out of established management recommendations in areas where multiple diseases could be present.

It must be recognised that we need to achieve deeper knowledge and ethics of forest ecosystem to reach a balance between the exploitation and conservation of forest resources, together with a greater diversification in land use and restoration of native forest.

References

Asiegbu F.A., Abu S., Stenlid J., Johansson M., 2004. Sequence polymorphism and molecular characterization of laccase genes of the conifer pathogen *Heterobasidion annosum*. *Mycological Research* 108: 136-148.

European Forest Institute, 2005. Datos Económicos Forestales de Euskadi. http://www.efi.int/portal/.

Eusko Jaurlaritza-Gobierno Vasco, 2002. EAEko ingurumeneko Esparru Programa (2002-2006). Ihobe, Vitoria-Gasteiz.

IKT, 2005. Inventario Forestal CAE 2005. http://www.nasdap.ejgv.euskadi.net/r50-15135/es/contenidos/informacion/inventario_forestal_index/es_dapa/inventario_forestal_index.html.

Martin de Agar P., de Pablo C.L., Schmitz M.F., Atauri J.A., Pineda F.D., 1995. Incidencias ambientales de los cambios de usos del suelo en la reserva de la Biosfera de Urdaibai. In: E. Angulo and I. Quincoces (eds.). Reserva de la Biosfera de Urdaibai: Investigación bâsica y aplicada, Servicio de Publicaciones del Gobierno Vasco. Vitoria., pp. 297-334.

Martín R., 2001. Caracterización ecológica del territorio histórico de Bizkaia. *Artadi* 1: 13-31.

Mitchell A., 2009. The ESRI Guide to analysis. Volume 2: Spatial Measurements & Statistics. ESRI Press, Redlands, California, USA.

Montero G, Cisneros O., Cañellas I., 2003. Manual de la selvicultura para plantaciones de especies productoras de madera de calidad. Mundi-Prensa, Madrid.

Oliva J., Colinas C., 2007. Decline of silver fir (*Abies alba* Mill.) stands in the Spanish Pyrenees: role of management, historic dynamics and pathogens. *Forest Ecology and Management* 252: 84-97.

Oliva J., Ibarra N., Colinas C., Martín E., 2008. Detección molecular de *Heterobasidion annosum s.s.* en claras de *Pinus nigra* en el Pirineo Aragonés. *Boletín Sanidad Vegetal. Plagas* 34: 415-416.

Puddu A., Luisi N., Capretti P., Santini A., 2003. Environmental factors related to damage by *Heterobasidion abietinum* in *Abies alba* forest in Southern Italy. *Forest Ecology and Management* 180: 37-44.

Ruiz Urrestarazu M.M., 1992. El Pino Radiata en el País Vasco dentro de la Perspectiva Forestal Mundial. In: Eusko Jaurlaritzaren Argitalpen Zerbitzu Nagusia, Vitoria-Gasteiz (ed.). *El Bosque en el Espacio Rural del Sur de Europa; Jornadas Foresta '91*, *Bibao* 1991: 41-52.

Saitou N., Nei M., 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.

Sánchez M.E., Luchi N., Jiménez J.J., de Vita P., Sánchez J.E., Trapero A., Capretti P., 2007. An isolated population of *Heterobasidion abietinum* on *Abies pinsapo* in Spain. *Forest Pathology* 37: 348-356.

Tamura K., Dudley J., Nei M., Kumar S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596-1599.

Worral J., 1991. Media for selective isolation of *Hymenomycetes*. *Mycologia* 83: 298-302.

Worral J.J., Harrington T.C., Conklin D.A., Fairweather M.L., 2010. *Heterobasidion annosum* and *H. parviporum* in the Southern Rocky Mountains and Adjoining States. *Plant Disease* 94: 115-118.

Susceptibility of stump heartwood and sapwood to *Heterobasidion annosum s.l.* infection in Norway spruce (*Picea abies*)

J. Oliva, M. Bernat, J. Stenlid

Dept. Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026 750 07 Uppsala (Sweden).
Corresponding author e-mail address: jonas.oliva@slu.se

Abstract. In Scandinavia, freshly created stump surfaces of Norway spruce (*Picea abies* (L.) Karst.) become often infected by *Heterobasidion annosum sensu stricto* (*s.s*) and *H. parviporum*. In an early experiment, it was shown that after stump infection, *H. parviporum* spread more often than *H. annosum s.s.* towards neighbouring trees. *H. parviporum* spread occurred mainly from big stumps. Big thinning stumps often have developed heartwood, thus *H. parviporum* could be more efficient at establishing on the heartwood than on the sapwood. Stump-to-tree spread not only depends on establishment but also on the progression through the root system. The observed pattern could also relate to a more efficient spread through heartwood than through sapwood. In order to test the effect of heartwood and sapwood on *Heterobasidion* establishment and spread, we randomly infected thinning stumps on the heartwood, the sapwood and on the border zone between the heartwood and the sapwood. The observed stump-to-spread differences between *Heterobasidion* species could also relate to differences in heartwood-sapwood preference, thus artificial infection included a set of four *H. annosum s.s.* and four *H. parviporum* isolates that were inoculated individually and in mixed cultures. To test whether sapwood-heartwood patterns could be dependent on the host species, we replicated the experiment in two spruce stands and in two pine (*Pinus sylvestris* L.) stands. Establishment was assessed two months after inoculation. One slice was extracted from each stump and was cultured in the dark at room temperature. The proportion of infection on each part (sapwood, border zone and heartwood) was measured and *ca.* thirty isolates were retrieved from every slice and were checked to be the original isolate by somatic compatibility. In the case of spruce stumps, preliminary results revealed a substantial establishment of *Heterobasidion* sp. on the heartwood. The original *H. parviporum* isolates were more commonly retrieved from heartwood than from sapwood. *H. annosum s.s.* was able to infect a lower number of spruce stumps than *H. parviporum*, supporting a role of the stumps in selecting between species already in the establishment stage. In pine stumps, artificial infections were less successful than in spruce stumps. The majority of isolates that succeeded establishing on pine stumps corresponded to *H. annosum s.s.*

In Scandinavia, freshly created stump surfaces of Norway spruce (*Picea abies* (L.) Karst.) become ofteninfected by *Heterobasidion annosum sensu stricto* (*s.s*) and *H. parviporum*. In an early experiment, it was shown that after stump infection, *H. parviporum* spread more often than *H. annosum s.s.* towards neighbouring trees (Oliva *et al.,* 2011). *H. parviporum* spread occurred mainly from big stumps. Big thinning stumps often have developed heartwood, thus *H. parviporum* could bemore efficient at establishing on the heartwood than on the sapwood. Stump-to-tree spread not only depends on establishment but also on the progression through the root system. The observed pattern could reveal not a more efficient establishment but a more efficient spread through heartwood than through sapwood. In order totest the effect of heartwood and sapwood on *Heterobasidion*

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

establishment and spread a long term experiment was established in 2010. We randomly infected thinning stumps on the heartwood, the sapwood and on the border zone between the heartwood and the sapwood. In order to test whether the observed stump-to-spread differences between *Heterobasidion* species could also relate to differences in heartwood-sapwood preference, both species were infected separately and in mixed cultures. Mixed cultures aim at reproduce real field conditions, where inoculum of both *H. parviporum* and *H. annosum s.s.* is likely to be present. Artificial infection included a set of four *H. annosum s.s.* and four *H. parviporum*. To test whether sapwood-heartwood patterns could be dependent on the host species, we replicated the experiment in two spruce stands and in two pine (*Pinus sylvestris* L.) stands. Establishment was assessed two months after inoculation. One slice was extracted from each stump and was cultured in the dark at room temperature. The proportion of infection on each part (sapwood, border zone and heartwood) was measured and *ca.*15-45 isolates were retrieved from every slice and were checked to be the original isolate by somatic compatibility. In the case of spruce stumps, preliminary results revealed a substantial establishment of *Heterobasidion* sp. on the heartwood. The original *H. parviporum* isolates could be retrieved from all infected tissues, although in the border zone a higher level of contamination presumably from basidiospores occurred. *H. annosum s.s.* was able to infect a lower number of spruce stumps than *H. parviporum* either when infected alone or when infected in a mixed culture supporting a role of the stumps in selecting between species already in the establishment stage. In pine stumps, artificial infections were less successful than in spruce stumps. The majority of isolates that succeeded establishing on pine stumps corresponded to *H. annosum s.s.* Future measurements at 10, 24 and 48 months after inoculations will follow the infection through the stump roots systems until neighbouring trees.

References

Oliva J., Bendz-Hellgren M., Stenlid J., 2011. Spread of *Heterobasidion annosum s.s.* and *Heterobasidion parviporum* in *Picea abies* 15 years after stump inoculation. *FEMS Microbiology Ecology* 75: 414-429.

Detection of *Armillaria tabescens* by bait method using old, freshly-cut logs and cherry seedlings

Y. Ota[1], H. Onozato[2], Y. Kawabe[3]

[1]*Forestry and Forest Products Research Institute, Ibaraki 305-8687, Japan.*
[2]*Gunma Prefecture Forestry Experiment Station, Gunma 370-3503, Japan.*
[3]*Forestry and Forest Products Research Institute, Ibaraki 305-8687, Japan.*
Corresponding author e-mail address: yuota@affrc.go.jp

Abstract. *Armillaria tabescens* is an important root rot pathogen of ornamental trees in Japan. We conducted an experiment to detect *A. tabescens* by the bait methods using old and freshly-cut logs and living seedlings. A total of 251 bait traps (85 old logs, 85 freshly-cut logs and 81 seedlings) were placed in contact with infected hardwood stamps and at distances of 0.5 and 5 m from the infected hardwood stamps. Eleven months later, the presence/absence of rhizomorphs and mycelial mats of *Armillaria* spp. in the logs and seedlings was investigated and *Armillaria* isolates were obtained from some of them. Species were determined by ITS sequences. *Armillaria* spp. were found on 14 seedlings (17%), 37 freshly-cut logs (44%) and 31 old logs (36%) and 11, 27 and 21 isolates were established, respectively. All isolates originating from rhizomorphs were identified as *A. gallica*. *Armillaria tabescens* was only recovered from mycelial mats. *Armillaria tabescens* was detected from 2 seedlings, 8 freshly-cut logs and 1 old log. These results show that *A. tabescens* does not produce rhizomorphs in the field and seems to prefer fresh substrates.

Introduction

Armillaria tabescens (Scop.) Emel is an important root rot pathogen of ornamental trees in Japan. The decline and death of such trees due to *A. tabescens* have been increasing among both hardwoods and conifers in gardens, arboreta and street margins in Japan. Fatal root rot caused by *A. tabescens* among cherry trees frequently reported (Onozato *et al.,* 2009) makes control of *Armillaria* disease an urgent matter. In a previous study, we conducted an experiment using oak logs and seedlings as bait traps to investigate the distribution of *A. tabescens* in soil at hardwood stands damaged by *A. tabescens* root rot. *Armillaria tabescens* was detected in one of 5 seedlings (20%) and one of 34 (3%) logs placed in contact with the infected stump, but never in logs placed randomly apart from the infected stump in the stands. In this study, we conducted an experiment to detect *A. tabescens* by bait method; using old and freshly-cut logs and living seedlings at various distances from the infected stumps.

Materials and Methods

The study was conducted in an arboretum damaged by *A. tabescens* in Gunma Pref., Japan. As bait traps, oak logs of about 70 cm in length and 3 cm in diameter were prepared one day before (freshly-cut logs) and 2 months before (old logs), and 2-year-old cherry seedlings *(Prunus* × *yedoensis* Matsumura) of 120 cm in height were used in this study. A total of 251 bait traps (85 old logs, 85 freshly-cut

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

logs and 81 seedlings) were placed in contact with infected hardwood stumps (at distance of 0 m) and at distances of 0.5 and 5 m, respectively. The stumps had recently died due to *A. tabescens* root rot and vigorous mycelial mats of *A. tabescens* under the bark had been confirmed. After eleven months, the logs and seedlings were dug up and examined for the presence of rhizomorphs and mycelial mats of *Armillaria* spp., and pure cultures obtained from them. The species were determined by sequence analysis of the ITS of the nrDNA and/or elongation factor 1α region.

Results and Discussion

Armillaria spp. were found on 14 seedlings (17%), 37 freshly-cut logs (44%) and 31 old logs (36%); with 12 (86%), 27 (73%) and 21 (68%) isolates respectively established. In this experiment, *A. tabescens* and *A. gallica* were obtained. *Armillaria tabescens* was detected in 2 seedlings, 8 freshly-cut logs and 1 old log: 25, 30 and 5% of the respective totals. In contrast, *A. gallica* was detected in more than 74% of seedlings, freshly-cut logs and old logs. *Armillaria tabescens* considered to prefer newer to old substrates compared with *A. gallica.* The detection rates of *A. tabescens* at distances of 0, 0.5 and 5 m from the infected stumps were 27, 24 and 0%, respectively, while those for *A. gallica* were 80, 76 and 100%, respectively. *Armillaria tabescens* was considered to infect only when in contact with the infected stumps or roots in the soil. 65 mycelial mats and 62 rhizomorphs were obtained from the seedlings and logs in this study, of which 52 and 65% were identified, respectively. From the mycelial mats identified, 38% were determined as *A. tabescens*. Conversely, no *A. tabescens* was determined from rhizomorphs.

From these results, *A. tabescens* was detected only from mycelial mats; mostly on seedlings and freshly-cut logs at 0 and 0.5 m from the infected stump. Infection by *A. tabescens* in the soil could be decreased by planting seedlings at intervals of 5m from the infected stump. To detect *A. tabescens* in the soil using bait methods, freshly-cut logs were more useful than old ones.

Figure 1. An experiment using seedlings (with yellow colored tapes), freshly-cut logs (with white colored tapes) and old logs (with red colored tape) as bait traps.

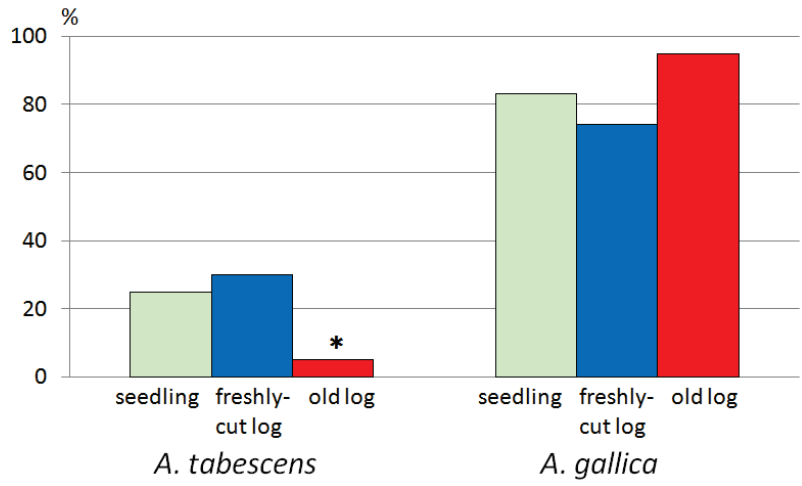


Figure 2. Detection rates of *A. tabescens* and *A. gallica* in seedlings, freshly-cut logs, and old logs. 12 seedlings, 27 freshly-cut logs and 21 old logs from which *Armillaria* spp. were obtained, were used.

References

Onozato H., Kawabe Y., Ota Y., Kikuchi T., Kanazawa Y., 2009. Detection of *Armillaria* spp. by the bait method using oak logs in a cherry stand damaged by *Armillaria tabescens*. *Journal of the Japanese Forestry Society* 91: 201-207 (in Japanese with English summary).

Survival of *Heterobasidion annosum* in buried pine roots

J.E. Pratt[1] and B.J.W. Greig[2]

[1]*Cross House, Mountain Cross, EH46 7DF, Scotland, UK.*
[2]*19 Jubilee Lane, Farnham, GU10 4SZ, UK.*
Corresponding author e-mail address: k.m.pratt@btinternet.com

Abstract. Some breakage inevitably occurs to stumps during mechanical excavation of diseased root systems for remedial control of *Heterobasidion annosum*, so that potential inoculum remains buried and in position to infect trees of the next rotation. We report an experiment to measure longevity of the fungus in broken Scots pine (*Pinus sylvestris* L.) root fragments in sandy soil (pH >7.0). *H. annosum* survived longest in roots which were totally decayed, or were partially decayed and partially resinous, when buried, and was still active after 13 years.

Introduction

On sandy soils with high pH in East England, the removal of pine stumps after clear-felling has been a successful remedial control measure against *H. annosum* for the past 40 years. Inevitably during stump extraction, some roots are broken and left in the ground. Although there is ample evidence that such roots act as sources of infection to replacement crops, no systematic investigation has been done on the fate of this diseased tissue. The experiment reported here was undertaken to determine the longevity of *H. annosum* in diseased pine roots in soils where de-stumping is routine, and to observe if colonisation of healthy roots would occur in a typical high disease-hazard site.

Methods

A trench, 10 m long, 1.5 m wide was dug down to the underlying chalk in a recently de-stumped area in Thetford Chase (52°24′ N, 0°40′ E) in September 1969.

The soil was a well-drained brown earth (sol lessivé) of the Worlington series, pH 8.1 at 230 mm, and pH 7.4 at 600 mm. Depth to the underlying chalky drift varied between 1.5 and 2.0 m. A thin clay-enriched Bt layer occurred at 600-800 mm depth.

During the excavation of the trench, the soil was removed in layers around 500 mm thick so that it could be back-filled in the original sequence.

Longitudinal segments, 150 mm long × 15-50 mm diameter, were cut from roots of excavated stumps of 43-year-old Scots pine trees that had been growing on a site infested with *H. annosum*. Four categories of roots were selected: healthy, resinous, decayed and resinous/decayed. Thirty samples of each category were buried. The healthy category consisted of live roots with no signs of *H. annosum*

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

infection. Resinous roots were dead or dying, suffused with resin, but without decay. Decayed roots were characterised by white pocket rot typical of H. annosum. Resinous/decayed roots combined features of the previous two categories. From each root, a 5mm thick slice was removed from one end, incubated and examined for the presence of H. annosum conidophores.

Root segments were pushed horizontally into narrow holes drilled in the sides of the trench at 230 mm and 600 mm depths below the surface. This method was adopted to reduce the disturbance of soil around each sample. The trench was backfilled with original soil.

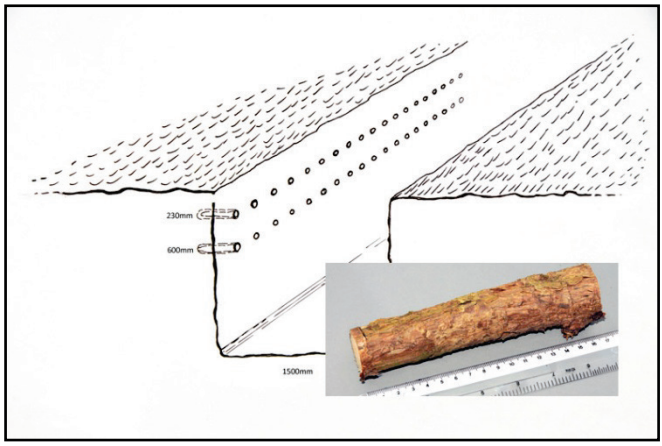


Figure 1. Cross-section of trench showing layout of buried roots and (inset) typical root sample.

Roots were destructively sampled at 13 and 31 months (5 samples per category) and 48 and 156 months (10 samples per category) after burial. After removal, each sample was brushed under running water and cut into 30 mm lengths that were incubated on moist vermiculite for eight days at 100°C. One cut length from each sample was split longitudinally. The presence or absence of *H. annosum* in each length was confirmed by examination conidiophores of the fungus.

Results

The weather over the 13-yr period of the trial was not recorded. However, meteorological records from 2 km distance show that soil temperatures at 200 mm and 500 mm vary by an average of 9.2°C and 5.6°C throughout the year, the average maximum temperature at each depth being 19.6°C and 18.1°C respectively. Rainfall averages 622 mm per year.

The fungus was not detected in the 5 mm slices removed at the start of the trial from any of the 30 roots designated as healthy. By contrast it was present in at least 80% of the slices removed from the roots in the other categories.

Depth of burial did not affect survival of *H. annosum* and the data from both depths have been amalgamated. Tab. 1 shows survival rates at each sampling.

Table 1. Frequency of survival of *H.annosum* in buried pine roots post burial.

Root category	Burial period (months)											
	13 (5 roots per category)			31 (5 roots per category)			48 (10 roots per category)			156 (10 roots per category)		
	Nos Ha +			Nos Ha +			Nos Ha +			Nos Ha +		
	Burial	Sample	%	Burial	Sample	%	Burial	Sample	%	Burial	Sample	%
Healthy	0	0	0	0	0	0	0	2	-	0	0	0
Resinous	5	3	60	3	1	33	8	5	62	8	0	0
Decayed	5	5	100	4	4	100	9	7	77	9	8	88
Resinous/ decayed	5	5	100	5	3	60	9	8	88	10	4	40

The fungus survived best in decayed roots where it was still viable in eight of nine roots after 13 years (156 months) burial, and in 24 out of 27 roots overall. In the resinous/decayed category, the fungus survived in 20 of the 29 roots infected on burial, and in four of the ten roots sampled after 13 years. *H. annosum* did not survive as well in the resinous roots, and typically it was replaced by other organisms so that none of these roots still contained active *H. annosum* when sampled after 13 years. Of the healthy roots, two out of 30 samples were colonised by *H. annosum* during the first four years (48 months) of the experiment, while the remainder quickly became colonised by *Trichoderma* spp. and other soil micro organisms.

Discussion

These results are of interest to those attempting remedial treatment of the disease by stump removal, since they demonstrate the capacity of the fungus to survive in small roots for extensive periods in high-risk soils in a hemioceanic climate. The data are also relevant to studies on the epidemiology of the disease, especially where dynamic changes in inoculum are being considered.

Previous studies have shown that *H. annosum* can survive for up to 110 weeks in naturally-infected roots buried in alkaline woodland soils, while on more acid soils *H. annosum* disappeared more quickly, and was apparently more rapidly replaced by other fungi (Rishbeth, 1951). Interestingly, and in contrast with our results, he also reported that *H. annosum* survived longer in naturally infected roots if they were resinous. *H. annosum* survived for at least 18 months in small (25 mm diameter) naturally-infected pine roots from Thetford buried in medium loam and

incubated in a greenhouse (Evans, 1965). In Evans' study, the fungus survived better in larger roots, and in roots with the bark attached and the ends sealed with wax.

Survival of up to 13 years reported here in decayed roots is surprising in view of the small dimensions of the root segments used and the advanced nature of the decay at the time of their burial. Such longevity in the dry, sandy soils of Thetford Chase which in most years are subject to ten months with a potential water deficit contrasts with poorly drained soils in the south of Scotland, where the fungus rarely survived more than seven years in roots of Sitka spruce (*Picea sitchensis* Bong (Carr.) (Redfern, 1984). However the influence of the host must be taken into account in making this comparison.

The mode by which healthy roots became infected during the trial was not determined. They were not protected from air-borne spores during the burial process, and this is a possible source. The potential for non-motile spores of *H. annosum* to infect wounded, live roots at depth in undisturbed soil is not thought to be high (Redfern and Stenlid, 1998). The low frequency of their infection in this trial suggest that healthy roots left in the soil after stump removal will not pose a measurable risk to replanted crops.

Stump removal is an expensive remedial treatment. When properly carried out, there is no doubt about its effectiveness (Greig, 1984). Roots remaining in soil after de-stumping comprise a small proportion of the total mass extracted (Pratt, unpublished). However, infected roots may act as inoculum for many years, and there is clear evidence from excavations elsewhere in Thetford Chase that short root segments of 2 cm diameter commonly lead to the infection and death of young Corsican pine at least five years after stump removal (Pratt, unpublished). The lesson for forest managers is that the fungus can survive within small roots for sufficient time to initiate infection in succeeding crops.

References

Evans E., 1965. Survival of *Fomes annosum* in infected roots in soil. *Nature* 207: 318-319.

Greig B.J.W., 1984. Management of East England pine plantations affected by *Heterobasidion annosum* root rot. *European Journal of Forest Pathology* 14: 392-397.

Redfern D.B. and Stenlid J., 1998. Spore dispersal and infection. In Woodward S., Stenlid J., Karjalainen R., Hütterman A. (eds). *Heterobasidion annosum* Biology Ecology, Impact and Control, CAB International, Wallingford, Oxon OX10 8DE, UK. pp. 105-124.

Redfern D.B., 1984. Factors affecting spread of *Heterobasidion annosum* in plantations. In: Kile G.A. (ed.). Proceedings of the 6th International Conference on Root and Butt Rots of Forest Trees, Melbourne, Victoria and Gympie, Queensland, Australia, 1983: 104-114.

Rishbeth J., 1951. Observations on the biology of *Fomes annosus*, with particular refer-ence to East Anglian pine plantations. II. Spore production, stump infection, and sapro-phytic activity in stumps. *Annals of Botany* 15: 1-21.

Stump removal trials to control root-rot in *Picea abies* stands in Scandinavia

N. Arhipova[1], I.M. Thomsen[2], J. Stenlid[1], R. Vasaitis[1]

[1]*Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, PO Box 7026, Se-750 07 Uppsala, Sweden.*
[2]*Forest and Landscape, University of Copenhagen.*
Corresponding author e-mail address: natalija.arhipova@slu.se

According to literature (Vasaitis, 2008), stump removal following clear-felling usually leads to root-rot (caused by *Heterobasidion* and *Armillaria*) reduction in next forest generation. Yet, only two trials to study this are available in Scandinavia: established in Sweden in 1958, and in Denmark in 1964. Both comprised heavily root-rot-infected clear-cuts of *Picea abies*. The sites were divided into several parcels, from which stumps were either removed or not-removed (control), replanting each parcel with *P. abies*. The aim of this study was to evaluate long-term (40-50 year) effect of stump removal on root-rot incidence. Swedish trials were preliminary evaluated in 1975-78 and in 1984-85. In first evaluation (after 18-20 years) the frequency of root-rot on de-stumped parcels was 0-0.7%, but in controls it was 8-11%. In second (after 26-27 years), corresponding figures were 0.6-2.3% and 11.4-16.7%, showing certain positive effect (Stenlid, 1987). Here, persistence of the effect was checked after yet another 25 years (in 2008). In Danish trial, from year 1967 to 1990 all parcels were regularly inspected for dead trees, and on those symptoms of *Heterobasidion* and *Armillaria* were recorded. Plantation was thinned three times, in years 1991, 1997 and 2005, and presence/absence on each stump of root-rot has been also recorded. Results show that stump removal reduced the occurrence of root-rot in next *P. abies* generation, although the effect decreased with stand age.

References

Stenlid J., 1987. Controlling and predictiong the spread of *Heterobasidion annosum* from infected stumps and trees of *Picea abies*. *Scandinavian Journal of Forest Research* 2: 187-198.

Vasaitis R., Stenlid J., Thomsen I.M., Barklund P., Dahlberg A., 2008. Stump removal to control root rot in forest stands. A literature study. *Silva Fennica* 42: 457-483.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Preliminary assessments to determine the potential risk of *Armillaria gallica* to healthy plants

L. Beal, I. Burdon, J. Denton, B. Henricot

The Royal Horticultural Society, Wisley, Woking, Surrey, GU23 6QB, UK.
Corresponding author e-mail address: beatricehenricot@rhs.org.uk

Armillaria spp. (honey fungus) is a root pathogen and has been identified as the principal cause of woody plant death in UK gardens (Henricot, 2011). A 4-year survey has shown that *A. mellea* and *A. gallica* are the two most common species in gardens. The former species is considered as a true pathogen whilst the latter is better known as a weak or opportunistic pathogen (Risbeth, 1982; Davidson and Risbeth 1988). However, the survey results showed that just under 23% of *A. gallica* isolates were recovered from dead material and 6% from basidiomes whilst the remaining 71% were found on standing trees with no other pathogens present. In order to assess the potential risk to plants from this species, a series of experiments have been designed. Experiments looking at the maximum temperature at which rhizomorphs or inoculum plugs could survive demonstrated that *A. gallica* was able to tolerate higher temperatures than *A. mellea*; rhizomorphs of both species were more sensitive to dry heat than mycelium embedded in hazel plugs. Factors that might influence the growth or survival of rhizomorphs once they are severed are currently being tested. Initial experiments with *A. gallica* and *A. mellea* have shown that severed rhizomorphs of *A. gallica* have a higher survival rate than *A. mellea* and loam is a better substrate than compost for their survival. Both species showed no increases in rhizomorph length over the 6 month trial period. This would suggest that in the absence of woody material the risk of severed rhizomorphs is not very significant. More work needs to be done to see whether severed rhizomorphs behave differently in the presence of a food source while further field trials are underway to determine if stress is necessary for *A. gallica* to infect live plants.

References

Davidson A.J. and Risbeth J., 1988. Effect of suppression and felling on infection of oak and Scots pine by *Armillaria*. *European Jouranl of Forest Pathology* 18: 161-168.

Henricot B., 2011. Honey Fungus. *The Garden*. November issue: 58-59.

Risbeth J., 1982. Species of *Armillaria* in Southern England. *Plant Pathology* 31: 9-17.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Occurrence of *Armillaria* root disease in Friuli Venezia Giulia Pine stands after a hailstorm

G. Frigimelica

Department of Agriculture and Environmental Sciences - Plant Pathology, Via delle Scienze, 208, 33100 UDINE, Italy.
Corresponding author e-mail address: frigimelica@hotmail.com

Abstract. In June 2009 a severe hail storm occurred in the high valley of Tagliamento river, in the Friuli Venezia Giulia Region (Northeast Italy), damaging both *Pinus nigra* Arnold and *Pinus sylvestris* L. stands. In July the trees exhibited die-back and crown reddening both related to hail damage, and, later, *Diplodia pinea* (Desm.) J.J. Kickx infections. In spring 2010, a biannual survey was started to evaluate the phytosanitary status of the hail damaged pines, in correlation with the succession of fungi and xylophagous insects. In autumn 2010, the pine mortality was 53% in the *P. sylvestris* plots severely damaged by hail, with *D. pinea* infections present on the main branches and upper part of the stems and high presence of xilophagous insects. In contrast, mortality was only 2% in the *P. nigra* plots, with *D. pinea* infections and xylophagus insects confined on smallest branches and trees showing signs of recovery. In spring 2011, however, the decline in *P. nigra* plots increased significantly, with mortality reaching 34% and new widespread crown reddening. The lower part of the stem of dead *P. sylvestris* and *P. nigra* trees were colonized by *Armillaria* (Fr.) Staude. The occurrence of expanding *Armillaria* disease foci may explain the recrudescence of *P. nigra* decline in stands where *D. pinea* and xylophagus insects colonization were still moderate, after the 2009 hail storm.

In June 2009 a severe hailstorm occurred in natural pine stands (Austrian pine, *Pinus nigra* Arnold and Scots pine, *P. sylvestris* L.) growing in north-eastern Italy (Friuli Venezia Giulia Region) along the upper part of the River Tagliamento (46.4°N; 12.9°E; 250-500 m a.s.l.). The hailstorm damaged an area of about 100 ha and the degree of crown loss varied from 26 to 90%, depending on the locations. One month after the hailstorm (July 2009) pine trees showed dieback and crown discoloration due to infections by *Diplodia pinea* (Desm.) J.J. Kickx that followed the hail damage. In 2010 and 2011 specific surveys were carried out in the damaged stands in order to evaluate pine tree conditions in relation to the occurrence of both fungi and xylophagous insects. Data reported in this note were obtained by selecting, in each stand, 3 areas (n=6) where the damage was higher than 60% and by monitoring 30 trees per area with the only exception of 2 Austrian pine areas where 40 trees were analyzed (n=200).

In summer 2010 Scots pine mortality (52%) was positively related to the degree of crown damage observed in 2009, whereas no relationships were found between Austrian pine mortality (2%) and crown damage level. In autumn 2010 the dead and dying Scots pine trees were infected by *D. pinea*, and showed sapstain and pycnidia spread on the main branches and on the upper part of the stems.

Moreover, they were colonised by a large number of xylophagous insects, mainly bark and ambrosia beetles and longhorn beetles. In addition, in 60% of the

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

dead trees the lower part of the stem was infected by *Armillaria* (Fr.) Staude. On the contrary, the most part of the Austrian pine trees showed signs of recovery, as *D. pinea* infections and attacks by xylophagous insects were confined mainly to the smallest branches. In spring and summer 2011 the crown decline greatly increased and the cumulate mortality (2010-2011) reached up to 35% in Austrian pine areas and 69% in Scots pine areas. Almost half the dead trees were colonized by *Armillaria* (57% and 41% of the Scots and Austrian pine trees, respectively), and signs of attacks by xilophagous insects were also observed (92% and 28% of the Scots and Austrian pine trees, respectively). Trees infected by *Armillaria* were grouped into six categories based on the occurrence of vegetative structures under the bark: young mycelium, aged mycelium, young and aged mycelium, mature mycelium, mycelium and rhizomorphs, young rhizomorphs, aged rhizomorphs. In addition, the presence of alive sapwood at the root collar and along the margins of the *Armillaria* colonization was recorded. Results revealed that 41% of the Scots pine dead trees showed only aged rhizomorphs, with melanized cortex, 10% only young mycelium, and 26% presence of still alive sapwood. In contrast, on Austrian pine dead trees, aged rhizomorphs occurred only in 2% of the cases, young mycelium in 43% and alive sapwood in 80%.

In Scots pine stands only secondary *Armillaria* infections occurred but they suffered a prompt and high mortality clearly related to the post-hail expansion of both new and latent *D. pinea* infections (Smith *et al.,* 2002) and to the severe insect attacks (Zwolinski *et al.,* 1995), as demonstrated by previous researches . The mortality that affected the Austrian pine stands was delayed and the expansion of the *Armillaria* disease foci may explain the recrudescence of decline in these stands where, one year after the hailstorm, the infection of *D. pinea* and the colonization by xylophagous insects were moderate.

Reference

Smith H., Wingfield M.J., Coutinho T.A., 2002. The role of latent *Sphaeropsis sapinea* infections in post-hail associated die-back of *Pinus patula*. *Forest Ecology and Management* 164: 177-184.

Zwolinski J.B., Swart W.J., Wingfield M.J., 1995. Association of *Sphaeropsis sapinea* with insect infestation following hail damage of *Pinus radiata*. *Forest Ecology and Management* 72: 293-298.

Decay and associated fungi in *Alnus glutinosa* in Latvia

N. Arhipova[1,2], T. Gaitnieks[1], J. Donis[1], J. Stenlid[2], R. Vasaitis[2]

[1]*Latvian State Forest Research Institute "Silava", LV2169, Salaspils, Latvia.*
[2]*Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, PO Box 7026, SE-75007, Uppsala, Sweden.*
Corresponding author e-mail address: natalija.arhipova@slu.se

Black alder (*Alnus glutinosa* L.) is considered as the most relevant alder species for forest industry in Europe. Currently, black alder stands comprise 5.1% (161200 ha) of the total forest area of Latvia. Studies about stem decay in *A. glutinosa* and decay-causing fungi have never been performed in Latvia before.

The aims of this study were to: i) estimate the incidence of stem decay in *A. glutinosa* stands, ii) measure the extent of decay within individual stems, iii) identify decay-causing fungi. In total, in four *A. glutinosa* stands (51-84 years old) 450 trees were sampled with increment borer and presence/absence of decay was recorded. As a result, 112 visually healthy and 338 decayed trees were sampled, and corresponding numbers of wood samples were collected. All wood samples, including sound looking, were subjected for fungal isolation. For fungal species identification morphological and molecular techniques were used. The incidence of decayed stems varied from 52.7% to 98% (75.1% in average). In addition, 34 decayed trees on two forest stands were cut to measure the extent of decay within the stems. The length of decay columns varied from 0.4-17.4 m, and that of spongy rot - from 0-14.7 m. In total, 1134 isolates representing 68 fungal taxa were obtained from living stems of *A. glutinosa*. The most important decay causing fungus was *Inonotus radiatus* (42.6% of decayed trees), following by *Armillaria* sp. (5.9% of decayed trees).

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Dynamics of *Heterobasidion* sporocarp formation on coarse woody debris retained on harvested *Picea abies* sites

D. Nitisa[1], T. Gaitnieks[1], B. Stivrina[1], J. Donis[1], K. Korhonen[2], R. Vasaitis[3]

[1]*Latvian State Forest Research Institute, Silava", 111 Rigas Street, Salaspils, LV-2169, Latvia.*
[2]*Finnish Forest Research Institute, PO Box 18, FI-01301 Vantaa, Finland.*
[3]*Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 Uppsala, Sweden.*
Corresponding author e-mail address: nitisa.dina@inbox.lv

In Latvian forests, during harvesting of wood a certain amount of large dimension logs are being retained on felled sites in order to sustain biodiversity of wood-inhabiting organisms. Those logs, together with cut stumps, comprise the major source of coarse woody debris (CWD) available in ecosystems of managed forests. It is no secret, however, that due to economical reasons harvesting manager would prefer to retain on a site rot-containing logs. In particular, this is the case for the most economically important *Picea abies*, about 20% stems of which in Latvian stands are butt-rot pre-infected, mainly by *Heterobasidion annosum s.l.* (Gaitnieks *et al.,* 2008). This results in a situation when large proportion of CWD on a country scale contains mycelia of the pathogen, active parts of which are suddenly exposed to the environment. The mycelia have a potential for sporocarp formation and subsequent dispersion by airborne basidiospores, thus increasing the probability of new primary infections. The aim of the present study was to investigate the dynamics of *H. annosum s.l.* sporocarp formation on CWD retained on harvested *P. abies* sites. During 3-5 year period following logging, the surface area of *H. annosum s.l.* sporocarps was found to be greatest on large size logs, comprising on average 4,074 cm^2 of sporocarps per 1 m^3 of retained rot-containing log. Interestingly, more than 90% of *H. annosum s.l.* sporocarps had developed on the logs with a diameter 10-40 cm, rather than on logs with the diameter larger than 40 cm. The sporocarps were most abundant in forests on drained peat soil. As for the windthrown trees, the surface area of *H. annosum s.l.* fruit bodies that had developed on stems was twice as large as that on roots (1,013 cm^2 and 442 cm^2, respectively). As for the stumps, the total surface area of *H. annosum s.l.* fruit bodies on the uplifted stumps was approximately 2.5 times larger than on the standing stumps (387 cm^2 and 155 cm^2, respectively). Results demonstrated that retaining *H. annosum s.l.* infected spruce wood on harvested sites favours spread of the pathogen in the forest ecosystem.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

References

Gaitnieks T., Arhipova N., Donis J., Stenlid J., Vasaitis R., 2008. Butt Root rot and related losses in Latvian *Picea abies* (L.) Karst. stands. In: Garbeletto M., Gonthier P. (eds.). Proceedings of the 12th Interantional Conference on Root and Butt Rots of Forest Trees. Barkeley, California – Medford, Oregon, 12th-19th August 2007. The University of California, Berkeley, USA, pp. 177-179.

Closure of *Picea abies* stem wounds: practical implications

R. Vasaitis[1], I. Vasiliauskaite[2], V. Lygis[2]

[1]*Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026 750 07 Uppsala, Sweden.*
[2]*Institute of Botany, Lithuanian Nature Research Centre, Vilnius, Lithuania.*
Corresponding author e-mail address: Rimvys.Vasaitis@slu.se

In forests of Europe, *Picea abies* suffer severely from mechanical damages inflicted to stems during forestry operations and made by wildlife when bark stripping. Wounds are colonized by decay fungi, which over 10-20 years spread over heartwood and 3-5 m in length, decomposing the most valuable part of a tree (butt log), and causing extensive finnancial losses (Vasiliauskas, 2001). Yet, the wounds vary in size within a very wide range, from a few to over 2000 cm^2 (Vasiliauskas, 1998). The question was therefore often asked, which of those are likely to result in wound decay development, and which are likely to escape the infection? The answer has certain practical implications, as stems with decay susceptible wounds could be then removed in next commercial thinning, while the ones expected to heal, left for further growth. Aim of this work was to estimate treshhold wound size in respect to likelihood of its closure / decay development in *P. abies* stems. The experiment was established in March 1991. Mechanical wounds of five size classes were artificially made at breast height to a total of 100 of *P. abies* stems (20 in each wound category): 1×15 (15 cm^2), 2×15 (30 cm^2), 3×15 (45 cm^2), 4×15 (60 cm^2) and 5×15 cm (75 cm^2). In October 2009 (after 19 vegetation seasons), the trees were felled, dissected, eventual wounds measured, wound closure rate and decay presence recorded, and isolations of fungi accomplished.

References

Vasiliauskas R., 1998. Ecology of fungi colonizing wounds of Norway spruce (*Picea abies* (L.) Karst.), with special emphasis on *Stereum sanguinolentum* (Alb. & Schw.:Fr.) Fr. Acta Universitatis Agriculturae Sueciae. Silvestria, 79. Swedish University of Agricultural Science, Uppsala, Sweden.

Vasiliauskas R., 2001. Damage to trees due to forestry operations and its pathological significance in temperate forests: a literature review. *Forestry* 74: 319-336.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Resistance of *Pinus contorta* and *Pinus sylvestris* to *Heterobasidion annosum*

A. Zaluma[1], N. Arhipova[1], L. Sisenis[2], A. Jansons[1], I. Baumanis[1], T. Gaitnieks[1], R. Vasaitis[3]

[1]*Latvian State Forest Research Institute „Silava", 111 Rigas Street, Salaspils, LV-2169, Latvia.*
[2]*Latvia University of Agriculture, 2 Liela Street, Jelgava, LV-3001, Latvia.*
[3]*Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 Uppsala, Sweden.*
Corresponding author e-mail address: astra_z@inbox.lv

Previous studies had suggested that in certain geographic areas *P. contorta* is faster growing and more productive than *P. sylvestris*. However, available data on their relative resistance to *Heterobasidion* root rot (main risk factor for pine stand productivity) is scarce and contradicting (Hagner, 1983; Karlman, 2001). The aim of the present work was to compare the resistance of *P. contorta* (three provenances: Summit Lake, Fort Nelson, Pink Mountain) and *P. sylvestris* to *Heterobasidion* under natural (field) conditions. The study was conducted in their plantations established on heavily infested *P. sylvestris* clear-cuts of previous generation. At first, the disease was mapped over the area, by collecting *Heterobasidion* isolates from 258 dead trees of current, and 244 stumps of previous generation, and performing with them somatic incompatibility and intersterility tests. Consequently, *Heterobasidion*-colonised areas were determined, and in those 2,421 *P. contorta* and 109 *P. sylvestris* trees were examined for *Heterobasidion* infections, and health condition of the trees was evaluated. *H. annosum sensu stricto* individuals were the most common. *P. sylvestris* exhibited higher level of resistance to *Heterobasidion* as compared to any provenance of *P. contorta*. In *P. contorta*, highest resistance was found in Summit Lake provenance. Plantations of *P. contorta* were highly susceptible to secondary *H. annosum* infections via root contacts, as indicated by high level of territorial clonality detected in local populations of the pathogen. Fruitbodies of *H. annosum* were commonly found on infected *P. contorta* trees.

References

Hagner S., 1983. *Pinus contorta*: Sweden`s third conifer. *Forest Ecology and Management* 6: 185-199.

Karlman M., 2001. Risks associated with the introduction of *Pinus contorta* in northern Sweden with respect to pathogens. *Forest ecology and managment* 141: 97-105.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

A comprehensive approach to investigate the invasion by *Heterobasidion irregulare* in Italy and its interaction with *H. annosum*

P. Gonthier[1], N. Anselmi[2], P. Capretti[3], F. Bussotti[3], M. Feducci[3], M. Garbelotto[4], L. Giordano[1], F. Guglielmo[1], T. Honorati[2], G. Lione[1], N. Luchi[5], V. Mancini[3], S. Michelotti[1], M. Michelozzi[6], G. Nicolotti[1], B. Paparatti[2], M. Pollastrini[3], S. Speranza[7], A.M. Vettraino[2]

[1]*DIVAPRA, University of Torino, Grugliasco (TO), Italy.*
[2]*DIBAF, University of Tuscia, Viterbo, Italy.*
[3]*DIBA, University of Firenze, Firenze, Italy.*
[4]*ESPM, University of California, Berkeley CA, USA.*
[5]*Istituto per la Protezione delle Piante, CNR, Sesto Fiorentino, Italy.*
[6]*Istituto di Genetica Vegetale, CNR, Sesto Fiorentino, Italy.*
[7]*DAFNE, University of Tuscia, Viterbo, Italy.*
Corresponding author e-mail address: paolo.gonthier@unito.it

Abstract. The invasive North American forest pathogen *Heterobasidion irregulare* was introduced in Italy in 1944. Little is known on its epidemiology, impact on native tree species, and interactions with the congeneric *Heterobasidion annosum*. The Italian Ministry of Education, University and Research, within the PRIN program, granted a research aimed at: i) investigating the ecology and epidemiology of *H. irregulare* in Italy, including its level of pathogenicity on native tree species, its spreading ability, its interactions with abiotic factors or related fungal species, and ii) defining, based on the above information, the contents of a proposal for its control. Preliminary data indicate that a northward expansion of its range in the last six years did not occur. Comparative inoculation experiments using *H. irregulare* and *H. annosum* genotypes failed to detect a disproportionate pathogenicity of the exotic pathogen on native pine species. However, preliminary results from pine logs inoculations indicate a higher saprotrophic ability of *H. irregulare* versus *H. annosum*, which may be consistent with the observed invasion process.

The North American forest pathogen *Heterobasidion irregulare* was introduced in an Italian stone pine (*Pinus pinea* L.) stand of central Italy (near Rome) in 1944 (Gonthier *et al.,* 2004). Subsequently, this exotic species became invasive (Gonthier *et al.,* 2007). It is currently distributed in forest stands and parks over about 105 km of coast approximately centered around Rome, somewhere in association with significant mortality of pine trees (Gonthier *et al.,* 2007; D'Amico *et al.,* 2007).

The Italian Ministry of Education, University and Research, within the PRIN program, granted a research aimed at: i) investigating the ecology and epidemiology of *H. irregulare* in Italy, including its level of pathogenicity on native tree species, its spreading ability, its interactions with abiotic factors and the related species *H. annosum sensu stricto* (*s.s.*), and ii) defining, based on information collected, the opportunity to perform a Pest Risk Analysis and the

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

contents of a proposal for its control. The project began in the Spring of 2010 and will end in the Fall 2012.

The tasks of the project can be grouped into three actions: 1- monitoring, 2- investigations on the impact of the exotic fungus on native forest ecosystems, and 3- on its ecology and epidemiology.

In order to determine the current distribution of *H. irregulare* in Italy and to assess if an expansion of its geographic range of colonization has occurred, spores have been collected and will be collected by using the wood disk exposure method in forests located inland and outside the boundaries of the coastal area currently colonized by the exotic fungus. Although a new stand has been found to be colonized by the pathogen, a northward expansion of its range in the last six years did not occur (Fig. 1).

Comparative inoculation experiments have been performed to assess the pathogenicity of *H. irregulare* and *H. annosum s.s.* on native pine species (*Pinus pinea*, *P. sylvestris*). The potential impact of the exotic fungus is also assessed by detecting through open-top chambers possible interactions between the exotic pathogen and abiotic stresses, i.e. drought and ozone.

The saprotrophic ability of *H. irregulare* and *H. annosum* has been compared by inoculating logs of *P. pinea* and *P. sylvestris*. While we failed to detect a disproportionate pathogenicity of *H. irregulare* on native pine species (Garbelotto *et al.,* 2010), preliminary results from pine logs inoculations indicate a higher saprotrophic ability of *H. irregulare* with respected *H. annosum s.s.* This may be consistent with the high fitness that *H. irregulare* seems to have in central Italy and may also explain, at least partially, its spreading ability.

Acknowledgements

The research was supported by MIUR within the PRIN project 2008.

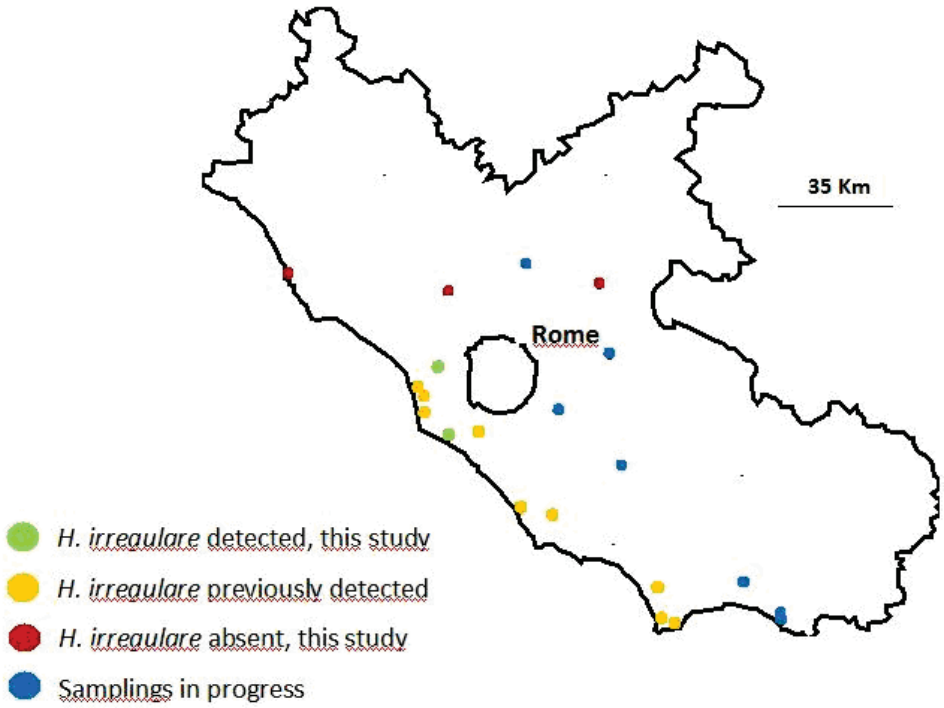


Figure 1. Map of current distribution area of *H. irregulare* and new sites of monitoring. The wood-disc exposure method was used to sample airborne spores in stands. Preliminary data indicate that a northward expansion of its range in the last six years did not occur.

References

D'Amico L., Motta E., Annesi T., Scirè M., Luchi N., Hantula J., Korhonen K., Capretti P., 2007. The North American P group of *Heterobasidion annosum s.l.* is widely distributed in *Pinus pinea* forests of the western coast of central Italy. *Forest Pathology* 37: 303-320.

Garbelotto M., Linzer R., Nicolotti G., Gonthier P., 2010. Comparing the influences of ecological and evolutionary factors on the successful invasion of a fungal forest pathogen. *Biological Invasion* 12: 943-957.

Gonthier P., Nicolotti G., Linzer R., Guglielmo F., Garbelotto M., 2007. Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. *Molecular Ecology* 16: 1389-1400.

Gonthier P., Warner R., Nicolotti G., Mazzaglia A., Garbelotto M., 2004. Pathogen introduction as a collateral effect of military activity. *Mycological Research* 108: 468-470.

A diverse community of viruses inhabiting *Heterobasidion parviporum* at a spruce-dominated forest plot in southern Finland

E.J. Vainio, T. Piri, J. Hantula

Finnish Forest Research Institute, Jokiniemenkuja 1, 01301 Vantaa, Finland.
Corresponding author e-mail address: eeva.vainio@metla.fi

We used CF11 cellulose chromatography, RT-PCR and sequencing to investigate the dsRNA virus community structure infecting the conifer pathogen *Heterobasidion parviporum* (Niemelä & Korhonen) at a forest plot dominated by Norway spruce in southern Finland. Among 18 genetically distinct *Heterobasidion* individuals (genets) represented by 66 fungal isolates, we found four different dsRNA virus species: *Heterobasidion* RNA virus 1 (HetRV1), HetRV2, HetRV6 and HetRV7. HetRV6 was represented by four distinct virus strains sharing 93-96% nucleotide sequence similarity, whereas each of the three remaining virus species lacked within-species variation. The distribution of the viruses was associated both to the *Heterobasidion* genet and location at the study site, and one large *Heterobasidion* genet harbored all the four virus species found. All the individual virus species occurred in multiple *Heterobasidion* genets, and double or triple virus infections were found from certain fungal isolates. Taxonomically, HetRV1, HetRV2 and HetRV7 represent diverse lineages of partitiviruses (*Partitiviridae*), whereas HetRV6 viruses are not related to any previously described viruses of *Heterobasidion* species, but show a low affiliation to the mutualistic *Curvularia* thermal tolerance virus infecting the grass endophyte fungus *Curvularia protuberata*.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Session 4

Population Genetics

Patterns of gene introgression between the invasive *Heterobasidion irregulare* and the native *H. annosum* in Italy

P. Gonthier[1] and M. Garbelotto[2]

[1]*University of Torino, Dept. of Exploitation and Protection of the Agricultural and Forestry Resources, Plant Pathology, I-10095 Grugliasco, Italy.*
[2]*University of California at Berkeley, Dept. of Environmental Science, Policy and Management, Ecosystem Sciences Division, Berkeley 94720, CA, USA.*
Corresponding author e-mail address: paolo.gonthier@unito.it

Abstract. The North American root rot fungus *Heterobasidion irregulare* invaded coastal pine stands of the Latium Region, in Italy, an habitat in which the native *H. annosum* is marginally present. Based on the outcomes of a 2-loci molecular assay, 4% of *Heterobasidion* genotypes collected in a single forest stand in the invasion area were previously found to be hybrid. Here we describe the patterns of hybridization and gene introgression between the two fungal species in the invasion area. A STRUCTURE analysis of AFLP data for 267 individuals identified gene introgression at all sites, with a frequency ranging from 8% to 42%. Data indicate that introgression is mostly occurring unilaterally from the native to the invasive species, and is responsible for 5-45% of genomes in admixed individuals. Sequence analysis of 11 randomly selected and unlinked loci for 30 individuals identified introgression at every locus. In 37 cases, we documented movement of entire alleles between the two species, but in 7 cases we also documented the creation of new alleles through intra-locus recombination. Sequence analysis did not identify enrichment of either transcriptionally different non-synonymous alleles or of transcriptionally identical synonymous alleles. These findings may suggest introgression is occurring randomly for extant alleles without an obvious enrichment process driven by selection.

The North American root rot fungus *Heterobasidion irregulare* invaded coastal pine stands of the Latium Region, in Italy, an habitat in which the native *H. annosum* is only marginally present (Gonthier *et al.,* 2007). In the invasion area, the native pathogen was reported to be frequent in a single forest, i.e. the Circeo National Park. Based on the outcomes of a 2-loci molecular assay, 4% of *Heterobasidion* genotypes collected in that forest were found to be hybrid (Gonthier *et al.,* 2007), while no hybrids were detected in other sites.
In this study we examined the levels and the direction of gene introgression between the invasive and the native fungal species using a large number of anonymous AFLP markers and comparative analysis of 11 individual gene genealogies, we assessed whether gene introgression is affected by genetic structure, density, and/or by time since sympatry of specie, and we determined whether introgression may have resulted in novel alleles. Finally, we performed a preliminary analysis to detect whether an enrichment of non-synonymous alleles through introgression, which would indicate the action of selection, has occurred.

Analyses were performed on 267 single spore colonies collected by using the wood disk exposure method from 9 forest sites, 3 of which located outside and 6

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

within the area of invasion of *H. irregulare*. Colonies were identified as previously described (Gonthier *et al.,* 2007). True species determination and presence of admixtures between *H. irregulare* and *H. annosum* was investigated by AFLP (Vos *et al.,* 1995) with 6 pairs of selective-base primers following a published protocol (Ivors *et al.,* 2004). AFLP data were analyzed by using the software STRUCTURE (Pritchard *et al.,* 2000) with k=4, which resulted in the maximum likelihood estimate among k ranging from 1 to 9. Genotypes were assigned to *H. irregulare* or *H. annosum* when the probability of membership of either group was at least 95%, otherwise they were assumed to be admixtures. Two independent analyses of molecular variance (AMOVA; Excoffier *et al.*, 1992) were performed to study the presence of significant genetic structure of the 2 species.

Admixed genotypes were found in all sites within the zone of infestation of *H. irregulare*, with a frequency ranging between 8% and 42% (Fig. 1). In the Circeo National Park, we further distinguished a central area representing the front of current sympatry, and a northern and southern area characterized by a dominance of *H. irregulare* and *H. annosum* spores, respectively. The highest frequency of introgression was recorded not at but rather behind the front of current sympatry, suggesting that the time since contact may have effects on the rate of hybridization. Although density of *H. annosum* differed significantly among the Circeo National Park and other sites (Gonthier *et al.,* 2007), the frequency of admixtures did not differ significantly among sites (χ^2= 7.476, *P*= 0.188). The large majority of all admixed genotypes had a predominant genetic background of *H. irregulare*, indicating that gene introgression is occurring mainly from the native to the invasive species (Fig. 1). Introgression levels of *H. annosum* into *H. irregulare* ranged between 5% and 45% depending on genotype (Tab. 1), with an overall mean of 19%. introgression is not likely to have been mediated by genetic differences among populations of either species, as based on AMOVA results they display no signs of significant genetic structure.

Eight *H. irregulare* and 8 *H. annosum* genotypes defined as pure by AFLP analysis and 14 admixtures were sequences in 1 mitochondrial locus (ATP) and in 10 nuclear loci (ACH, BTUB, CAM, EFA, EPG, GPD, GST1, ITS, OMP and TF). While 3 loci were previously characterized (ATP, EFA, GPD), remaining loci were identified and characterized in this study by using the recently released *H. irregulare* genome. Maximum parsimony phylogenetic trees were calculated in PAUP using a heuristic search.

Putatively pure *H. annosum* and *H. irregulare* genotypes consistently fell into two different clades, with significant bootstrap support (73 to 100) for at least one of them at all 11 loci. Genotypes putatively defined as hybrid through AFLP analysis displayed incongruent placement in one or more individual gene genealogies. While inter-locus recombination was detected in 37 cases, intra-locus recombination was exemplified by the presence of 7 chimeric alleles in four loci (ACH, CAM, ITS and TF). Genotypes with complicated recombination patterns

(i.e., several alternate portions of sequences from each of the two species) were identified.

Manual annotation of multiple sequence alignments was employed to determine whether introgressed alleles were synonymous or non-synonymous to those already found in the receiving species. Considering all 11 sequenced loci (Tab. 2), the cumulative frequency of synonymous *H. annosum* alleles introgressed into *H. irregulare* was significantly higher than that of non-synonymous alleles (20 vs. 9; Yates corrected χ^2= 6.90, *P*= 0.009). The actual frequency of synonymous alleles between the two species, excluding introgressed alleles, was also significantly higher than that of non-synonymous ones (89 vs. 54; Yates corrected χ^2= 16.17, *P*< 0.001). The proportion of synonymous and non-synonymous introgressed alleles is not different than the actual proportion of synonymous and non-synonymous alleles present between the two species (χ^2= 0.54, *P*= 0.765). Thus we failed to indentify an enrichment of non-synonymous alleles through introgression.

In conclusion, results from AFLP analyses clearly indicate that a massive, mostly unidirectional introgression of genes is occurring from *H. annosum* into *H. irregulare*. Sequence analysis has confirmed this massive introgression, has allowed the detection of intra-locus recombined alleles, and has shown a lack of enrichment of non-synonymous alleles through hybridization. These findings may suggest introgression is occurring randomly for extant alleles and may be driven by population expansion without an obvious enrichment process driven by selection.

Acknowledgements

The research was supported by the Ministry of Education, University and Research within the PRIN Program.

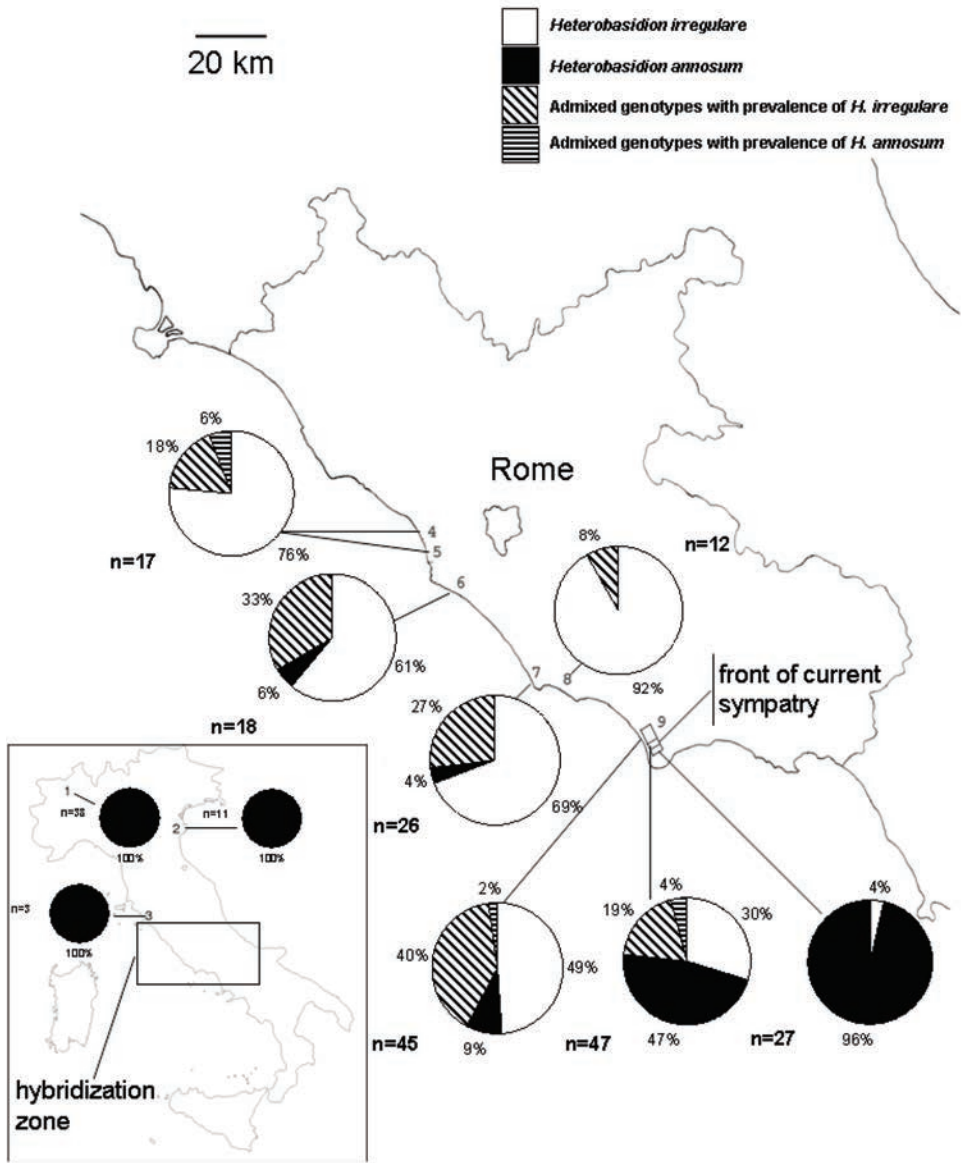


Figure 1. Relative frequency of *Heterobasidion annosum*, *H. irregulare* and admixed genotypes between the two species in the zone of invasion of *H. irregulare* in Italy based on STRUCTURE analysis of AFLP data. For population codes see tab. 1. Population 9 (Forest of Sabaudia at the Circeo National Park) was further subdivided in northern, central and southern areas (see text). The location of the front of current sympatry is indicated.

Table 1. Mean level and range of gene introgression between *H. annosum* and *H. irregulare* for each study site.

Collection site	N. of genotypes analyzed	N. of pure *H. annosum* genotypes	N. of pure *H. irregularare* genotypes	Mean percentage (range) of *H. annosum* genome introgressed in *H. irregulare*	Mean percentage (range) of *H. irregulare* genome introgressed in *H. annosum*
1. Alps	38	38	0	0 (-)	0 (-)
2. Mesola Forest	11	11	0	0 (-)	0 (-)
3. Feniglia Pinewood	3	3	0		
4. Fregene Monumental Pinewood - 5 Coccia di Morto Estate	24	0	18	12.4 (5.0-22.4)	17.3 (-)
6. Castel Fusano Pinewood Urban Park	18	1	11	15.3 (6.8-27.1)	0
7. Gallinara Pine Plantation - Anzio	27	1	19	25.8 (5.3-44.8)	0
8. La Campana Pine Plantation - Nettuno	12	0	11	41.0 (-)	0
9. Forest of Sabaudia, Circeo National Park - nothern area	57	4	24	18.8 (5.5-41.2)	44.6 (-)
9. Forest of Sabaudia, Circeo National Park - central area	48	22	14	19.7 (7.5-41.6)	26.4 (22.5-26.4)
9. Forest of Sabaudia, Circeo National Park - southern area	29	28	1	0 (-)	0 (-)

Table 2. Patterns of genic introgression of synonymous and non-synonymous alleles between the native *Heterobasidion annosum* and the invasive *H. irregulare* in Italy.

Loci	N. of *H. irregulare* genotypes with synonymous *H. annosum* alleles	N. of *H. irregulare* genotypes with non-synonymous *H. annosum* alleles	N. of *H. annosum* genotypes with synonymous *H. irregulare* alleles	N. of *H. annosum* genotypes with non-synonymous *H. irregulare* alleles
ACH	2	1	1	0
ATP	3	0	1	0
BTUB	2	0	1	0
CAM	3	0	0	0
EFA	0	2	0	2
EPG	2	0	1	0
GPD	4	0	1	0
GST1	0	2	0	2
ITS	2	0	2	0
OMP	0	3	0	2
TF	2	1	2	0
TOT	20	9	9	6

References

Excoffier L., Smouse P.E., Quattro J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

Gonthier P., Nicolotti G., Linzer R., Guglielmo F., Garbelotto M., 2007. Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. *Molecular Ecology* 16: 1389-1400.

Ivors K.L., Hayden K.J., Bonants P.J.M., Rizzo D.M., Garbelotto M., 2004. AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum*. *Mycological Research* 108: 378-392.

Pritchard J.K., Stephens M., Donnelly P., 2000. Inference of population structure using multilocus genetype data. *Genetics* 155: 945-959.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., Zabeau M., 1995. AFLP: a new tecnique for DNA fingerprinting. *Nucleic Acid Research* 23: 4407-4414.

Determining the actual area of introduction and the dispersal potential of *Heterobasidion irregulare* in Italy through genetic analyses

M. Garbelotto[1], F. Guglielmo[2], S. Mascheretti[1], P. Gonthier[2]

[1]*University of California, Dept. of ESPM, Berkeley, CA, USA.*
[2]*University of Torino, DIVAPRA, Grugliasco, Italy.*
Corresponding author e-mail address: matteog@berkeley.edu

Abstract. Circumstantial evidence points to an introduction of *Heterobasidion irregulare* in Italy in an area spanning from Castelfusano-Castelporziano to Anzio. Based on accepted population genetics theory, allelic diversity is bound to be higher in older compared to younger infestations. Unfortunately, factors such as variable population size among infested sites and interspecific gene introgression with congeneric native species may confound the analysis. It has been recently shown that the exotic *H. irregulare* is being massively introgressed by native *H. annosum* genes. In this study, we use AFLPs to exclude genotypes of *H. irregulare* that show any signs of genic introgression from *H. annosum* and we use sequence, SSR, and AFLP analyses to investigate allelic diversity, genetic structure, and spatial autocorrelation of genetic markers among pure *H. irregulare* genotypes from different populations. AFLPs show no genetic structure among sites, with the exception of Castelfusano and the Circeo National Park, suggesting these two sites may represent the oldest and the youngest infestations. Sequence and SSR unambiguously confirm the Circeo site to have the least diversity and indicate this is the youngest infestation in Italy. Sequence analysis indicate diversity to be highest in Castelfusano-Castelporziano, intermediate in areas that surround it such as Fregene to the North and Anzio to the South, and lowest in the Circeo. Additionally, spatial autocorrelation analyses may provide information on the dispersal potential of this pathogen.

Circumstantial evidence points to an introduction of the North American *Heterobasidion irregulare* in Italy in the Latium region in Central Italy (Gonthier *et al.,* 2007). Castelporziano-Castelfusano, west of Rome, displays the greatest extent of mortality and is thus the possible original site of introduction. Conversely, the Circeo National Park in Southern Latium displays limited mortality and has not been entirely colonized by the exotic species, suggesting a recent arrival of the North American species.

Based on accepted population genetics theory, allelic diversity is bound to be higher in older compared to younger infestations. Unfortunately, factors such as variable population size among infested sites and interspecific gene introgression with congeneric native species may confound the analysis. It has been recently shown that the exotic *H. irregulare* is being massively introgressed by native *H. annosum* genes (Gonthier and Garbelotto, 2011). In this study, we use AFLPs to exclude genotypes of *H. irregulare* that show any signs of allelic introgression from *H. annosum* and we use sequence and SSR analyses to investigate allelic diversity, genetic structure, and spatial autocorrelation of genetic markers among pure *H. irregulare* genotypes from different populations. Sequences of 11 loci (Gonthier *et al.,* 2011) were obtained for approximately 90 haploid genotypes

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

displaying only *H. irregulare* AFLP markers from the following locations: Fregene-Coccia di Morto, Castelfusano-Castelporziano, Anzio, Nettuno, and the Circeo National Park. All sequences clearly belonged to *H. irregulare* and all loci were polymorphic. ARLEQUIN (Schneider *et al.,* 2000) was used to calculate allelic diversity, genic diversity (a measure of allelic diversity that includes frequency of each allele per site), and nucleotide diversity for each site. All three indices were highest for Castelfusano-Castelporziano and lowest for the Circeo National Park, supporting the notion that the first site may be the oldest infestation, while the latter may be the youngest (Tab. 1).

Table 1. Diversity indices determined by sequence analysis at 11 loci.

Site	Average Haplotype Diversity	Average Gene Diversity	Average Nucleotide Diversity
Fregene-Coccia di Morto	0.51	0.58	0.0049
Castelfusano-Castelporziano	**0.53**	**0.73**	**0.0058**
Anzio	0.40	0.66	0.0048
Circeo	0.35	0.57	0.0042

A total of 13 polymorphic SSR loci were employed to describe the genetic structure of the invasive pathogen by performing three analyses: i)- AMOVA implemented by ARLEQUIN was performed to study how genetic variance is partitioned within and among populations; ii)- definition of number of discrete genetic clusters and their distribution was investigated using the program STRUCTURE (Pritchard *et al.,* 2000); iii)- spatial autocorrelation was studied using the program SPAGeDI (Hardy and Vekemans, 2002). Results indicated that the vast majority of the genetic variance (93%) was found within populations, but that a small but significant amount of genetic variance (7%, P= 0.03802) was also found among populations. In pairwise comparisons, the only significant difference (PHIst= 0.28, P= 0.003) was found between Castelfusano-Castelporziano and the Circeo National Park. It should be noticed that these two sites are not the two furthest ones in the analysis, hence the observed genetic structure may be driven by the limited migration between the oldest infestation (Castelfusano-Castelporziano) and the youngest one (Circeo). STRUCTURE analysis revealed the presence of three genetically distinct clusters (Fig. 1). All three clusters are present everywhere, indicating a single introduction, however the frequency of the three clusters is different when comparing the Northern sites (Castelfusano-Castelporzano and Fregene-Coccia di Morto), the Central sites (Anzio, Nettuno) and the Southern Circeo site, suggesting each area has been infested for different periods of time. Spatial autocorrelation analysis indicated that genetic similarity is much greater

than expected by random dispersal up to 2.5 km, is greater than expected up to 10 km, and is lower than expected at 90 km (Fig. 2). We deduct that fragmentation among the forests currently infested (20-30 Km on average) has slowed down the spread of the pathogen, but that if gaps in between forests were to be less than 10 km, or less than 2.5 km, spread should accelerate considerably.

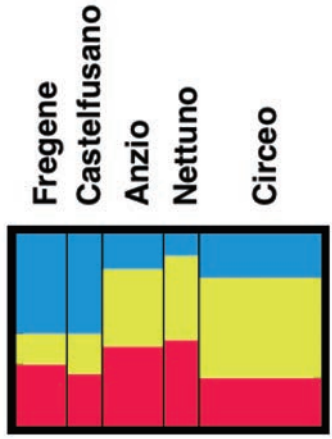


Figure 1. Barplot of genetic clusters (k=3) identified by STRUCTURE analysis of SSR alleles at 13 loci.

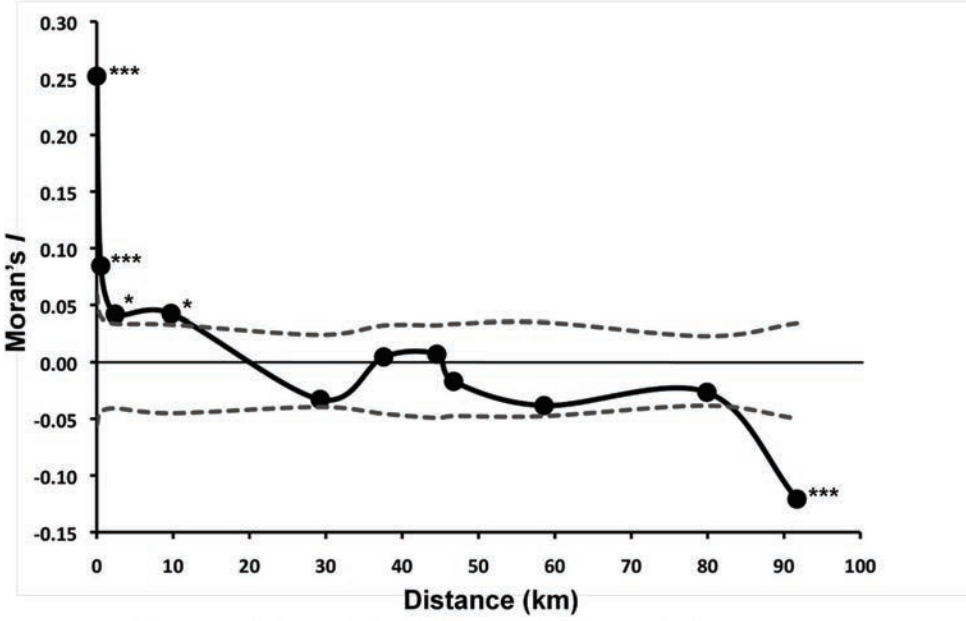


Figure 2. Spatial autocorrelation analysis results on SSR alleles at 13 loci.

References

Gonthier P., Garbelotto M., 2011. Amplified fragment length polymorphism and sequence analyses reveal massive gene introgression from the European fungal pathogen *Heterobasidion annosum* into its introduced congener *H. irregulare*. *Molecular Ecology* 20: 2756-2770.

Gonthier P., Nicolotti G., Linzer R., Guglielmo F., Garbelotto M., 2007. Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. *Molecular Ecology* 16: 1389-1440.

Hardy O.J., Vekemans X., 2002. SPAGeDi: a verstile computer program to analyse spatial genetic structure at the individua or population levels. *Molecular Ecology Notes* 2: 618-620.

Pritchard J.K., Stephens M., Donnelly P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Schneider S., Roessli D., Excoffier L., 2000. Arlequin ver. 2.000 A software for population genetics data analysis. http://cmpg.unibe.ch/software/arlequin/.

Variation in *Heterobasidion occidentale* on different hosts in western Washington USA and growth at different temperatures

R.L. Edmonds

School of Forest Resources, University of Washington, Seattle, WA 98195, USA.
Corresponding author e-mail address: bobe@u.washington.edu

Abstract. *Heterobasidion occidentale (*formerly *H. annosum* S strain*)* occurs in forests of western Washington on a variety of conifer and hardwood hosts. Physiological specialization of *H. occidentale* occurs on conifer hosts in Washington, both at the interspecific and intra-specific levels. However, there has been little study of the genetic variation in *H. occidentale* populations in Washington's forests. There is also concern that global climate change, particularly global warming, may increase the incidence of this disease. Objectives of this study were to determine: (1) the genetic variability of *H. occidentale* populations from different hosts in western Washington - western hemlock, Douglas-fir, and red alder, and (2) the linear growth rates of different isolates at different temperatures in culture. Fruiting bodies were obtained from downed hemlock and red alder logs and Douglas-fir stumps in western Washington. DNA oligonucleotide primers were used to conduct an arbitrary-primed polymerase chain reaction analysis. There was considerable variation in the *H. occidentale* populations. However, there appeared to be no clustering by tree species. Linear growth rates of cultures taken from fruiting bodies were determined on 2% malt agar at one degree intervals from 20 to 29 C. The optimum temperature was 23 C for all cultures with growth rates varying from 6.9 to 9 mm day^{-1}. Currently temperatures in woody substrates in western Washington are below optimum most of the year. Thus growth rates of *H. occidentale* and disease expression could be increased by global warming. Furthermore, moisture is predicted to increase.

Introduction

Heterobasidion occidentale (formerly the S-strain of *Heterobasidion annosum)* is common in western Washington forests on a variety of conifer and hardwood hosts. The dominant conifer hosts are western hemlock (*Tsuga heterophylla*), Sitka spruce (*Picea sitchensis*) and grand fir (*Abies grandis*), but it has also been recorded on Douglas-fir (*Pseudotsuga menziessii*), Ponderosa pine (*Pinus ponderosa*) and other conifers. Hardwood hosts include red alder (Alnus rubra), western cottonwood (*Populus trichocarpa*), bigleaf maple (*Acer macrophyllum*), and Pacific madrone (*Arbutus menziesii*).

Hsiang and Edmonds (Hsiang and Edmonds, 1989) demonstrated physiological specialization of S-strain of *H. annosum* on conifer hosts in Washington relative to production of conidia on wood discs from different hosts. Dart et al. (Dart *et al.,* 2007) studied genetic variability in Christmas tree plantations in western Washington and found that isolates from different trees had distinct genotypes, but root systems of individual trees were dominated by 1 or 2 genotypes. In California forests Garbelotto et al. (Garbelotto *et al.,* 1999) also found considerable variability

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

in the genetic structure of *H. annosum- S type* in white fir mortality centers; each mortality center was characterized by several fungal genotypes.

There is great concern that global climate change, particularly global warming may increase the rate of growth of *H. occidentale* and its impact. Thus one of the reasons for studying genetic variation in western Washington populations variability in *H. occidentale* populations is to assess variability in growth of different isolates from different hosts and geographic locations in Washington, including coastal areas, the Puget Sound and east slopes of the Cascades. These areas have different climates.

Data from the Western Regional Climate Center (WRCC, 2001) show that temperatures in western Washington have been rising since the mid 1950s (from 1955 to 2005 average summer temperatures in June, July and August rose from 14.1-15.7°C, 15.8-17.9°C and 16.1-18.3°C, in the Coastal, Puget Sound, and East Cascade Provenances of Washington.

Objectives

The objectives of this study were to determine: 1) the genetic variability of *H. occidentale* populations from different hosts in western Washington - western hemlock, Douglas-fir, grand fir, red alder, and western cottonwood, 2) basidiocarp pore density and shape, and 3) the linear growth rates in culture of different isolates from coastal Washington, the Puget Sound lowlands, and east slopes of the Cascade Mountains at different temperatures.

Methods

Basidiocarps were collected from western hemlock trees and logs in Olympic National Park in the Hoh River Valley, from stumps, logs and roots of blowndown western hemlock trees, dead red alder trees, western cottonwood logs and Douglas-fir stumps in the Puget Sound area near Seattle and from grand fir and Ponderosa pine stumps on the east slopes of the Cascade Mountains near Cle Elum Washington. DNA was extracted from basidiocarps and the following DNA oligonucleotide primers were used for the arbitrary-primed polymerase chain reaction analysis (Garbelotto *et al.,* 1999) NS3-NS1.5R, NS3-Ctb6,ITS4b-Mb2, KimQ-KJ2, CNS3.6-Mb2, NS6-ITS2, and ML5-ML6.

Isolations were also made from the basidiocarps and cultures were grown in 2 percent malt agar.
Temperature growth data were collected by measuring diameter growth on the plates and expressing it as radial growth in mm day^{-1}. Growth data were determined between 20°C and 29°C at 1°C intervals.

Results and Discussion

There was considerable genetic variability in *H. occidentale* populations from the Puget Sound area, with each population being different from each other. However, there was no clustering by host species. In California forests Garbelotto et al. (Garbelotto *et al.,* 1999) also found considerable variability in the genetic structure of *H. annosum- S type (H. occidentale)* in white fir mortality centers and found that each mortality center was characterized by several fungal genotypes. Dart et al. (Dart et *al.,* 2007) also found considerable variability in Christmas tree plantations in western Washington and isolates from different trees had distinct genotypes.

Basidiocarp pore densities and shapes were similar in fruiting bodies from different tree species and averaged 11.9 ± 0.6 cm^{-2} (Tab. 1).

Table 1. Pore density and shapes in *Heterobasidion occidentale* basidiocarps from different tree species in Washington (Mean ± SD).

Tree species	Pore density (No/cm^2)	% elongated	% irregular
Western hemlock	12.5 (2.8)	12.8 (9.5)	5.8 (7.9)
Douglas-fir	12.3 (2.9)	8.4 (4.7)	2.5 (0.0)
Grand fir	12.0 (3.8)	12.0 (4.7)	13.2 (3.3)
Ponderosa pine	11.3 (1.0)	24.5 (6.4)	4.8 (6.8)
Average	**11.9 (0.6)**	**14.8 (6.1)**	**5.9 (4.3)**

However, basidiocarps from western hemlock tended to be larger than those from other tree species. Ostrosina and Garbelotto (Ostrosina and Garbelotto, 2010) found an average pore density of 8.6 cm^{-2} in *H. occidentale* basidiocarps from *Abies concolor* in California. Average elongated pores was 14.8 ± 6.1 percent, lower than the 53 percent found in California by Ostrosina and Garbelotto (Ostrosina and Garbelotto, 2010). However, the average irregular pores 5.9 ± 4.3 percent was the same as found by Ostrosina and Garbelotto (6 percent) (Ostrosina and Garbelotto, 2010).

Linear radial growth rates of nine Puget Sound cultures at temperatures between 20°C and 29°C had maximum growth rates ranging from 6.8-9.0 mm day^{-1} mostly at 23°C; one isolate had a maximum at 24°C. At 20°C growth rates ranged from 4.4-6.0 mm day^{-1} and 1.0-2.8 mm day^{-1} at 29°C. Preliminary data for growth at 24°C of isolates from different tree species showed they were very similar (averaging 6.2 mm day^{-1}) except for the grand fir isolate from the east slopes of the Cascades which had the slowest growth rate (3.3 mm day^{-1}).

Conclusions

1) There was considerable genetic variability in *H. occidentale* genotypes, but no clustering by tree species, 2) pore densities varied little among basidiocarps from different tree species, and were similar to that found on basidiocarps from *Abies concolor* in California, 3) percent elongated pores was lower than in basidiocarps from California, but percent irregular pores was similar, 4) basidocarps on western hemlock tended to be larger than those on other species, 5). radial growth in culture at different temperatures showed some variability, with maximum growth between 23°C and 24°C, and, 6) warming temperatures could potentially increase the impact of *H. occidentale* in Washington's forests (particularly on the east slopes of the Cascades), contributing to tree death and increasing wildfire problems.

References

Dart N.L., Chastagner G.A., Peever T.L., 2007. Spread of *Heterobasidion annosum* in Christmas tree plantations of the United States Pacific Northwest. *Phytopathology* 97: 551-556.

Garbelotto M., Cobb F.W., Bruns T.D., Otrosina W.J., Popenuck T., Slaughter G., 1999. Genetic structure of *Heterobasidion annosum* in white fir mortality centers in California. *Phytopathology* 89: 546-554.

Hsiang T., Edmonds R.L., 1989. Physiological specialization of *Heterobasidion annosum* on conifer hosts. *Canadian Journal of Botany* 67: 2396-2400.

Otrosina W.J., Garbelotto M., 2010. *Heterobasidion occidentale sp. nov.* and *Heterobasidion irregulare nom. nov.*: A disposition of North American *Heterobasidion* biological species. *Fungal Biology* 114: 16-25.

Western Regional Climate Center (WRCC), 2011. 2215 Raggio Parkway Reno, NV. wrcc@dri.edu.

Genetic Structure of three French populations in *Armillaria ostoyae*

C. Dutech, N. Leymarie, X. Capdevielle, O. Fabreguettes, B. Lung-Escarmant

INRA, UMR 1202 BIOGECO, Domaine de l'Hermitage, Cestas F-33662, France.
Corresponding author e-mail address: cdutech@bordeaux.inra.fr

Armillaria ostoyae, the fungal plant pathogen causing the root rot disease on maritime pine, is in expansion in the South-Western French forest of Landes de Gascogne for about few ten years. The origin and processes of this expansion have been little explored yet. Previous genetic studies suggested that the coastal area of this forest is one of the source populations of new reported disease foci. The genetic structure of three populations in this area was investigated to infer reproductive and local dispersal processes, using 14 microsatellite loci. In the three population, a high excess in homozygotes was estimated (Fis> 0.20) suggesting null alleles, inbreeding or genetic substructure. This genetic substructure could be due to spore or mycelium restricted dispersal, in agreement with a pattern of isolation by distance detected in one of the three populations where this pattern was tested. This limited dispersal could also explain the genetic differentiation estimated among the three coastal populations, and significantly higher than 0.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Geographic population structure of *Armillaria cepistipes* in Switzerland

R. Heinzelmann, D. Rigling, S. Prospero

Swiss Federal Research Institute WSL, Zuercherstrasse 111, 8903 Birmensdorf, Switzerland.
Corresponding author e-mail address: simone.prospero@wsl.ch

Armillaria species are significant components of forest ecosystems worldwide. The preferentially saprotrophic *A. cepistipes* is the most frequent species in Norway spruce (*Picea abies*) forests in Switzerland, where it usually forms dense networks of rhizomorphs in the soil. In this study, we investigated the genetic structure of the Swiss *A. cepistipes* population. Specifically, we aimed to test whether the current population is geographically structured because of (1) the presence of a main geographic barrier (Alps), (2) possible re-colonisation from different genetic pools after the last glaciations, and (3) past forest management practices. For this, we genotyped about 170 *A. cepistipes* isolates from all over Switzerland at eight polymorphic microsatellite loci and eight neutral single-nucleotide polymorphisms (SNPs) at four loci. Genotyping with the selected markers showed that all isolates belonged to different genotypes. Preliminary analyses seem to exclude a clear geographic structure in the *A. cepistipes* population in Switzerland. In this presentation we will present and discuss in detail the results obtained.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Field studies agree and extend greenhouse study results of host resistance trials of Douglas-fir to *Armillaria* root disease

M.G. Cruickshank[1] and B. Jaquish[2]

[1]*Natural Resources Canada, Canadian Forest Service - Pacific Forestry Centre, 1219 506 W. Burnside, Victoria, BC, Canada V8Z 1M5.*
[2]*BC Ministry of Forests and Range, Research Branch, Kalamalka, BC 3401 Reservoir Rd. Vernon, BC, Canada V1B 2C7.*
Corresponding author e-mail address: Mike.Cruickshank@NRCan-RNCan.gc.ca

Abstract. Plants attacked by an enemy can either limit the damage they receive or cope with the damage; these traits are called "resistance" and "tolerance", respectively. In a preliminary nursery study, resistance or tolerance was determined in 70 half-sib Douglas-fir families challenged with *Armillaria ostoyae*. Samples of the best and worst surviving families were subsequently inoculated in a 17-year-old progeny test of the same families to compare the studies and to gain understanding about host tolerance and resistance. Paper birch blocks containing *A. ostoyae* were placed against the root collar of the field trees from five half-sibling families and left for 5 years. Resistant families exhibited lesions with lower average proportionate collar girdling (range 0.35-0.06) and lower average lesion size (range 175-18 cm^2) than the tolerant families. Field tree proportionate collar girdling correlated closely with the nursery tree survival since collar girdling is a prerequisite of mortality (r= -0.80). Tolerant families had better 5-year average radial growth after infection for a given level of damage (F significant P= 0.04) compared to healthy trees, but also greater average lesion size. Results suggested a tradeoff between families with resistance or tolerance. One family demonstrating high tolerance and intermediate resistance restricted horizontal lesion spread but not vertical lesion spread or lesion size. Both resistance and tolerance may be present in the population allowing trees an advantage where either trait is more effective under changing ecological scenarios.

Selection for positive breeding traits for conifers mostly considers tree height growth as the dominant trait for early selection. More recently, tree breeders recognize the need to incorporate insect and disease resistance traits into selection programs. In this study, resistance to *Armillaria* root disease was evaluated in an Interior Douglas-fir nursery trail that consisted of seedlings of 70 select half-sib families from 4 seed planning zones in the BC Interior. Results showed families from drier subzones had higher seedling survival rates than families from cooler and wetter subzones; however, considerable variation existed among families. In the present study, five good and poor surviving families from one zone (West Kootenay Low-WKL) were used to determine whether the nursery screening results were valid under field conditions. A 22-year-old progeny test of WKL of the same families was used for comparison by inoculating fifteen trees within each family at the root collar with *Armillaria ostoyae* (Romagn.) Herink, and left for five years. The trees were subsequently excavated, lesions measured, and increment cores were taken at breast height from the infected trees and uninfected trees of the same family. Tolerant families (larger lesions) had lower growth

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

impacts for given level of damage than families with resistance (smaller lesions) compared to healthy trees (p=0.026). Family 423 with moderate levels both traits was able to limit the horizontal lesion spread to about half that of the other tolerant family 421, but still with comparable lesion area (Tab. 1). Although the sample size was small, it suggested a tradeoff between resistance and tolerance so that larger sized lesions were evident on tolerant families and smaller lesions on resistant families. Unfortunately having smaller lesions after infection appears to require energy at a cost in growth. Infected trees appear to loose crown length in proportion to tree height with increasing length of time since infection; however, there was variation in the magnitude of this response that may relate to resistance and tolerance. The results suggest that families respond differently to disease and breeding for Armillaria resistance might also consider including tolerance in programs of multi-trait index selection. Disease tolerance to disease is not well studied in conifers but it may play an important role at the population level.

Table 1. Comparison between families involving in the present study for different lesions considered

Half - sib family	**421**	**422**	**423**	**514**	**620**
Number or field trees (healthy, infected)	5, 14	4, 7	6, 13	9, 4	6, 6
Mean proportionate collar girdling - diseased (min, max)	0.35 (0.01, 0.6)	0.06 (0.01, 0.14)	0.2 (0.01, 0.7)	0.15 (0.01, 0.4)	0.12 (0.03, 0.3)
Mean horizontal degrees collar lesion spread (min, max)	50 (7,93)	18 (1,42)	33 (10,83)	34 (3,76)	23 (5,36)
Collar lesion area (cm^2) (min,max)	147.5 (2.0, 363.7)	18.2 (0.7, 42.1)	175.2 (7.9, 552.6)	43.03 (3.6, 138.5)	21.59 (1.8, 52.4)
Proportion of 6 year old seedlings surviving	0.12	0.47	0.35	0.07	0.44

Heterobasidion in conifer forests: genetic diversity across the Italian peninsula

N. Luchi[1], D. Paffetti[2], A. Santini[1], P. Capretti[3]

[1]*Plant Protection Institute, National Research Council (CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), (Italy).*
[2]*Department of Agricultural and Forest Economics, Engineering, Sciences and Technologies (DEISTAF), University of Florence, Via San Bonaventura 13, 50145 Florence (Italy).*
[3]*Department of Agricultural, Food and Environmental Science, University of Florence, Piazzale delle Cascine 28, 50144 Florence (Italy).*
Corresponding author e-mail address: n.luchi@ipp.cnr.it

Abstract. Conifers represent one of the main component of Italian forests particularly in the Alps and along the Apennine mountains. During the last years the research showed that tree species in plantations as Pine, Fir and Spruce, have been damaged respectively by *Heterobasidion annosum* s.s., *H. abietinum* and *H. parvipurum*. In this context the genetic diversity of Heterobasidion populations have been studied using molecular markers such as M13 and RAMs. Differences between populations were found within Heterobasidion species, comparing isolates collected from the Alps and some refuge area along the Apennines. These divergences were probably related to the host populations but also to the morphology of the territory that reduces opportunities of gene flow between different regions.

Introduction

According to the Italian National Inventory of Forests and Carbon Stocks (INFC, 2009) Italian forests cover about 25% of peninsula. Due to the morphology of territory most forests (60%) are located in the mountains at 700 - 2,500 m. a.s.l., one part in the hills (35%) and just 4% on the low lands. The north-south orientation of the peninsula, offers different environmental conditions and soil diversity, allowing the growth of dissimilar forest tree species. Among conifers the most common are represented by Norway spruce (*Picea abies*), Silver Fir (*Abies alba*) and Pine species (*Pinus nigra*, *P. sylvestris*, *P. pinea* and *P. pinaster*).

The origin and the distribution of conifers is related to the geographical landscape of peninsula: in the northern part Norway spruce, Silver Fir, Larch (*Larix decidua*) and Pine are mainly naturally regenerated while in the central part, mostly along the Apennine mountains, woods, generally constituted by Silver Fir, Pine and Douglas fir, are usually planted.

In northern part of the country, where clear cutting is not adopted, most of harmful fungal parasites affect trees in juvenile age. Damages on mature trees are generally present in pure stands. They are related to *Armillaria* sp. and *Heterobasidion annosum* s.l., root and butt rot fungi that cause losses in wood production. Both of these fungi are relatively common and can be found on stumps

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

and wood logs abandoned in the forest in saprophytic habit (Woodward *et al.,* 1998).

In the regions characterized by Mediterranean climate, along the Apennines, root rot disease are often found in Silver Fir, Austrian pine and occasionally Douglas fir plantations particularly on formerly agricultural abandoned sites (Puddu *et al.,* 2003).

In Italy all three European species (*H. annosum s.s*., *H. abietinum* and *H. parviporum*) have been found (Capretti *et al.,* 1998). The last species is confined in the North in the Alpine area whilst the other two are present in the North and along the peninsula. Recently also the invasive *H. irregulare,* introduced from North America, was found along Tyrrhenian coast (Gonthier *et al.,* 2004; D'Amico *et al.,* 2007).

Genetic diversity of *H. annosum s.l.* populations have been studied in different periods by using molecular markers such as M13 and RAMs (Petta *et al.,* 2001; D'Amico *et al.,* 2007; Zamponi *et al.,* 2007). Differences between populations were found within each species of *H. annosum*, especially among *H. abietinum* isolates collected from Alps and some refuge area along the Apennines (La Porta *et al.,* 1997a, b; Zamponi *et al.,* 2007; Luchi *et al.,* 2011). These divergences were probably related to host populations but also to the morphology of the country that may not allow a gene flow between different areas. Studies on genetic population of *Heterobasidion* can been useful to understand possible differences in pathogenicity, that may also be related to environmental factors.

Materials and Methods

Fifty-one isolates collected along the Italian peninsula from different hosts species, have been use to study the genetic diversity of *H. annosum s.l.*. The study included 22 isolates from pine hosts (Petta *et al.,* 2001) and 29 isolates from Silver fir (Luchi *et al.,* 2011) (Tab. 1).

Fungal isolates maintained at DiBA (Department of Agricultural Biotechnologies, University of Florence, Italy) were used for DNA extraction. Mycelium was grown at room temperature on 1.5% malt extract agar covered with cellophane membrane at 20°C for 10 days. It was then scraped off and homogenized in 1.5 ml tube with glass rod and quartz. DNA extraction was performed following the method described by Vainio *et al.* (1998). PCR amplification using M13 was carried out according to Luchi *et al.* (2011).

The electrophoretic amplified profiles transformed in presence/absence vectors were used for following analyses.

We inferred population structure using a Bayesian Monte Carlo Markov Chains method implemented in the Geneland package vers 3.0 (Guillot *et al.,* 2009) under the R Language and Environment for Statistical Computing software as described by Guillot *et al.* (2005a, b) and Guillot (2008).

Table 1. List of isolates of *H. annosum* s.l. used in the investigation, grouped according their geographical origin (Petta *et al.,* 2001; Luchi *et al.,* 2011).

Isolate	Geographical origin	Isolate	Geographical origin
	H. annosum s.s.		*H. abietinum*
3-t	Monte Amiata, Central Italy	ItA1	Val Bormida, Savona, North Italy
4-t	Monte Amiata, Central Italy	ItA2	Val Bormida, Savona, North Italy
5-t	Monte Amiata, Central Italy	ItA3	Chiusa Pesio, Cuneo, North Italy
8-t	Tirrenia, Central Italy	ItA4	Chiusa Pesio, Cuneo, North Italy
9-t	Tirrenia, Central Italy	ItA5	Cadore, Belluno, North Italy
10-t	Tirrenia, Central Italy	ItA6	Cadore, Belluno, North Italy
13-c	Mercurella, South Italy	ItA7	Pinzolo, Trento, North Italy
14-c	Mercurella, South Italy	ItA8	Lavarone,Trento, North Italy
15-c	Mercurella, South Italy	ItA9	Lavarone,Trento, North Italy
16-c	Mercurella, South Italy	ItA10	Lavarone,Trento, North Italy
17-c	Mercurella, South Italy	ItNA1	Macchia Antonini, Pistoia, Central Italy
18-c	Mercurella, South Italy	ItNA2	Sestaione, Pistoia, Central Italy
19-c	Mercurella, South Italy	ItNA3	Bocca di Rio, Prato, Central Italy
25-L	Bressanone, North Italy	ItNA4	Abetone, Pistoia, Central Italy
26-L	Bressanone, North Italy	ItNA5	Vallombrosa, Firenze, Central Italy
30-L	Centocroci, North Italy	ItNA6	Vallombrosa, Firenze, Central Italy
31-L	Centocroci, North Italy	ItNA7	Vallombrosa, Firenze, Central Italy
34-L	Marina di Ravenna, North Italy	ItNA8	Monte Amiata, Grosseto, Central Italy
35-L	Marina di Ravenna, North Italy	ItNA9	Monte Amiata, Grosseto, Central Italy
55-c	Gambarie, South Italy	ItNA10	Monte Amiata, Grosseto, Central Italy
56-c	Gambarie, South Italy	ItSA1	Monte Taburno, Benevento, South Italy
57-c	Gambarie, South Italy	ItSA2	Monte Vulture, Potenza, South Italy
		ItSA3	Monte Vulture, Potenza, South Italy
		ItSA4	Marsico Nuovo, Potenza, South Italy
		ItSA5	Foresta Umbra, Foggia, South Italy
		ItSA6	Serra San Bruno, Vibo Valentia, South Italy
		ItSA7	Garbari, Reggio Calabria, South Italy
		ItSA8	Monte Cocuzzo, Cosenza, South Italy
		ItSA9	Sersale, Catanzaro, South Italy

Ten independent Monte Carlo Markov Chains runs were performed by Geneland with the following settings: 1,000,000 iterations with 100 thinning interval and a burning of 250,000, using the correlated allele frequencies model. The maximum number of populations was set to 20. A map of posterior probabilities (membership) was obtained by PostProcessChain and PostTessellation functions into Geneland by tesselling the landscape. F-statistics were calculated using SPAGeDi program (Hardy and Vekemans, 2002).

Results and Discussion

H. annosum s.l. isolates from pine and Silver fir showed high polymorphism (Fig. 1a, 2a). In spite of that it was possible to separate the two populations in different clusters with a clear geographic structure along the Italian peninsula (Fig. 1b, 2b). These results are in accordance with La Porta *et al.* (1997a,b) that found

geographical variation among isolates collected in Italy, and particularly between samples collected from Apennines and from the Alps.

Three clusters obtained from *H. annosum* s.s. (pine isolates) have a strong genetic divergence as demonstrated by F_{ST} value (Tab. 1, 2), this reflect some geographical isolation. High level of inbreeding (Tab. 2) show that probably fungal samples analyzed in this study originated from a reduced number of ancestral individuals. Only isolates included in cluster 3 have a low level of inbreeding by F_{IS} index (Tab. 2).

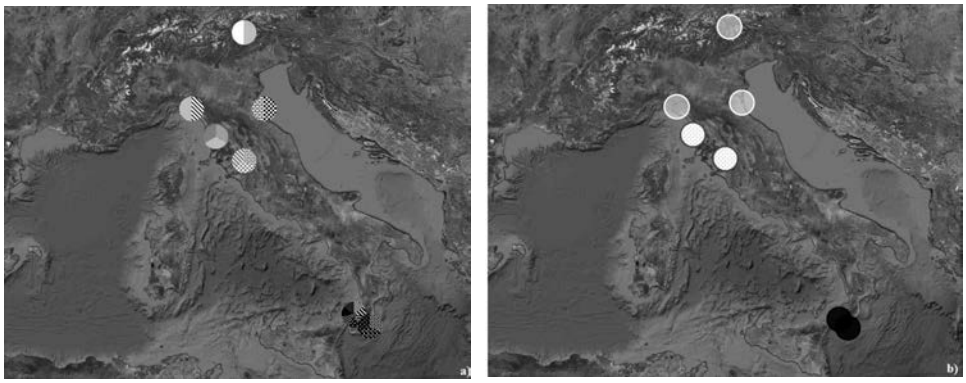


Figure 1. *H. annosum* s.l. isolates from Pinus. a) Haplotype distribution. b) main population clusters: 1 grey, 2 white, 3 black.

Table 2. F_{ST} and F_{IS} in the population of isolates from pine.

	cluster 1	**cluster 2**	**cluster 3**
cluster 1 (F_{IS} = 0.355)	-		
cluster 2 (F_{IS} = 0.671)	0.532	-	
cluster 3 (F_{IS} = 0.080)	0.546	0.631	-

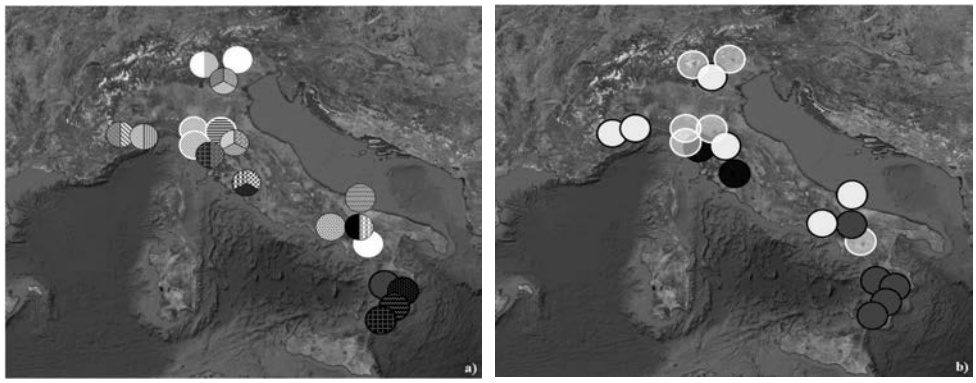


Figure 2. *H. annosum s.l.* isolates from Silver fir hosts. a) Haplotype distribution; b) main population clusters: 1 white bordered black, 2 clear white bordered white, 3 black, 4 dark grey.

3. F_{ST} and F_{IS} in the population of isolates from Silver fir.

	cluster 1	**cluster 2**	**cluster 3**	**cluster 4**
cluster 1 (F_{IS} = 0.257)	-			
cluster 2 (F_{IS} = 0.349)	0.000	-		
cluster 3 (F_{IS} = 0.000)	0.000	0.000	-	
cluster 4 (F_{IS} = 0.019)	0.000	0.015	0.000	-

Silver fir isolates were grouped in four different clusters showing a high gene flow level. Only clusters 1 and 2 exhibit inbreeding by F_{IS} index (Tab. 3).

The geographic structure of *H. annosum* populations, from pine and silver fir, is probably related to the morphology of the Italian peninsula and to glaciations and post-glaciations events. During the last ice age forest trees refuged in small areas from where, after retreat of ice, re-colonized the Italian peninsula (Vettori *et al.,* 2004).

The evolution of *Heterobasidion* species have probably followed similar spreading history as tree host species, reflecting genetic variation of fungal populations (Johannesson and Stenlid, 2003). In case of *Heterobasidion* from pine, host isolation reflect establishment of isolated fungal subpopulations. Differently *Heterobasidion* subpopulations from Silver fir, like its main host, were never completely isolated showing re-colonization from glacial refuges.

Aknowledgements

The research was supported by MIUR within the PRIN number 2008SBCC9S

References

Capretti P., 1998. Impact, control and management of Heterobasidion annosum root and butt rot in Europe and North America. Italy. In: *Heterobasidion annosum*: Biology, Ecology, Impact and Control. Ed. by Woodward, S.; Stenlid, J.; Karjalainen, R.; Hüttermann, A. Wallingford, UK: CAB International, pp. 377-385.

D'Amico L., Motta E., Annesi T., Scirè M., Luchi N., Hantula J., Korhonen K., Capretti P., 2007. The North American P group of *Heterobasidion annosum* s.l. is widely distributed in *Pinus pinea* forests of the western coast of central Italy. *Forest Pathology* 37: 303-320.

Gonthier P., Warner R., Nicolotti G., Mazzaglia A., Garbelotto M., 2004. Pathogen introduction as a collateral effect of military activity. *Mycological Research* 108: 468-470.

Guillot G., Estoup A., Mortier F., Cosson J.F., 2005a. A Spatial Statistical Model for Landscape Genetics. *Genetics* 170: 1261-1280.

Guillot G., Leblois R., Coulon A., Frantz A.C., 2009. Statistical methods in spatial genetics. *Molecular Ecology* 18: 4734-4756.

Guillot G., Mortier F., Estoup A., 2005b. Geneland: a computer package for landscape genetics. *Molecular Ecology Notes* 5: 712-715.

Guillot G., Santos F., Estoup A., 2008. Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. *Bioinformatics* 24: 1406-1407.

Hardy O.J., Vekemans X., 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2: 618-620.

INFC, 2009. I caratteri quantitativi 2005 – parte 1, vers. 2. Autori P. Gasparini, F. De Natale, L. Di Cosmo, C. Gagliano, I. Salvadori, G. Tabacchi e V. Tosi. Inventario Nazionale delle Foreste e dei Serbatoi Forestali di Carbonio. MiPAAF – Ispettorato Generale Corpo Forestale dello Stato, CRA-MPF, Trento.

Johannesson H., Stenlid J., 2003. Molecular markers reveal genetic isolation and phylogeography of the S and F intersterility groups of the wood-decay fungus *Heterobasidion annosum*. *Molecular Phylogenetics and Evolution* 29: 94-101.

La Porta N., Capretti P., Kammiovirta K., Karjalainen R., Korhonen K., 1997a. Geographical cline of DNA variation within the F intersterility group of *Heterobasidion annosum* in Italy. *Plant Pathology* 46: 773-784.

La Porta N., Capretti P., Kammiovirta K., Korhonen K., Karjalainen R., 1997b. The relatedness of the Italian F intersterility group of *Heterobasidion annosum* with the S group, as revealed by RAPD assay. *Mycological Research* 101: 1065–1072.

Luchi N., Paffetti D., Korhonen K., Hantula J., Capretti P., 2011. Genetic variation of *Heterobasidion abietinum* in southern Europe and the Mediterranean Basin. *Forest Pathology* 41: 270-280.

Petta M., Capretti P., Tegli S., Maresi G., 2001. Variations among isolates of *Heterobasidion annosum* P group detected using the M13 minisatellite. *Phytopatologia Mediterranea* 40: 55-60.

Puddu A., Luisi N., Capretti P., Santini A., 2003. Environmental factors related to damage by *Heterobasidion abietinum* in *Abies alba* forests in Southern Italy. *Forest Ecology and Management* 180: 37-44.

Vainio, E.; Korhonen, K.; Hantula, J., 1998. Genetic variation in *Phlebiopsis gigantea* as detected with random amplified microsatellite (RAMS) markers. *Mycological Research* 102, 187-192.

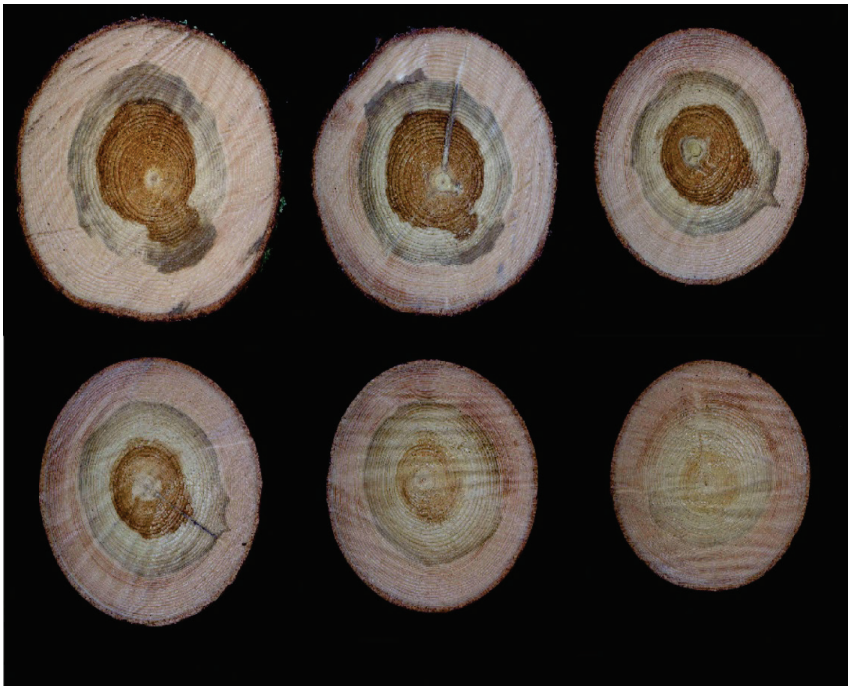
Vettori C., Vendramin G., Anzidei M., Pastorelli R., Paffetti D., Giannini R., 2004. Geographic distribution of chloroplast variation in Italian populations of beech. *Theoretical and Applied Genetics* 109: 1-9.

Woodward S., Stenlid J., Karjalainen R., Hüttermann A., 1998. *Heterobasidion annosum*: Biology, Ecology, Impact and Control. Wallingford, UK: CAB International.

Zamponi L., Paffetti D., Tegli S., Łakomy P., Capretti P., 2007. Genetic variation in *Heterobasidion abietinum* populations detected with the M13 minisatellite marker. *Forest Pathology* 37: 321-328.

Session 5

Aetiology and Epidemiology



Pathogenicity of virus infected and virus-free *Heterobasidion abietinum* isolates on *Abies* species

A.G. Aday[1], A. Lehtijärvi[2], E.J. Vainio[3], H.T. Doğmuş-Lehtijärvi[2], J. Hantula[3]

[1]*Süleyman Demirel University, Yenişarbademli Vacational School, 32260 Isparta, Turkey.*
[2]*Süleyman Demirel University, Faculty of Forestry, 32260 Isparta, Turkey.*
[3]*Finnish Forest Reserach Instutite, Vantaa, Finland.*
Corresponding author e-mail address: guldenaday@sdu.edu.tr

Abstract. *Heterobasidion abietinum*, one of the three species identified in the *Heterobasidion annosum* complex in Eurasia, causes root rot predominantly on species of *Abies*. European and western Asian *Heterobasidion* isolates were found to contain dsRNA viruses. In this study the effect of a dsRNA virus on the virulence of *H. abietinum* was investigated. For this purpose, 3-year-old *Abies cilicica* and 5-year-old *Abies bornmülleriana* seedlings were inoculated on the lower stem with four isogenic virus-infected and virus-free isolates of *H. abietinum* originating from different regions of Turkey. Ten replicate seedlings per treatment were incubated in growth chamber for seven weeks, where after infection incidence, lesion length in the inner bark and fungal growth in the sapwood were investigated. There were significant differences between lesion lengths on both host and isolate combinations. Clear differences in virulence between viruse-infected and viruse-free *H. abietinum* isolates were found. The results indicate that the virus affects the virulence of its host fungus.

Heterobasidion abietinum, one of the three species identified in the *Heterobasidion annosum* complex in Eurasia, causes root rot predominantly on species of *Abies*. European and western Asian *Heterobasidion* spp. isolates was found to contain dsRNA viruses which are member of *Partitiviridae* family (Ihrmark 2001; Vainio *et al.,* 2010).

The aim of this work was to investigate the effect of a dsRNA virus on the virulence of *H. abietinum.*

Five-year-old seedlings of *A. bornmülleriana* and 2-year-old seedlings of *A. cilicica* were used in the inoculation. Four previously identified isogenic virus-infected and virus- free isolates of *Heterobasidion abietinum* originating from different regions of Turkey were used in the inoculation experiments.

Virus-infected isolates were cured from viruse with the aid of temperature treatments. For this purpose, virus-infected isolates were cultured on petri plates containing 2% Malt-extract and 2% agar and incubated at 33°C for 3 days. After incubation, single hyphal tips were cut and placed onto 2% MEA petri plates and incubated at 33°C for one week. This treatment was repeated for 3 times. After curing the fungal cultures, the isolates were grown on MEA covered with cellophane membrane. The mycelia were harvested and ground in liquid nitrogen using sterile mortar. The isolates were tested for presence of dsRNA with both CF11 chromatography and RT-PCR.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Pathogenicity test was conducted as described by Stenlid and Swedjemark (1988) with minor modifications. Inocula were prepared by growing *H. abietinum* on autoclaved 5-mm diameter fir plugs placed on malt extract agar (2%, w/v; Merck KGaA, Darmstadt, Germany) for 4 weeks at 23°C. On each seedling, a 5-cm section of the main stem at the inoculation point was cleaned with 70% (v/v) ethanol.

Using a sterilized cork borer, a circular 5-mm wound was made by removing the bark about 8 cm (*A. bornmülleriana*) or 5 cm (*A. cilicica*) above the soil level. A wooden plug colonized by *H. abietinum* was attached to the wound by wrapping Parafilm around the stem. Controls were conducted with non-colonized, autoclaved wooden plugs. The pathogenicity test was carried out in a growth chamber. Each isolate-host-virus combination was repeated ten times. Including control seedlings (20), the total number of seedlings was 180. In growth chamber, a 16-h photoperiod was provided and the mean temperature was 18.6°C. During the 7 weeks incubation period, seedlings were irrigated with tap water once or, when necessary, twice a day.

After incubation period, the seedlings were harvested, branches and the root system removed and the lesion length measured in the inner bark. The stem was cut into 0.5 cm discs from the upper and lower part of inoculation point and the discs were placed on Petri dishes containing wet filter paper. After 1 week incubation at 23°C, the discs were examined under a dissecting microscope for the conidial stage of *Heterobasidion* and the extension of the fungus in each stem was determined (Stenlid and Swedjemark, 1988).

Data were analysed using the SPSS GLM procedure and differences among mean values determined using Duncan's multiple range test.

All isolates were found to be pathogenic when compared with controls ($P< 0.05$). There were significant differences between virus-infected and virus-free isolates in lesion length (Tab. 1).

The results from pathogenicity experiments demonstrate that *H. abietinum* isolates which were virus- free had effect on lesion length and fungal growth of both inoculated *Abies* species. Further studies are needed to confirm this inoculation results.

1. Comparison between virus-free and virus-infected isolated of *H.abietinum*

	Virus-free *H. abietinum*		**Virus-infected *H. abietinum***	
	A. bornmülleriana	***A. cilicica***	***A. bornmülleriana***	***A. cilicica***
Seedling height (cm)	51.0	13.0	48.0	14.1
Seedling diameter (cm)	10.0	6.0	10.0	6.0
Mortality (%)	0	2.5	7.5	22.5
Lesion length (mm)	10.2 a	30.7 b	11.3 a	38.6 b
Fungal growth (mm)	15.5 a	60.0 b	21.0 a	61.0 b

References

Ihrmark K., 2001. Double-stranded RNA Elements in the Root Rot Fungus *Heterobasidion annosum*. PhD dissertation, Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala. ISSN 1401-6230, ISBN 91-576-6094-8.

Stenlid J., Swedjemark G. 1988. Differential growth of S- and P-isolates of *Heterobasidion annosum* in *Picea abies* and *Pinus sylvestris*. *Transactions of the British Mycological Society* 90: 209-213.

Vainio E.-J., Korhonen K., Tuomivirta T.T., Hantula J. 2010. A novel putative partitivirus of the saprotrophic fungus *Heterobasidion ecrustosum* infects pathogenic species of the *Heterobasidion annosum* complex. *Fungal Biology* 114: 955-965.

Variation in pathogenicity and virulence of four ophiostomatoid fungi and *Heterobasdion irregulare* in *Pinus taeda* and *Pinus elliottii*

L. Eckhardt[1] and G. Matusick[2]

[1]*Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL.*
[2]*Centre of Excellence for Climate Change Woodland and Forest Health, Murdoch University, School of Biological Sciences and Biotechnology, Murdoch, Western Australia.*
Corresponding author e-mail address: eckhalg@auburn.edu

Root disease is an important natural disturbance in pine-dominated systems around the world including the southeastern U.S. In some instances, both *H. irregulare* and root-inhabiting ophiostomatoid fungi have been observed infecting the same dying southern pine. Studies with *P. taeda* and *P. elliottii* were conducted to determine the relative pathogenicity and virulence of *H. irregulare* and four root-inhabiting ophiostomatoid fungi in healthy tree roots, and to characterize the local symptomology following infection. All fungal species tested were found capable of infecting healthy pine roots and inducing a significant lesion following inoculation. In both *Pinus* species, *G. huntii* caused a significantly greater lesion when compared to *H. irregulare*, *L. terebrantis*, and *L. procerum*. *H. irregulare* caused lesions comparable to *L. serpens*, *L. terebrantis*, and *L. procerum* in *P. taeda* and was significantly larger than *L. procerum* in *P. elliottii*. *L. procerum* caused the smallest lesions when compared to all other ophiostomatoid fungi.

These studies illustrate that important differences exist in the relative pathogenicity and virulence of the fungi tested. *G. huntii* and in some cases *L. serpens*, appear to cause significantly greater lesions following infection and may be contributing more to the symptomology observed in diseased trees.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Interaction of Norway spruce and *Heterobasidion parviporum* in xylem

A.M. Hietala, N.E. Nagy, I. Yakovlev, C.G. Fossdal, H. Solheim

Norwegian Forest and Landscape Institute, P.O. Box 115, NO-1431 Ås, Norway.
Corresponding author e-mail address: ari.hietala@skogoglandskap.no

Abstract. In Norway spruce, a fungistatic reaction zone with high pH and enrichment of phenolics is formed in the sapwood facing heartwood colonized by the white-rot fungus *Heterobasidion parviporum*. Fungal penetration of the reaction zone eventually results in expansion of this xylem defence. Despite the fact that Heterobasidion is able to breach the reaction zone, not much is known about the ways this is achieved. To gain knowledge about mechanisms operative at reaction zone colonization by the pathogen, by using various approaches we have examined naturally colonized trees showing extensive stem decay and signs of host-pathogen interaction at the reaction zone region. Hyphal self-defence, detoxification of host defence compounds and utilization of various carbon sources, themes central to breaching of reaction zones, are discussed based on our long-term working experience on this topic.

Heterobasidion parviporum Niemelä & Korhonen is a white-rot fungus primarily restricted to Norway spruce (*Picea abies* (L.) Karst.). Stem colonization of Norway spruce by this facultative parasite usually initiates in 25-40 year old trees, where heartwood formation has already started. In stems, the fungus spreads rapidly in an axial direction. Since the fungus is confined to heartwood, the stem decay column may eventually be up to ten-meter-long while the tree is still alive. In colonized trees xylem defense response is characteristically seen as a production of a fungistatic reaction zone (RZ), phenol-rich pathological heartwood forming at the border between sapwood and colonized heartwood. Besides accumulation of phenols, RZ shows elevated pH, element and water content in relation to normal wood (Shain, 1971). Pathogen penetration into RZ is followed by expansion of this xylem defense to inner sapwood. Finally, this process terminates in death of the tree due to a reduced amount of conductive tissue or stem failure. The mechanisms utilized by *Heterobasidion* and other xylem associated fungi that are able to breach through host RZ have long remained poorly understood.

Incubation of spruce wood discs from trees with advanced decay by *H. parviporum* results in luxurious production of conidiophores at the colony edge facing reaction zone, whereas elsewhere in colonized heartwood conidiogenesis is regular but relatively sparse (Hietala *et al.*, 2009). As conidiophore production depends on transport of nutrients from assimilative hyphae, the observed dense belt of conidiophores suggests that an efficient translocation channel is in place at the colony margin. The locally high fungal biomass at the interface between reaction zone and colonized heartwood has also been confirmed by spatial profiling of pathogen DNA levels in wood (Hietala *et al.*, 2009). In areas with incipient decay

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

by *H. parviporum,* a carbohydrate-rich hyphal sheath characteristic to wood decay fungi is spatially restricted to the hyphal surface facing the tracheid wall, whereas upon exposure to RZ phenolics the pathogen hyphae are fully encased by a carbohydrate-rich sheath (Hietala *et al.*, 2009). In the sheath material of spores of the ascomycete *Colletotrichum graminicola*, a maize anthracnose pathogen, the glycoprotein components bind phenols and allow spore germination under conditions that are otherwise toxic (Nicholson *et al.*, 1989). Similarly, a prerequisite for fungal takeover of the RZ is the ability to tolerate exposure to polyphenols. The hyphal sheath that encapsulates *H. parviporum* hyphae upon their exposure to phenols may thus prevent membrane permeabilization by organosolvents. This would allow maintenance of cellular activities in a phytotoxic environment. The increased production of a hyphal sheath has been observed upon exposure of artificially grown wood decay fungi to an adverse pH and fungicides (Vesentini *et al.*, 2006).

Hemicelluloses and pectin are the preferred carbon sources of fungi capable of selective delignification. In consistency, both ligninolytic enzymes and hemicellulases have been immunolocalized in the hyphal sheath of white-rot fungi during wood decay (e.g. Ruel and Joseleau, 1991), this sheath forming the functional interface between the organisms. Degradation of reaction zone polyphenols by *H. parviporum* has been suggested by the appearance of lumen areas in which the phenolic deposit is cleared in the presence of fungal hyphae (Hietala *et al.*, 2009). Since in general the ligninolytic enzymes of white-rot fungi are nonspecific, it could be expected that they participate in the oxidation of polyphenols associated with RZ. For example, laccases that are induced particularly upon fungal exposure to reaction zone (Fig. 1) form good candidates to future knock-out, enzyme activity and in-situ immunolocalization studies. Regarding fungal feeding, spatial profiling of structural carbohydrates of Norway spruce in naturally colonized heartwood shows that pathogen utilization of middle lamella associated pectin is very pronounced at the colony edge interacting with RZ (Fig. 2).

Taken together, the currently available data indicate that the interaction of *H. parviporum* with RZ of Norway spruce involves a local concentration of fungal biomass that forms an efficient translocation channel for nutrients. The enhanced production of hyphal sheath may be instrumental in lateral expansion of the decay column beyond the RZ boundary. Ligninolytic enzymes such as laccases presumably contribute to detoxification of xylem defense, pectin providing an important energy source for the fungus at this stage. We anticipate that knowledge gain on pathogen responses to xylem defense will now be accelerated owing to the completed genome sequencing of *Heterobasidion* species, whereas rapid progress on conifer defense remains hampered by the lack of knowledge on host genomes.

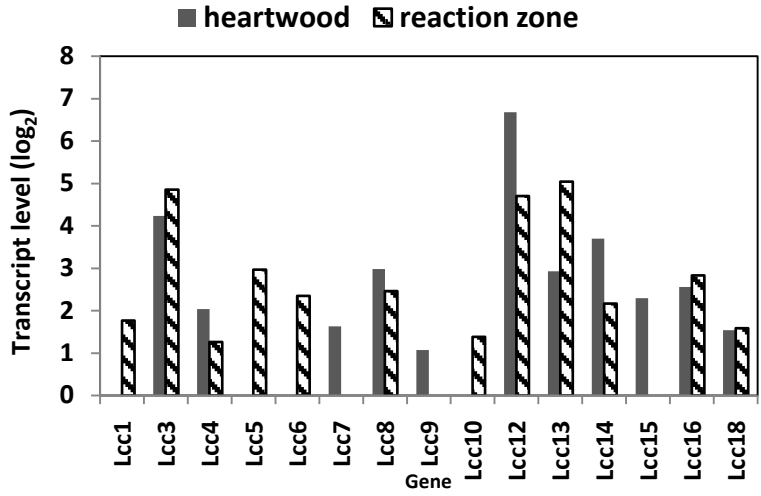


Figure 1. Laccase genes of *H. irregulare* induced upon growth on heartwood or reaction zone of Norway spruce in a Petri dish experiment. Transcript level indicates the induction level in relation to the corresponding transcript level on liquid Hagem medium. The absence of transcript level value for a substrate indicates that the gene was not induced in relation to liquid Hagem medium (Igor Yakovlev, Ari M. Hietala, Pierre-Emmanuel Courty, Taina Lundell, Halvor Solheim, Carl Gunnar Fossdal, manuscript in preparation).

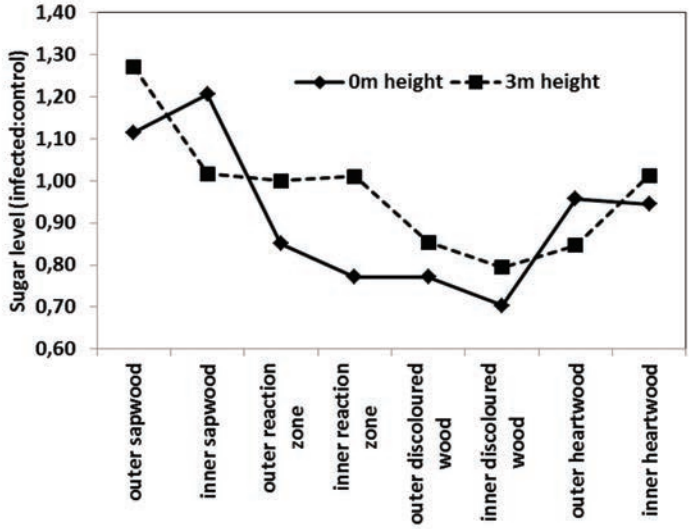


Figure 2. The relative amount of galacturonic acid at stump level and 3 meter height in stems of Norway spruce trees naturally infected by *H. parviporum* and showing advanced decay columns. The sugar amount is expressed in relation to spatially corresponding xylem tissue in similar-sized healthy trees (Nina Nagy, Simon Ballance, Harald Kvaalen, Carl G. Fossdal, Halvor Solheim, Ari M. Hietala, a manuscript in preparation.

References

Hietala A.M., Nagy N.E., Steffenrem A., Fossdal C.G., Kvaalen H., Solheim H. 2009. Spatial patterns in hyphal growth and wood degradation within Norway spruce stems colonized by the pathogenic white-rot fungus *Heterobasidion parviporum*. *Applied Environmental Microbiology* 75: 4069-4078.

Nicholson R.L., Hipskind J., Hanau R.M. 1989. Protection against phenol toxicity by the spore mucilage of *Colletotrichum gramicola*, an aid to secondary spread. *Physiological Molecular Plant Pathology* 35: 243-252.

Ruel K., Joseleau J.-P. 1991. Involvement of an extracellular glucan sheath during degradation of Populus wood by *Phanerochaete chrysosporium*. *Applied Environmental Microbiology* 57: 374-384.

Shain L. 1971. The response of sapwood of Norway spruce to infection by *Fomes annosus*. *Phytopathology* 61: 301-307.

Vesentini D., Dickinson D.J., Murphy R.J. 2006. Fungicides affect the production of extracellular mucilaginous material (ECMM) and the peripheral growth unit (PGU) in two wood-rotting basidiomycetes. *Mycological Research* 110: 1207-1213.

Susceptibility of lodgepole pine to *Heterobasidion annosum* and *H. parviporum* in central Sweden.

J. Rönnberg and S. Svensson

Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences, Alnarp, Sweden.
Corresponding author e-mail address: Jonas.Ronnberg@slu.se

Abstract. The susceptibility of lodgepole pine (*Pinus contorta*) to attack by *Heterobasidion* spp. was investigated in: i) Three unthinned stands established on former Norway spruce sites infested by *Heterobasidion*, ii) Stumps from thinning exposed to natural spore infection, and iii) Stumps artificially infected with *H. parviporum* and *H. annosum*. In the first study, discs were collected from roots of trees and in the second and third studies from stumps. All discs were analyzed for the presence of *Heterobasidion*. *Heterobasidion* was found on the roots of four out of 15 symptomatic lodgepole pine trees while one of the 15 asymptomatic trees was infected. Stumps from thinnings had no infection. The thinnings were though accidentally conducted ca two years prior to the sampling during cold weather conditions. All lodgepole pine stumps artificially infected with *H. annosum* (n=30) and all except one with *H. parviporum* (29 out of 30) were infected 51 days post-inoculation. Conclusively there is a potential problem with *Heterobasidion* infection in lodgepole pine stands in central Sweden. Furthermore lodgepole pine stumps are susceptible to infection by both *H. annosum* and *H. parviporum*. Before new recommendations for silvicultural practices can be given measurements to quantify the problem and studies on e.g. the effect from stump treatment or removal are needed.

Introduction

Heterobasidion spp. are common root and butt rot causing fungi in the northern hemisphere (Hodges, 1969) and cause large economic losses to the Swedish forestry sector. Two species of *Heterobasidion spp.* can be found in Sweden, *Heterobasidion parviporum Niemelä & Korhonen*, and *Heterobasidion annosum*. While *H. annosum* has been found in southern Sweden up to 61°N, *H. parviporum* is present throughout the country (Korhonen *et al.*, 1998). *H. parviporum* generally attacks Norway spruce (*Picea abies* (L.) Karst.) but can also infect saplings of Scots pine (*Pinus sylvestris L.*). *H. annosum* mainly infects pines in all ages but has also been found in spruce and on other species e.g. birch and alder. Trees are infected by airborne basidiospores landing on fresh cut stumps surfaces when temperature are greater than 0°C and subsequent mycelia transfer at root contact between infected stumps and adjacent healthy trees (Hodges, 1969). The fungus can remain alive in stumps for decades (Piri, 1996) and trees planted on sites where the previous stand was infected by *Heterobasidion spp.* may become infected (Rönnberg *et al.,* 1999) e.g. lodgepole pine *(Pinuscontorta var. latifolia)* (Piri, 1996).

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

The forestry sector in Sweden is exceptionally important for the country's economy. Increased demands for wood fibreand other goods from forests e.g. ecosystem services, has resulted in discussions on how to increase wood production in Sweden. Already during the 1970s a prognosticated timber supply shortage initiated the search for faster growing species than Scots pine and Norway spruce. One of the major forestry companies in Sweden (SCA) therefore started extensive planting of exotic lodgepole pine primarily north of 60°N (Hagner, 1983). Also today's discussion includes a wider use of lodgepole pine to boost the timber production in some areas. Many of the plantations were and will be established on former forest land where *Heterobasidion* spp. may have been present to various degrees. Very little research, especially for Swedish conditions, has though been done on the susceptibility of lodgepole pine to *H. parviporum*. Some of the earliest plantations are furthermore now subjected to thinning. Consequently, a pilot study was initiated to see if *Heterobasidion spp.* infects lodgepole pine through recently cut stumps or through root contacts with infected stumps and to what extent the two species of *Heterobasidion* in Sweden would grow on stumps created during summer thinnings.

Materials and Methods

The study was established in two main areas in the middle north of Swedenat 62-63°N, one located near the coast and one more inland. The susceptibility of lodgepole pine to attack by *Heterobasidion* spp. was investigated in: i) Three unthinned inland stands established on former Norway spruce sites infested by *Heterobasidion*, ii) Stumps from thinning exposed to natural spore infection in five stands closer to the coast, and iii) Stumps artificially infected with *H. parviporum* and *H. annosum* in three of the previously mentioned stands. In the first study, discs were collected from roots of trees and in the second and third studies from stumps. Stumps artificially infected after thinning were sprayed with a suspension of conidiospores from either *H. parviporum* or *H. annosum* at a concentration of ca 50 spores per cm^2. Artificially infected stumps were sampled after 51 days. All sample discs were analyzed for the presence of *Heterobasidion* conidiophore colonies.

Results

Heterobasidion was found on the roots of four out of 15 symptomatic lodgepole pine trees while one of the 15 asymptomatic trees was infected. One sample with infection was 10 cm from the butt of the tree, two samples 50 cm from the butt and three samples 100 cm from the butt. Infections of *Armillaria* could be found but were not quantified or species determined. Stumps (n=100) from the practical thinning had no infection. It later turned out that thinnings were though accidentally conducted two years prior to sampling during cold weather conditions.

All lodgepole pine stumps artificially infected with *H. annosum* (n=30) and all except one with *H. parviporum* (29 out of 30) were infected 51 days post-inoculation (Fig. 1, 2).

Conclusions

Conclusively there is a potential problem with *Heterobasidion* infection in lodgepole pine stands in central Sweden. The study did not show the extent of damage but it seems prudent to monitor and follow up the potential development not to cause the same problem as we have with Norway spruce today. Furthermore lodgepole pine stumps are susceptible to infection by both *H. annosum* and *H. parviporum*. In parts of the area where lodgepole pine is planted in Sweden only *H. parviporum* is supposed to be present. It is however not known how global warming might affect the spread of *H. annosum* further north in the country (Witzell *et al.*, 2011). Before good recommendations for silvicultural practices can be given measurements to quantify the problem and studies on e.g. the effect from stump treatment or stump removal are needed.

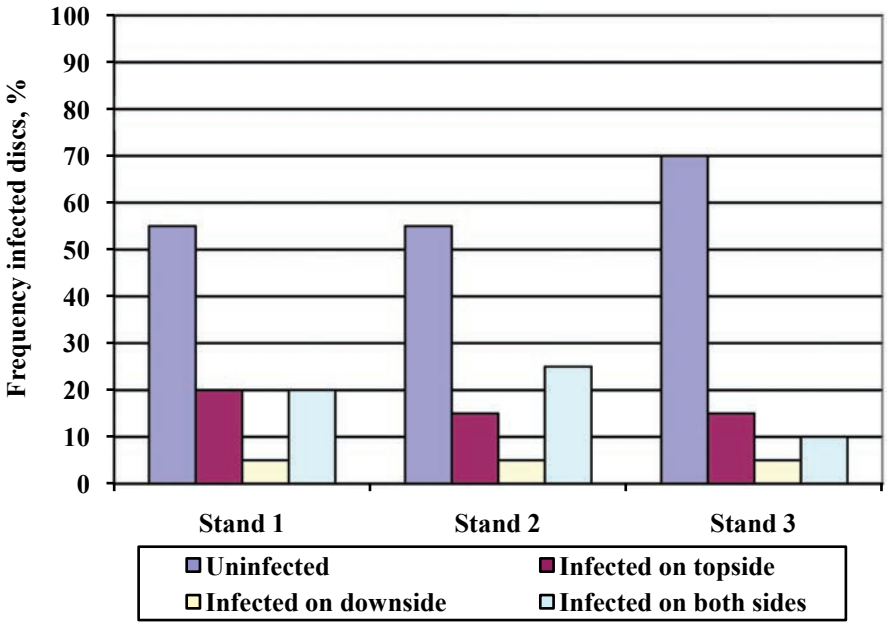


Figure 1. Frequency of uninfected and infected stump discs (on the top of the disc, the downside of the disc and on both sides of the discs) from thinning before the artificial infection, in each of the three sites.

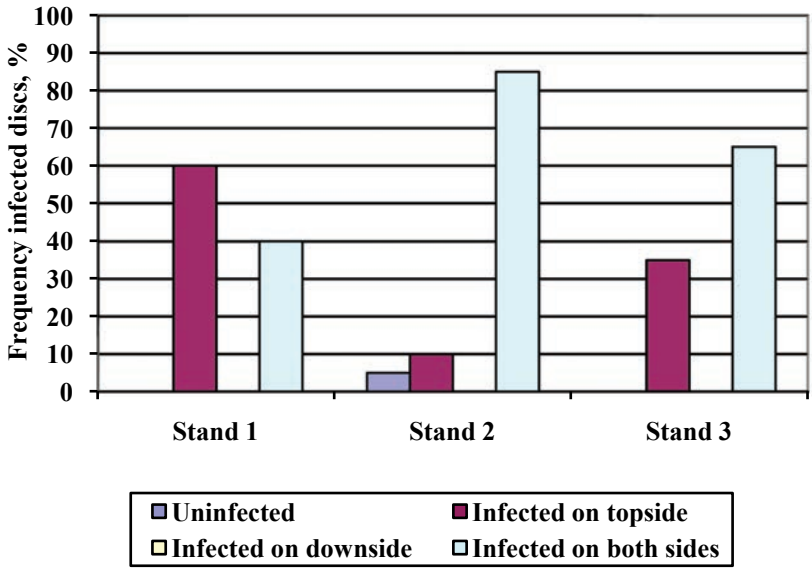


Figure 2. Frequency of uninfected and infected stump discs (on the top of the disc, the downside of the disc and on both sides of the discs) from thinning after the artificial infection, in each of the three sites.

References

Hagner S., 1983. *Pinus contorta*: Sweden's third conifer. *Forest Ecology. Managment* 6: 185-199.

Hodges C.S., 1969. Modes of infection and spread of *Fomes annosus*. *Annual review of Phytopathology* 7: 247-266.

Korhonen K., Capretti P., Karjalainen R., Stenlid J., 1998. Distribution of *Heterobasidion annosum* intersterility groups in Europe. In: Woodward S., Stenlid J., Karjalainen R., Hüttermann A. (eds.). *Heterobasidion annosum* Biology, Ecology, Impact and Control, CAB International, Wellingford, Oxon, OX10 8DE, UK. pp 93-104.

Piri T. 1996. The spreading of the S type of Heterobasidion annosum from Norway spruce stumps to the subsequent tree stand. *European Journal of Forest Pathology* 26: 193-204.

Rönnberg J., Vollbrecht G., Thomsen I.M. 1999. Incidence of butt rot in a tree species experiment in northern Denmark. *Scandinavian Journal of Forest Research* 14: 234-239.

Witzell J., Berglund M., Rönnberg J. 2011. Does temperature regime govern the establishment of *Heterobasidion annosum* in Scandinavia? *International Journal of Biometeorology* 55: 275-284.

Distribution of *Heterobasidion parviporum* genets in Norway spruce forests in Serbia

N. Keča and L. Keča

Faculty of Forestry, University of Belgrade, Kneza Viseslava 1, 11030 Belgrade, Serbia.
Corresponding author e-mail address: nenad.keca@sfb.bg.ac.rs

Abstract. Three European *Heterobasdion* species are present in forest ecosystems in Serbia. Aim of this study was to identify *Heterobasidion* genets, and present distribution of genets in Norway spruce selection forests. Three study plots, 100×50 m in size, were established in May 2006. Trees showing chlorosis, declining processes, and all wind - and snow - snapped trees were mapped and the diameter of the stumps and trees (breast height) was recorded. For isolation of the decay fungus, wood samples were taken aseptically from the stem base and main roots of all mentioned categories of trees with an increment borer. All obtained isolates were identified as *Heterobasidion parvipurum*. Size of genets ranged from 75 m^2 (separate tree) to 800 m^2. Even number of genets was almost the same at three sites, size of individua was greatly influenced by impact of forest management.

Introduction

During last 25 years our picture of the root-rot fungus *H. annosum* and the honey fungus *Armillaria mellea* has changed considerably because both of these fungi have been split into several species (Korhonen, 2004).

History of study of *Heterobasidion* in Serbia goes back in 1963, when strong attack of *Fomes annosus* on mountain Kopaonik was reported. At the same time, the disease was found in natural forests of spruce on Zlatar, Durmitor (Montenegro), Jahorina (Bosnia and Herzegovina) (Marinković *et al.,* 1990). Most recent studies showed that all three species are present in coniferous forests in Serbia (Keča, 2008). Studies about population structure, size of genets, spread and distribution of *Heterobasidion* haven't been performed earlier in Serbia. Fifty to 60% of the *Heterobasidion* genets identified in the final cutting Norway spruce stands had infected only one tree (Piri *et al.,* 1990). Most of available data are obtained from cultures and from Scandinavian countries. There are almost no data about structure and size of *Heterobasidion* genets from South-East Europe and also from Balkan region.

The aim of present case study was to obtain detailed information about the presence, size and spatial distribution of *Heterobasidion* genets in a selection Norway spruce forest. *Heterobasidion* genets are mapped and data about tree condition are collected.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Materials and Methods

This study was carried out in the most important forest types of a Norway spruce in Serbia. First was the *Picetum excelsae oxalidetosum* (Miš. *et* Pop.) on a brown podzolic soil, second by *Abieti-Picetum oxalidetosum* on brown podzolic soil. According to the forest inventory data average density is 493 trees ha^{-1}, volume 409.5 m^3 ha^{-1}, diameter 29 cm and height is 20.5 m.

Three study plots, 90×40 m in size, were established in May 2006. Trees showing chlorosis, decline, and all wind- and snow-snapped trees were mapped and the diameter of the stumps and trees (breast height) was recorded. Isolates were collected with increment borer - stem base and main roots. Isolation was also performed from all carpophores found on site.

Heterobasidion were isolated onto a modified selective MEA medium (5 mg l^{-1} benzomidazol, 4 mg l^{-1} dichloran and 100 mg l^{-1} of streptomycin sulphate).

The genets were identified with somatic incompatibility test, by pairing the isolates in all possible combinations and recording the line of demarcation. The isolates were identified by pairing with 4 haploid tester isolates from the three European *Heterobasidion* species.

Results

Eighty *Heterobasidion* isolates and 12 isolates of rotting fungi were collected, each from a separate tree, stump, or other substrate. All *Heterobasidion* isolates found on the study sites belong to the species *H. parviporum*. There was no significant diference ($P > 0.15$) between isolation of *H. parviporum* from healthy (visually), chlorotic, declining or snapped trees (Tab. 1).

Fourteen genets (50% of all identified) occupied only one tree, 9 genets were present on two trees, while only 5 genets were present on more than two trees (Tab. 2). Occupied area ranged between 75 and 800 m^2. Distribution of genets on study site no. 2 was shown on Fig. 1.

Discussion

Twenty-nine *Heterobasidion* genets were present in the three relatively small studied plots in spruce stands through Serbia. Fifty to 60% of the *Heterobasdion* genets identified in stands at the end of rotation had infected only one tree (Vasiliauskas and Stenlid, 1998). In present study two genets had spread on five neighbouring trees and occupied area of about 550 and 800 m^2, respectively. Both of genets were present on the same site where cutting was intensive in last 10 years. Cutting of trees with big stumps enabled fast vegetative spread through root contact on neighboring trees.

Management in selection forests should provide conditions which will favor stump decomposition. This study showed that even relatively small stumps

(diameter ~30 cm) produced sporophores 27 years after tree was cut (Keča, unpublished).

Young regenerated trees may not necessarily decline and die, but infection could spread into the central part of trunk and cause rot. Artificial infections, carried out on spruce showed that central rot can spread up to 12 m in height (Marinković *et al.,* 1990), which means that such tree can not be, used a timber. Economic loss is highly infected stands could reach up to 25% of volume (Keča, unpublished).

According to the presented results forest managers need to be very careful during management of selective spruce forests. It is necessary to enable conditions which will favour stump decomposition and reduce vegetative spread in neighbouring both older and regenerated trees by root contact.

Aknowledgements

This research was supported by grant from the Ministry of Education and Science of the Republic of Serbia. We would like to thank to the projects TR 37008 and TR 31041. Dr. Kari Korhonen, METLA donated haploid tester strains of *Heterobasidion* species.

Table 1. Distribution of number of controled, infected, chlorotic and healthy trees on studied sites.

Sudy site	No. of control	No. of infected	No. of healthy	No. of chlorotic	% of infected
I	46	21	13	12	46
II	49	15	22	12	31
III	34	22	8	4	65

Table 2. Distribution of *Heterobasidon parviporum* genets and occupying area on three studied sites.

Study site	Total genets/site	No. of genets occupying tree(s)			Max. area (m^2)/genet	Infected stumps
		one	two	more		
I	11	5	5	1	~200	3
II	9	4	3	2	~800	11
III	9	5	1	3	~550	0
Total	29	14	9	6		

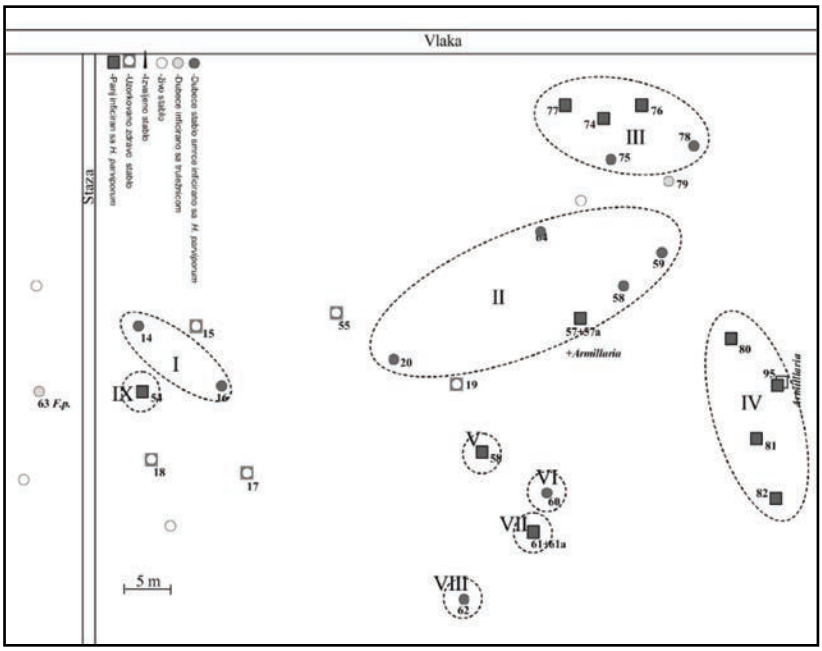


Figure1. Distribution of *Heterobasidion parviporum* genets on study site no. 2.

References

Keča N. 2008. Identification of *Heterobasidion* species in Serbia and possibility of its control. *Plant Doctor* 35: 44-50.

Korhonen K. 2004. Fungi belonging to the genera *Heterobasidion* and *Armillaria* in Eurasia. In: *Fungal Communities in forest ecosystems, Coordinating Investigation*, Vol. 2, (Storozhenko V.G., Krutov V.I., eds) Moscow - Petrozavodsk, pp. 89-113.

Marinković P., Šmit S., Popović J. 1990. Disease of the root of spruce, *Fomes annosus* (Fr.) Cooke, the importance of this phenomenon in maintaining and restoring spruce forests on Kopaonik. In: *Proceedings Nature of Kopaonik - Protection and use*, Institute for Tourism PMF, Belgrade pp. 235-240 (in Serbian with English summary).

Piri T., Korhonen K., Sairanen A. 1990. Occurrence of *Heterobasidion annosum* in pure and mixed spruce stands in southern Finland. *Scandinavian Journal of Forest Research* 5: 113-125.

Vasiliauskas R., Stenlid J. 1998. Spread of S and P group isolates of *Heterobasidion annosum* within and among *Picea abies* trees in central Lithuania. *Canadian Journal of Forest Research* 28: 961-966.

Armillaria root rot occurrence in Norway spruce (*Picea abies*) stands of Kolbudy Forest District

M. Mańka[1] and A. Juźwiak[2]

[1]*Department of Forest Pathology, Poznań University of Life Sciences, ul. Wojska Polskiego 71c, 60-618 Poznań, Poland.*
[2]*Museum in Kwidzyn, Branch of the Malbork Castle Museum ul. Katedralna 1, 82-500 Kwidzyn, Poland.*
Corresponding author e-mail address: mmanka@up.poznan.pl

Abstract. Kolbudy Forest District is situated in middle north of Poland, near Gdańsk, in a region considered a spruce disjunction area. The aim of the work was analysis of health condition of four Norway spruce stands (age 17, 62, 82 and 109 years in 2007) in Kolbudy Forest District (Regional Directorate of the State Forests in Gdańsk). All the stands are situated on fresh mixed broadleaf forest site (according to Polish forest site classification) and they all are affected by *Armillaria*. In 2007-2010 the four/three Norway spruce stands were monitored for tree health condition. All the isolates of *Armillaria* spp. collected in the stands were identified in with the Korhonen intersterility test and proved *A. ostoyae*. The monitoring was performed twice in 2007 (at the beginning and at the end of the vegetation season). In following years the stands were monitored in autumn. The health status of trees was estimated according to a 5-grade scale. The stands were affected by *Armillaria* to various extent. The youngest stand (17-year-old in 2007) was the least affected one - both from the point of view of mortality and disease symptoms development. The older stands suffered much more from the root rot. The reasons of the bad heath status of the stands in question nowadays are numerous. Norway spruce is a susceptible host to *Armillaria* and the species composition of the stands does not fit the forest site, frequent changes in water regime are a considerable stress factor, etc. Nevertheless, the most important factor seems to be inappropriate forest management in the past century, driven more by profit than by ecology. A possible role of the stands location within the disjunction in the range of Norway spruce (contested recently) is also considered.

Kolbudy Forest District is situated in middle north of Poland, near Gdańsk, in Pomerania region (considered earlier a Norway Spruce disjunction area).

The aim of the work was analysis of health condition of four Norway spruce stands affected by Armillaria in Kolbudy Forest District (Regional Directorate of the State Forests in Gdańsk) in the context of their location in spruce disjunction area. All the stands are situated on fresh mixed broadleaf forest site (according to Polish forest site classification), typical of Pomerania beech forest.

In 2007 the age, area, species composition and growing stock of the stands were as follows: A - 19 yrs: 2.61 ha, spruce 60% + larch 40%, growing stock 0.9, B - 62 yrs: 0.63 ha, spruce 80% + beech 20%, growing stock 0.7; C - 82 yrs: 1.25 ha, spruce 80% (locally: pine, beech, hornbeam), growing stock 0.5; D - 107 yrs: 2.47 ha, spruce 60% + pine 20% + beech 20%, growing stock 0.5.

From 2007 on the four stands were monitored for Norway spruce tree health condition (A, B and D were monitored in 2007, 2009 and 2010, and C was removed in 2008).

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

The monitoring was performed twice in 2007 (spring and autumn) and only in autumn in the following years, as the autumn symptoms were more distinct. The health status of trees was estimated according to a 5-grade scale (Tab. 1).

All the isolates of *Armillaria* spp. collected in the stands, identified with the Korhonen intersterility test (Korhonen, 1978), proved *A. ostoyae*.

The stands were affected by *Armillaria* to various extent. The youngest stand (17-year-old in 2007) was the least affected one - both from the point of view of mortality and disease symptoms development. The older stands suffered much more from the root rot.

The reasons of the bad heath status of the stands in question nowadays are numerous. Norway spruce is a susceptible host to *Armillaria* and the species composition of the stands does not fit the forest site, frequent changes in water regime and strong northern winds are a considerable stress factor, too. Nevertheless, the most important factor seems to be inappropriate forest management in the past century, driven more by profit than by ecology.

A possible role of the stands location within the Central European disjunction in the range of Norway Spruce is being contested recently (Boratyński, 2007), so it is hard to ascribe the poor health condition of the stands to the factor.

Table 1. Tree damage scale applied in the work

Damage degree	Trees under 20 years A	Trees over 20 years B C D
0	NO DISEASE SYMPTOMS	NO DISEASE SYMPTOMS
1	needles chlorotic, side shoot length reduced	defoliation up to 40%, white mycelium under bark, occasionally resin outflow
2	needles chlorotic to yellow, shorter; side shoot reduced and dying, occasionally resin outflows and/or mycelium fans	defoliation up to 60%, side shoot necrosis, resin outflows and mycelium fans
3	defoliation up to 75%, needles browning and dying, rhizomorphs, mycelium fans and resin outflows	defoliation up to 75%, needles brown and necrotic, rhizomorphs, mycelial fans and fruit bodies, numerous resin outflows and bark cracks
4	defoliation up to 90%, needles necrotic, main shoot dead	defoliation up to 90%, needles necrotic, main shoot dead, bark dropped off

Table 2. Percentage of trees in particular damage degrees in 2007-2010

Observation time	Damage degree (percent)					
	0	**I**	**II**	**III**	**IV**	**Lacking trees**
Stand A - 19 years (100 trees)						
Spring 2007	48	40	7	1	4	0
Autumn 2007	21	58	14	2	5	0
Autumn 2009	9	47	22	6	14	0
Autumn 2010	11	40	28	5	16	0
Stand B - 62 years (258 trees)						
Spring 2007	27	53	12	2	7	0
Autumn 2007	2	41	36	11	10	0
Autumn 2009	1	39	22	6	5	26
Autumn 2010	4	35	22	3	4	31
Stand C - 82 years (221 trees)						
Spring 2007	36	52	10	1	2	0
Autumn 2007	2	40	43	10	6	0
Autumn 2009	0	0	0	0	0	100
Autumn 2010	0	0	0	0	0	100
Stand D - 119 years (119 trees)						
Spring 2007	12	63	23	3	0	0
Autumn 2007	1	31	49	18	1	0
Autumn 2009	3	31	19	2	2	62
Autumn 2010	6	20	13	1	2	58

References

Boratyński A., 2007. The Central European disjunctions in the range of *Norway Spruce*. In: *Biology and Ecology of Norway Spruce* (Tjoelker M.G., Boratyński A., Bugała W. eds),. Springer, Dordrecht. 37-47.

Korhonen K., 1978. Interfertility and clonal size in the *Armillaria mellea* complex. *Karstenia* 18: 31-42.

Spread of *Heterobasidion irregulare* in eastern Canada towards northern natural forests of *Pinus banksiana*

G. Laflamme

Laurentian Forestry Centre, 1 055 du P.E.P.S., Québec, QC, Canada G1V 4C7.
Corresponding author: gaston.laflamme@RNCan-NRCan.gc.ca

Abstract. In eastern Canada, the forest pathogen *Heterobasidion irregulare* was first detected in southern Ontario in 1955. It was then reported under the name *H. annosum*. In 1989, we have found *H. irregulare* in the province of Quebec, near the Ontario border. The disease was there since 1981. During the following years, the disease has been found in few plantations in the same area. It has recently progress to Drummondville, Qc, North-East of the previous infection centres, as well as in eastern township. It is also progressing towards north with two infection centres at Saint-Jean-de-Matha, Qc. This last locality is not far from the natural stands of *Pinus banksiana* which extend from eastern Canada up to Yukon. Trials are being conducted to find out if fresh *P. Banksiana*'s stumps can be affected by the disease.

In eastern Canada, the forest pathogen *Heterobasidion irregulare*, known until recently under the name *H. annosum*, was first detected in 1955 on red pine (*Pinus resinosa*) at St. Williams (Lat. 42°40'N; Long. 80°24'W) in southern Ontario (Jorgensen, 1956). It was reported under the name *Fomes annosus* and the diseased plantation had been thinned 26 years before. Further north in Rockland (Lat. 45°33'N; Long. 75°17'W), near Ottawa, one of the oldest plantations of red pine in Ontario (planted in 1914) was thinned in 1938, 1951, 1961 and 1971; until age 67, mortality was confined to small suppressed trees (von Althen and Stiell, 1990). Thus the disease caused by *H. irregulare* was not present in the Rockland area when it was first detected at St. Williams. *H. irregulare* was detected in several neighbouring localities near St. Williams after 1955. But in 1968, the root disease was found in the Rockland region for the first time in this part of Ontario, at Larose forest (Sippell *et al.,* 1968). As that location was near the Ontario-Quebec border, I selected thinned red pine plantations in 1983 for annual inspection. The disease was found in 1989 for the first time in the province of Quebec; the diseased plantation had been thinned for the second time in 1981 (Laflamme and Blais, 1995) and the root disease was not present at that time. During the following years, *H. irregulare* was found in a few red pine plantations in the same area. The disease has recently spread to Drummondville (Lat. 45°53'N; Long. 72°29'W), northeast of the previous infection centres, as well as to the nearby Eastern Townships. *H. irregulare* is also progressing northward, with two infection centres in red pine plantations at Saint-Jean-de-Matha (Lat. 46°14'N; Long. 73°32'W). This last locality is not far from large natural stands of *Pinus banksiana* which extend from eastern Canada up to Yukon. Preliminary data from inoculation trials with *H.*

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

irregulare show that fresh *P. banksiana* stumps can be colonized by *H. irregulare* (M. Dumas, personal comm.). This pathogen probably colonized red pine stumps at St. Williams after the thinning in 1929. Eighty years later, it was found at Saint-Jean-de-Matha, 800 km north of St. Williams. At this rate, the risk of seeing an extension of this root disease in natural *P. banksiana* stands is very high. This extension of *H. irregulare* is related to forest activities.

References

Jorgensen E. 1956. *Fomes annosus* (Ft.) Cke. on red pine in Ontario. *Forestry Chronicle* 32: 86-88.

Laflamme G., Blais R. 1995. Détection du *Heterobasidion annosum* au Québec. *Phytoprotection* 76: 39-43.

Sippell W.L., Gross H.L., Rose A.H. 1968. "Ontario region". In: *Annual report of the Forest Insect and Disease Survey,* Department of Fisheries and Forestry Ottawa, ON 51-75.

von Althen F.W., Stiell W.M. 1990. A red pine case history: development of the Rockland plantation from 1914 to 1986. *Forestry Chronicle* 66: 606-610.

A first generation *Heterobasidion* hybrid discovered in *Larix lyalli* in Montana.

B. Lockman[1], S. Mascheretti[2], M. Garbelotto[2]

[1]*USFS, State and Private Forestry-Forest Health Protection, Missoula, MT, USA.*
[2]*Department of ESPM, University of California, Berkeley, CA, USA.*
Corresponding author e-mail address: mail: matteog@berkeley.edu

A sample was collected from heavily decayed roots of a downed but alive alpine larch (*Larix lyallii*) on September 25, 2010. The tree was among alpine larches that were either dead or were displaying thin and narrow crowns, indicating the possible presence of a root disease pocket. An older alpine larch root ball was also found with what appeared to be very old *Heterobasidion*-like fruiting bodies. Chips displaying a white rot with white pockets were placed from the decayed roots on 2% malt agar, and cultures displaying the typical *Heterobasidion* anamorph (*Spinickellus*) were visible after 7 days. The site (elev. 8,300 ft.) is along the shores of Gem Lake in the Bitterroot Mountains south of Darby, Montana. The stand is composed of alpine larch, whitebark pine and a few subalpine fir. DNA was extracted from two distinct cultures and the sequences of three loci, namely the Internal Transcribed Spacer, Elongation Factor alpha, and Glyceraldehyde 3-phosphate dehydrogenase were analyzed. Sequences of all three loci unequivocally indicated both isolates to be first generation hybrids between *H. irregulare* and *H. occidentale*. This is the second natural *Heterobasidion* hybrid ever discovered in North America, and indicates *L. lyalli* may be a host for both species, as described for pine stumps in California. In Europe, Interestingly, *Larix* is reported as a host for all three Eurasian *Heterobasidion* species.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

The length of decay column in Norway spruce stems infected by *Heterobasidion parviporum*

P. Łakomy[1], K. Flis[1], M. Glura-Molińska[2]

[1]*Poznan University of Life Sciences, Department of Forest Pathology.*
[2]*Poznan University of Medical Sciences, Department of Informatics and Statistics.*
Corresponding author e-mail address: plakomy@up.poznan.pl

Abstract. In this study the analysis of decay in stems of fifty 80-year-old Norway spruce infected by *Heterobasidion parviporum* were done. Each tree with visible decay at the cut surface were divided on 1.20 long section up to the end of decay. In addition the last section was split in length to measure the real end of decay column. The diameter of decay at the top and bottom of each section were measured and the decay volume was also calculated. Most of trees (60%) were probably infected through the wounds, which were done on the butt. The decay was spread up and down. The longest decay column reach 10.05 m in the stem and the shortest 0.2 m. Only in five Norway spruce stems decay column were longer than 5 m. The column length was multiplication of diameter from 3.53 to 25.84 (the longest column). In 30% cases the decay column were smaller than 1 m. The volume of decayed wood was more than 20 m^3, what equal 30.15% of the whole wood volume.

Heterobasidion parviporum occurs in the north-east and south part of Poland, in the whole range of Norway spruce appearance. This pathogen attacks mainly Norway spruce and sometimes colonizes European fir stumps or logs. *Heterobasidion parviporum* colonizes the hardwood and spread inside the stems up to 4-8 and even 12 meters (Bruchwald, 1984; Stenlid and Wästerlund, 1986).

The study (40 ha) was done in two 80-year-old mixed Norway spruce and Scots pine stands growing as a first generation in the post arable soil (cambic arenosol soil), in the south part of Poland (50°,65'N, 17°01'E).

Fifty trees were randomly chosen to the study from whole area of these stands. The size of decay and its length in spruce stems were analyzed. Each tree was cut on 1.2 m section and the decay was measured. In addition the last section was split in length to notice the decay tip. In this section the decay was assessed on the base of wood discoloration. The volume of tree and decay were calculated for separate tree. Moreover the volume of decay was calculated for each section.

In all chosen spruces the decay was found after felling. In laboratory *Heterobasidion parviporum* was proved of decay reason. In addition sporocarps of pathogen were found in stands, but only on old decayed stumps. Most of trees (60%) were infected through wounds, which were done on the butt, probably during the thinning operations in the past. The length of decay in spruce stems varied from 20 cm to 1,005 cm (Fig. 1). In 85% of cases the decay column didn't exceed 5 meters of stems length and in this group 40% of decays didn't exceed 1 m (51% of all analyzed decay). The longest decay was found in 15% of spruce' stems. In average the decay reach the 15% of trees' height and the tip of maximal

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

decay was found in half of the tree height. The diameter of decay in root collar varied from 4 cm to 42 cm, what makes 5% and 85% of stem surface. In forty percent of trees the decay covered more than 50% surface of root collar.

The decay volume in all analyzed trees was calculated on 3,2 m^2. The biggest decay was amounted on 37% of stem volume. Similar decay was found in 10% of analyzed stems. The losses of high quality timber was also calculated. Average losses in all 50 trees was amounted to 30% and varied from 12% to 67%. The volume of decayed wood was more than 20 m^3, what equal 30.15% of the whole wood volume of analyzed trees. The relationships between width of decay and its length was confirmed ($P< 0.05$, $r= 0.78$). The decay length was higher than diameter in 3.53-25.84 times, in average 10.

This study is an example of very high impact of *Heterobasidion parviporum* in Norway spruce stand growing as a first generation of forest on the post agricultural land. The analyze of decay column was similar with other studies in Europe (e.g. Stenlid and Wästerlund, 1986).

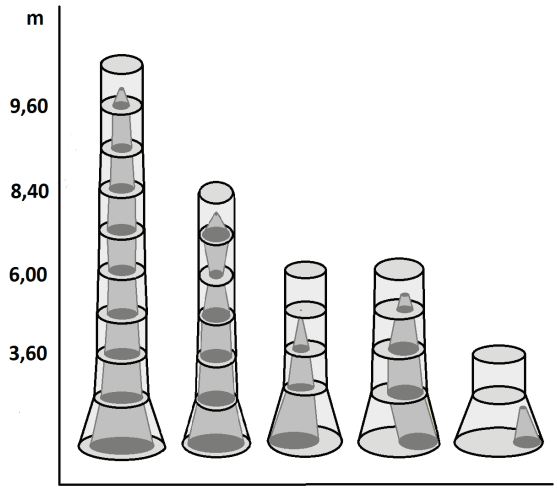


Figure 1. Diagram of trees representing different decay development

References

Bruchwald A. 1984. Estimation of attacking spruce trees by root rot (*Fomes annosus* Fr.) in spruce-pine stands of Puszcza Romnicka. Annals of Warsaw Agricultural University SGGW-AR, *Forestry and Wood Technology* 32: 7-11 (in Polish, English summary).

Stenlid J., Wästerlund I. 1986. Estimating the frequency of stem rot in *Picea abies* using and increment borer. *Scandinavian Journal of Forest Research* 1: 303-308.

Reaction zone and sapwood reduction in Norway spruce(*Picea abies*) attacked by *Heterobasidion annosum*

J. Oliva[1], J.J. Camarero[2], J. Stenlid[1]

[1]*Dept. Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026 750 07 Uppsala, Sweden.*
[2]*ARAID, Instituto Pirenaico de Ecología, CSIC. Avda.Montañana, 1005 Zaragoza E-50192, Spain.*
Corresponding author e-mail address: jonas.oliva@slu.se

Heterobasidion annosum causes butt rot and root rot toNorway spruce (*Picea abies* (L.) Karst.) trees. Trees with decay in the stem often present a lower growth than healthy neighbours (Bendz-Hellgren and Stenlid, 1995; Oliva *et al*., 2010).

Norway spruce trees createin the sapwood a reaction zone with fungistatic properties in order to compartmentalize decayed tissues (Shain, 1971). Building a reaction zone has energy costs that could explain up to 50% of growth reductions. Reaction zone may not fully prevent *Heterobasidion* advance towards sapwood. As the fungus progresses, it depletes the reaction zone, and the tree reacts converting more sapwood into reaction zone.

As a result, while the fungus increases the size of decay column, the sapwood of the tree becomes narrower. Narrow sapwood may reduce the capacity of the tree to pump water from the roots to the crown with negative effects on tree growth. As opposed to the role of the reaction zone, the observed association between decay and lower growth could be due to the effect of the fungus reducing the sapwood and therefore strangulating the tree. In order to test whether reaction zone of sapwood reductions were responsible of reduced growth in infected trees, we reconstructed the radial growth of 101 trees during the period 1980-2007.

The amount of sapwood, heartwood, reaction zone and decay in the section was measured at stump level and at breast height. The association between decay and growth was studied by structural equation models (SEM). The SEM model with the highest fit supported an indirect effect of decay on tree growth mediated by the formation of reaction zone.

Decay progression was associated with a smaller sapwood size, but a negative effect of sapwood size on tree growth was poorly supported. The best fit was obtained when sapwood size was incorporated in the model as a result of a low growth period.

Our observations suggest that once Norway spruce trees respond to decay by creating a reaction zone, they derive photosynthates that otherwise would have

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

been invested in radial growth. In the long run, the resulting reduction in radial growth translates into a smaller sapwood size.

References

Bendz-Hellgren M., Stenlid, J. 1995. Long-term reduction in the diameter growth of butt rot affected Norway spruce, *Picea abies*. *Forest Ecology and Management* 74: 239-243.

Oliva J., Thor M., Stenlid J. 2010. Reaction zone and periodic increment decrease in *Picea abies* trees infected by *Heterobasidion annosum* s.l. *Forest Ecology and Management* 260: 692-698.

Shain L. 1971. The response of sapwood of Norway spruce to infection by *Fomes annosus*. *Phytopathology* 61: 301-307.

Spatial distribution of *Heterobasidion abietinum* genets on *Abies cilicica* in a mixed stand

A. Lehtijärvi, H.T. Doğmuş-Lehtijärvi, A.G. Aday, F. Oskay

Süleyman Demirel University, Faculty of Forestry, 32260 Isparta, Turkey.
Corresponding author e-mail address: askolehtijarvi@sdu.edu.tr

Abstract. *Heterobasidion abietinum* is the dominant species in the genus on *Abies* species in Turkey. We investigated the spatial distribution of the genets of *H. abietinum* in a 20 × 50 m^2 sample plot in a mixed, thinned stand consisting of *Abies cilicica* (45%), *Cedrus libani* (15%) and *Pinus nigra* ssp. *pallasiana* (40%) with mean diameters of 42.1, 26.0 and 52.8 cm, respectively. All standing and windthrown firs were sampled with increment bore from one side at the butt. From stumps, samples were taken with the bore only if basidiocarps could not be found. All the obtained isolates were heterokaryons and identified as *H. abietinum* in pairings with known homokaryotic tester strains. In heterokaryon × heterokaryon pairings totally six genets were found among the isolates; while each of five genets occupied a single standing or windthrown tree one genet was found to have colonized three adjacent stumps. The frequencies of the fungus on living trees, stumps and windthrows were 33, 100 and 50%, respectively.

Heterobasidion abietinum (Fr.) Niemelä & Korhonen is the dominant species in the genus on *Abies* species in Turkey (Doğmuş-Lehtijärvi *et al.*, 2006). Disease incidence on freshly-cut stumps of approximately 90-year-old *A. cilicica* (Ant. & Kotsch.) Carr. and 75- and 120-year-old *A. bornmülleriana* Mattf. was 11.5, 18.8 and 28.2%, respectively (Doğmuş-Lehtijärvi *et al.,* 2008). As basidiocarps of the fungus are abundant on stumps (Doğmuş-Lehtijärvi *et al.*, 2006) and logging is performed predominantly during the growing season when the weather promotes sporulation and infection the disease incidence can be expected to increase in the future. In the present study, we investigated the spatial distribution of the genets of *H. abietinum* in a 20 × 50 m^2 sample plot in a mixed, thinned stand consisting of *A. cilicica* (43%), *Cedrus libani* A. Rich (14%), *Pinus nigra* Arn. ssp. *pallasiana* (Lamb.) Holmboe (38%) and *Juniperus excelsa* L. with mean diameters at breast height (DBH) of 42.1, 26.0, 52.8 and 30.0 cm, respectively. The study site was located approximately 1,475 m above sea level in Konya province in southwestern Turkey. The bedrock consisted mainly of hard, recrystallized Mesozoic limestone (Özkan *et al.*, 2010). The forests were managed by selective cuttings. The prevailing climate in the study area is a transitional one between the Mediterranean and Continental climates characterized by a strong water deficit in summer (Özkan *et al.*, 2010). Mean annual temperature and precipitation are approximately 11°C and 800-1,000 mm, respectively.

All standing firs with DBH larger than 20 cm were sampled with an increment bore from one side at the butt (approximately 30 cm above ground). From fir stumps and logs, samples were taken with the bore only if basidiocarps could not

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

be found. The bore was cleaned with 70% ethanol before each sampling. The samples were placed into sterile test tubes and kept in a cold box. On the same day, in the laboratory, the wood samples were flamed briefly and placed on Petri dishes containing 2% malt extract agar. All the obtained isolates were heterokaryons and identified as *H. abietinum* in pairings with known homokaryotic tester strains. In heterokaryon × heterokaryon pairings totally five genets were found among the isolates. Two of the genets occurred on a single standing tree, one on a stump and a neighboring tree, and the remaining two genets occupied a stump-stump and a stump-log domain, respectively. The infection frequencies of the fungus on living trees, stumps and logs were 33, 100 and 50%, respectively.

References

Doğmuş-Lehtijärvi H.T., Lehtijärvi A., Korhonen K. 2006. *Heterobasidion abietinum* on *Abies* species in western Turkey. Forest Pathology 36: 280-286.

Doğmuş-Lehtijärvi H.T., Lehtijärvi A., Oskay F., Aday A.G., Karadeniz M., 2008. Annosum kök ve alt gövde çürüklüğünün *Abies bornmülleriana* ve *Abies cilicica* meşcerelerinde yoğunluğunun belirlenmesi. Artvin Çoruh Türkiye I. Orman Entomolojisi ve Patolojisi Sempozyumu 23-25 Kasım 2011 - Antalya Universtity, Faculty of Forestry Journal 9: 111-120. (in Turkish, English abstract).

Özkan K., Gulsoy S., Mert A., Ozturk M., Muys B. 2010. Plant distribution - altitude and landform relationships in karstic sinkholes of Mediterranean region of Turkey. *Journal of Environmental Biology* 31: 51-60.

Armillaria ostoyae associated with dying sixty-year-old Scots pines in northern Turkey

A. Lehtijärvi, H.T. Doğmuş-Lehtijärvi, A.G. Aday, F. Oskay

Süleyman Demirel University, Faculty of Forestry, 32260 Isparta, Turkey.
Corresponding author E-mail: askolehtijarvi@sdu.edu.tr

Armillaria root disease can cause significant damage in commercial forests. We investigated a disease center in a sixty-year-old, naturally regenerated *Pinus sylvestris* stand located at 1,250 m altitude in Sinop province in the Black Sea region of Turkey. The position of each tree within a 30×70 m plot was mapped, the breast height diameter measured and the health status of the tree noted. The bark at the butt of symptomatic, dying and dead trees was removed and samples taken from the mycelial fans when present. Based on sequence analysis of the internal transcribed spacer (ITS) region of the rDNA the mycelial fans belonged to *Armillaria ostoyae*, which is a primary pathogen and frequently encountered on *P. nigra* and *P. sylvestris* in southern Europe. Randomly amplified microsatellite sequence (RAMS) analysis indicated that the fans belong to a single genet. There was no extreme long-term drought in the province during the three preceding years that would have weakened the trees. However, weather in the study area in May, June and August in 2009 as well as in August and September in 2010 was dry with 50-70 and 25-50% of the normal precipitation, respectively.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Session 6

Disease Management and Control

Immuno-fluorescence approach to distinguish *Phlebiopsis gigantea* hyphae from the conifer pathogen *Heterobasidion annosum* with confocal microscope

F.O. Asiegbu

Department of Forest Sciences, University of Helsinki, Finland.
Corresponding author e-mail address: fred.asiegbu@Helsinki.fi

Abstract. The saprotrophic fungus *Phlebiopsis gigantea* has for several years been used as a biocontrol agent against the conifer pathogen (*Heterobasidion annosum*). However, very little is known about the mechanism behind *P. gigantea* mode of action. At cellular level, there have been suggestions that hyphal interference may be involved during the combative interaction. A major challenge for basic cellular understanding of the mechanistic basis for the antagonistic interaction is the lack of suitable tools to distinguish the hyphae of these two fungi in vivo. The present study explored the feasibility of using a combined dual immunofluorescence labelling and confocal microscopy for detection of the two fungal species. Over 48 monoclonal antibodies were screened using ELISA (Enzyme Linked Immunosorbent Assay) for their ability to specifically recognize hyphae of either *P. gigantea* or *H. annosum*. Two monoclonal antibodies met these criteria and were further investigated. The result revealed that with the aid of the dual immune-fluoresence labeling technique it was possible to distinguish the hyphae of the two fungi within the barrage zone. Using this approach, we found that a potential mechanism for the superior antagonistic capability of *P. gigantea* over *H. annosum* is by hyphal thinning. The potential application of this method for mechanistic understanding of the interaction at both cellular levels and during competitive growth in wood is discussed.

Introduction

The wood decay fungus *Phlebiopsis gigantea* has been used for several years as the sole biocontrol agent against the conifer pathogen (*Heterobasidion annosum*) (Rishbeth, 1952; Holdenreider and Greig 1998; Asiegbu *et al.,* 2005). However, very little is known about the molecular mechanism of *P. gigantea* biocontrol activity. Unlike other biocontrol agents (e.g. *Trichoderma viride*), much of what is known about mode of action of *P. gigantea* is largely hypothetical. Hyphal interference was reported by Ikediugwu (1976) and Adomas *et al.* (2006) observed a comparatively high transcript levels of genes important for nutrient acquisition with *P. gigantea*. Recently, Sun *et al.* (2011) reported induced resistance as a possible mechanism for the efficacy of the biocontrol agent. No formation of inhibition zone has however been reported. An additional problem is that within woody tissues it can be difficult to differentiate hyphae of the two fungal species. This further complicates mechanistic understanding of the interaction at cellular level. The primary objective of this study was to use a combination of monoclonal

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

antibody technique and confocal microscopy for specific distinction of *H. annosum* and *P. gigantea* hyphae in vitro.

Materials and Methods

Dual labellling by Wheat germ agglutinin, WGA-FITC and goat anti-mouse IgG conjugated to Texas-Red Technically, the Hagem (Stenlid 1985) agar glass slides containing both fungi were rinsed in PBST for 10 minutes. Thereafter, immersed in 0.2% bovine serum albumin (BSA) + 1% normal goat serum for 30 minutes in PBST. Then, rinsed 2× in PBST for 15 minutes. Monoclonal antibody specific for either H. annosum hyphae diluted 1:5 in PBST-BSA was applied and incubated at 4°C. Thereafter rinsed 3× in PBST for 30 minutes. This was followed by additional labelling with Texas red goat antimouse (2 mg ml^{-1}, molecular probes, T-862) (1:80) together with non-specific WGA-FITC (Sigma, L-4895) (1:80) mixed in equal volumes and applied to the glass slides. Incubated for 1 hr at 20°C. Rinsed 3× in PBST and examined under confocal microscope (Zeis LSM 510) at excitation 535/emission 610 for Texas Red and BP 450-490/RKP 510 for FITC. In this dual labelling, WGA-FITC which binds to chitin on fungal cell walls will label both hyphae of the two fungi and will emit greenish fluorescence. But the goat anti-mouse IgG conjugated to Texas Red will bind to MAb (BC-AB9) specific only for *H. annosum* and will give a reddish fluorescence.

Results and Discussion

The interaction between *P. gigantea* and *H. annosum* on surface Hagem agar cultures was found to be a deadlock and independent of inoculum size. The same observation was made on samples grown on Hagem agar glass slides. For convinience and ease of identification, all dual labelling samples were conducted on samples grown on glass slides. Differential screening with ELISA led to identification of two MAb, BC-AB9 and SN-EB8 that were specific and have high affinity for *H. annosum* and *P. gigantea* hyphae respectively. Direct FITC-labelling using the BC-AB) antibodies confirmed binding to *H. annosum* hyphae. Intense labelling of *P. gigantea* hyphae was equally documented following labelling with SN-EB8 antibody. In the dual labelling experiment (Fig. 1), images were documented from different sections of the glass slide classified as a) *P. gigantea* inoculum side or niche b) *H. annosum* inoculum side or niche c) *H. annosum* region close to interaction zone d) *P. gigantea* region close to interaction (barrage) zone and e) the interaction (barrage) zone where both hyphae meet. With dual labelling with WGA-FITC + anti-mouse-Texas Red, *H. annosum* hyphae was not detected in *P. gigantea* niche (Fig. 1) but *P. gigantea* hyphae was visible. Similarly, *H. annosum* hyphae was found to be visible in *H. annosum* niche (Fig. 1; red channel). Traces of *P. gigantea* hyphae was found to be visible within *H. annosum* area close to barrage or interaction zone but the most dominant hyphae in

this region originated from *H. annosum*. On the other hand, traces of *H. annosum* hyphae was detected within *P. gigantea* area close to barrage zone but the most predominant hyphae originated from *P. gigantea*. Furthermore, at the barrage zone, *P. gigantea* hyphae was found to dominate with only traces of *H. annosum* hyphae documented (Fig. 1). Morphologically, we documented some changes in *H. annosum* hyphae during contact with *P. gigantea* mycelia. One of the major morphological changes is thinning of *H. annosum* hyphae growing in the midst of dense *P. gigantea* mycelia. These antibodies could have potential practical application for specific distinction of the hyphae of *P. gigantea* and *H. annosum*. Understanding the molecules which the antibody binds to will hopefully facilitate protein tagging and its consequent application in environmental monitoring.

Acknowledgements

Prof. Sarah Gurr and Dr. Molly Dewey are gratefully acknowledged for making available monoclonal antibodies.

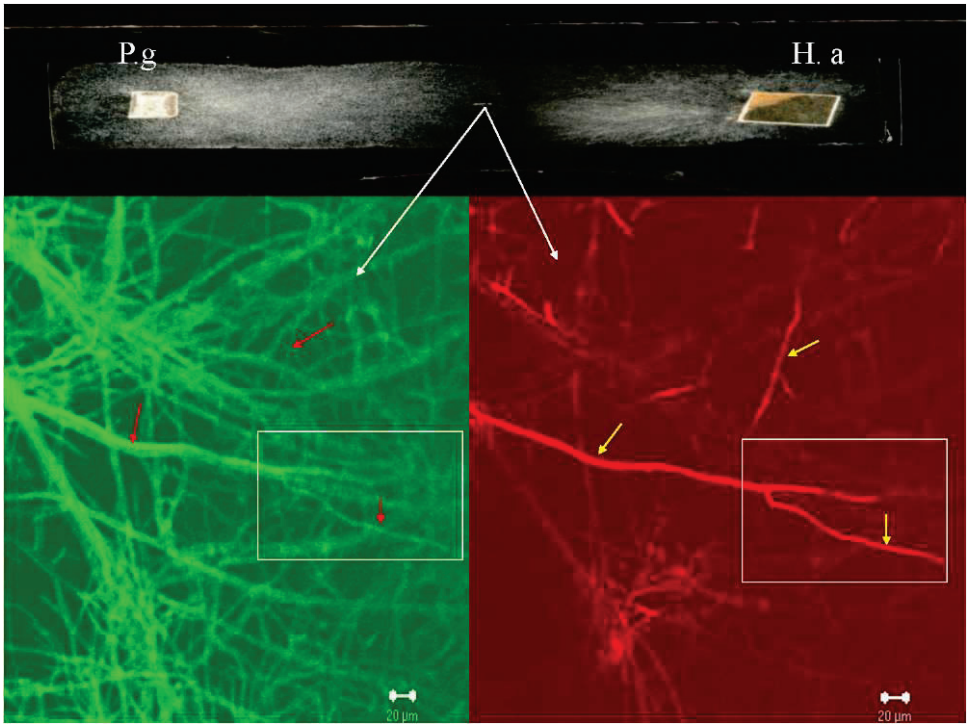


Figure 1. Dual labelling by Wheat germ agglutinin (WGA-FITC) and goat anti-mouse IgG conjugated to Texas-Red. Both *P. gigantea* (P.g) and *H. annosum* (H.a) hyphae under confocal microscope had greenish fluorescence. Only *H. annosum* hyphae (arrows) was visible in the red channel.

References

Adomas A., Eklund M., Johansson M., Asiegbu F.O., 2006. Identification and analysis of differentially expressed cDNA's during non-self competitive interaction between *Phlebiopsis gigantea* and *Heterobasidion parviporum*. *FEMS Microbiology Ecology* 57: 26-39.

Asiegbu F.O., Adomas A., Stenlid J., 2005. Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. s.l. *Molecular Plant Pathology* 6: 395-409.

Holdenrieder O., Grieg B.J.W., 1998. Biological methodes of control. In: Woodward S., Stenlid J., Karjalainen R., Hüttermann A. (eds.). *Heterobasidion annosum* Biology, Ecology, Impact and Control,. CAB International, Wallingford, Oxon OX10 8DE, UK. pp. 235-258

Ikediugwu F.E.O., 1976. The interface in hyphal interference by *Peniophora gigantea* against *Heterobasidion annosum*. *Transactions of the British Mycological Society* 66: 291-296.

Rishbeth J., 1952. Control of *Fomes annosus*. *Forestry* 25: 41-50.

Stenlid J., 1985. Population structure of *Heterobasision annosum* as determined by somatic incompatibility, sexual incompatibility and isozyme patterns. *Canadian Journal of Botany*. 63: 2268-2273.

Sun H., Paulin L., Alatalo E., Asiegbu F.O., 2011. Response of living tissues of *Pinus sylvestris* to the saprotrophic biocontrol fungus *Phlebiopsis gigantea*. *Tree Physiology*. 31: 438-451.

Effects of biocontrol agent (*Phlebiopsis gigantea*) against *Heterobasidion annosum* on the bacterial communities of *Pica abies* stumps

H. Sun[1], E. Terhonen[1], K. Koskinen[2], L. Paulin[2], R. Kasanen[1,3], F.O. Asiegbu[1]

[1]*Department of Forest Sciences, University of Helsinki, P.O. Box 27, FIN-00014 Helsinki, Finland.*
[2]*DNA Sequencing and Genomics Lab, Institute of Biotechnology, University of Helsinki, P.O. Box 56, FIN-00014 Helsinki, Finland.*
[3]*Finnish Forest Research Institute (Metla), Vantaa Research Unit, P.O. Box 18, FIN-01301 Vantaa, Finland.*
Corresponding author e-mail address: hui.sun@helsinki.fi

Abstract. The biocontrol agent *Phlebiopsis gigantea* has been intensively applied on the surface of *Picea abies* stumps against *Heterobasidion* root rot. However, very little is known on the possible impact of such treatment on the resident bacteria community in the stumps. We have for the first time used high throughput DNA bar-coded pyrosequencing to characterize the diversity as well as the successional dynamics of bacteria in the stumps of *P. abies* after 1-, 6- and 13-year post treatment with *P. gigantea*. The sequences were classified into 12 bacterial phyla and 160 genera, in which *Proteobacteria* and *Acidobacteria* were the most abundant groups in the stumps. Successionally, *Proteobacteria* were the most abundant at the initial stages of stump decay but were selectively replaced by *Acidobacteria* at advanced stages of the decay. Treatment with *P. gigantea* led to significant increase of the genus GP1after 1-year treatment. The analysis of observed and estimated OTUs as well as diversity indices revealed that *P. gigantea* treatment significantly decreased the bacterial richness at initial decay stage in the stumps. Over time, the bacterial community in the stumps gradually recovered and the negative effects of *P. gigantea* was attenuated. These results provide additional insight on the risk assessment as well as environmental impact on the long-term use of *P. gigantea* in the control of *Heterobasidion* root rot in conifer forests.

Root rot caused by *Heterobasidion annosum* sensu lato (Fr.) Bref. is one of the most destructive diseases of coniferous trees in the Northern Hemisphere (Asiegbu *et al.*, 2005). Protection against *Heterobasidion* has been focused on preventing germination and growth of the fungus after spore deposition on the stump surface. Many methods have been used to prevent the infection, such as silviculture, stump removal, chemical and biocontrol methods. For biocontrol method, *Phlebiopsis gigantea* is currently used as a biological control agent and has been intensively applied on the stumps of conifer trees. Large-scale applications of a biological control agent in forest ecosystems may involve a potential risk and could have adverse impact on non-target organisms. However, the effect of these treatments on non-target organisms has received little attention. None has examined the influence of Rotstop *P. gigantea* (commercial product manufactured in Finland) on the

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

bacterial community in conifer stumps. In this study, the high-throughput bar-coded Titanium pyrosequencing was used (Malausa *et al.*, 2011) to amplify the hyper-variable regions of the 16S rRNA gene (V1-V3), to analyze the composition and diversity of the bacterial communities on *P. abies* stumps with or without pre-treatment by the Rotstop *P. gigantea* and to evalutate the impact of such treatment on the bacterial community over time (1, 6 and 13 years after treatment; site 1, 2 and 3).

The rarefaction curves indicated that estimates of OTUs increased with the number of sequences in all samples. However, the sequencing effort was not large enough to capture the complete diversity of these communities, as the curves did not reach the plateau phase. The number of observed OTUs in treated stumps differs from that in control stumps within each site (Tab. 1). At a genetic distance of 3%, the Shannon index showed high bacterial diversity across all the samples, and ranged from 5.1 to 6.9. The highest bacterial diversity was found at site 3, followed by site 2 and site 1. Within site 1 and site 3, the bacterial diversity in treated stumps was lower than that in control stumps. Moreover, the number of observed and estimated OTUs as well as Shannon index between the three replicates (individual sampled stumps) from each treatment showed high variation within each site.

The number of unique OTUs in both treated and control stumps increased over time along with the decay process, except the lower number on the control stumps after 6 years. The numbers of unique OTUs after 1, 6 and 13 years (corresponding to initial, intermediate and advanced decay) in treated and control stumps were 1,608 and 1,931, 1,921 and 1,011, 2,423 and 3,017, respectively.

The 123,562 sequences were classified below domain level, 92% of the total sequences were classified into bacterial phyla and were affiliated to 12 bacterial phyla, 24 classes and 160 genera. The most abundant phyla were *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Cyanobacteria* and *Actinobacteria* (Fig. 1). Analysis of the *Acidobacteria* phylum revealed dominance of the genus *Acidobacteria_Gp1* in all treatments. The pattern of succession of the bacterial groups differed between the stages of decay. In treated stumps, the *Proteobacteria* population declined with time whereas *Acidobacteria* increased. The abundances of the bacterial group at the initial stage of decay was as follows: *Proteobacteria* > *Acidobacteria* > *Bacteroidetes* > *Cyanobacteria* > *Actinobacteria*, intermediate stage of decay: *Proteobacteria* > *Acidobacteria* > *Actinobacteria* > *Cyanobacteria* > *Bacteroidetes* and advanced stage of decay: *Acidobacteria* > *Proteobacteria* > *Actinobacteria* > *Cyanobacteria* > *Bacteroidete* (Fig. 1). In control stumps, the successional pattern of abundance for each bacterial group was similar to that in treated stumps between year 1 and year 6, but differences were observed for both *Proteobacteria* and *Acidobacteria* between year 6 and year 13.

The populations of bacteria in woody substrates may vary in size and may be influenced by the abiotic environment within the wood as well as by the presence or absence of other microorganisms (Woods *et al*., 2006). The right environmental conditions (such as vegetation and location) seem to be essential for bacteria to shape the local diversity in terms of richness and abundance (Teixeira *et al*., 2010). The differences in the number of OTUs and diversity as well as structure of bacteria between replicates within each site suggest that even at the same site, the within-stump environments could differ from each other and might have a strong influence on the bacterial community structure. Therefore, to fully understand how the bacterial communities in the stumps are influenced by *P. gigantea*, it is also necessary to draw a bigger picture, in which all the factors involved in the decay process and affected by *P. gigantea* are taken into account.

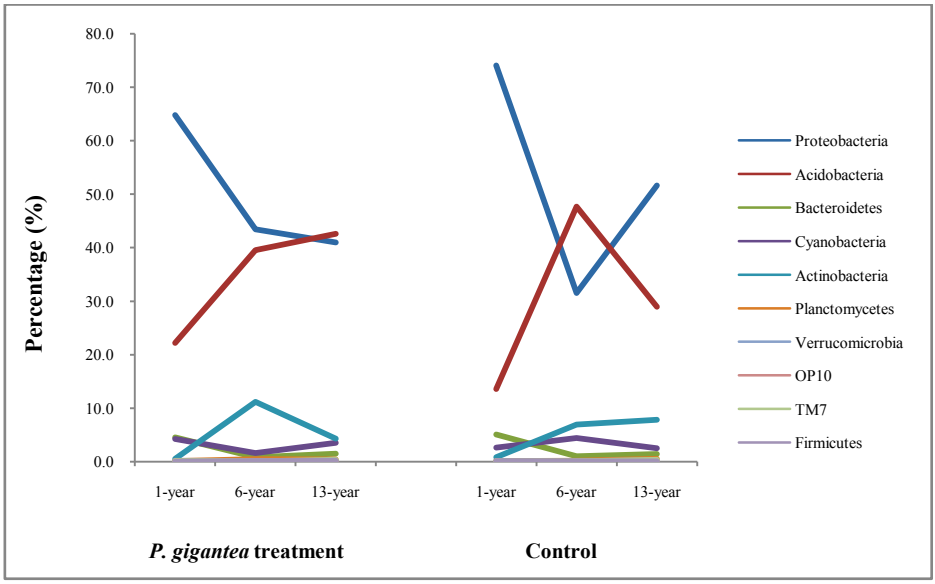


Figure 1. Successional changes of the relative abundance of bacterial phylum in *P. gigantea* treated and control stumps over time

Table 1 Observed and estimated OTU richness and diversity index for 16S rRNA gene libraries of samples from *P. abies* stumps after *P. gigantea* treatment at three different sites.

Sample	Replicate	No. of sequence	shannon index	Observed OTUs	Chao 1	ACE
site 1 treatment	1	4386	5.3	847	2206	3169
	2	6469	6.0	1308	2709	4129
	3	6946	5.3	953	1976	2928
site 1 control	1	5154	5.8	1056	2536	4084
	2	5896	6.2	1436	3897	6082
	3	6174	5.9	1233	2957	4503
site 2 treatment	1	5703	6.0	1283	3077	4960
	2	8454	6.5	1852	4121	6185
	3	8185	5.5	1171	2497	3748
site 2 control	1	7499	5.6	1041	2217	3242
	2	7322	6.1	1339	2577	3699
	3	5799	5.1	665	1224	1535
site 3 treatment	1	8649	6.0	1514	3370	4778
	2	7384	6.1	1344	3400	5120
	3	7365	6.9	2103	3537	4980
site 3 control	1	8902	6.7	2075	4676	6551
	2	6953	6.5	1880	4422	6930
	3	6323	6.7	1903	4563	7122

OTUs were calculated with Mothur at the 3% genetic distance level

References

Asiegbu F.O., Adomas A., Stenlid J. 2005. Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. sl. *Molecular Plant Pathology* 6: 395-409.

Malausa T., Gilles A., Meglécz E., Banquart H., Duthoy S., Costedoat C., Dubut V., Pech N., Castagone-Sereno P., Délye C., 2011. High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. *Molecular Ecology Resources* DOI: 10.1111/j.1755-0998.2011.02992.x

Teixeira L.C R.S., Peixoto R.S., Cury J.C., Sul W.J., Pellizari V.H., Tiedje J., Rosado A.S., 2010. Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. *The ISME Journal* 4: 989-1001.

Woodward S., Stenlid J., Karjalainen R., Hüttermann A., 1998. *Heterobasidion annosum* Biology, Ecology, Impact and Control. CAB International, Wallingford,UK.

A field trial testing *Phlebiopsis gigantea* as a biocontrol agent for Heterobasidion root disease in the southeastern United States

S. Covert[1], J. Brown[1], M. Cram[2]

[1]*Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA, 30602 USA.*
[2]*USDA Forest Service-FHP, Athens, GA, 30602 USA.*
Corresponding author e-mail address: covert@uga.edu

Abstract. Concern over Heterobasidion root disease has been on the rise in the southeastern United States in recent years. To determine if a *Phlebiopsis gigantea* isolate from Alabama is capable of controlling *H. irregulare* infections in the field, even if *P. gigantea* application is delayed until several days after thinning, loblolly pine stumps were inoculated with *H. irregulare* and treated with *P. gigantea* 0, 3 or 7 days later. The treatments were applied to 100 stumps in a blocked design at each of 6 different sites. Three to four months after the stumps were treated, disks were cut from each stump and the amount of *H. irregulare* colonization in each was recorded after 7 days incubation in the lab. The results for all disks, across all sites, were analyzed with a linear mixed-effects model, with treatment included as a fixed effect while block and site were considered random effects. The Day 0 and Day 3 *P. gigantea* treatments both significantly reduced *H. irregulare* colonization relative to the no-*P. gigantea* control treatment at all sites except for one, which had very low *H. irregulare* colonization in its control stumps. The Day 7 *P. gigantea* treatments were inconsistent, having a significant effect on *H. irregulare* colonization at only half of the sites. These results suggest that *P. gigantea* stump treatments can be used to control *Heterobasidion* root disease in the southeastern U.S., even if application of *P. gigantea* occurs 3 days after a thinning, thus enabling its safe application once harvesting machinery has left the forest.

As mid-rotation thinning of pine plantations has become a common practice in the southeastern United States (U.S.), concern over *Heterobasidion* root disease has been on the rise. In Europe, spore suspensions of the wood decay fungus *Phlebiopsis gigantea* are routinely applied to fresh stumps to control Heterobasidion root disease, but in the U.S., the Environmental Protection Agency (EPA) has not approved the use of *P. gigantea* for this purpose. Specialized sprayers mounted on logging equipment have made immediate stump treatment with *P. gigantea* a standard practice in Europe (Jim Pratt, UK Forestry Commission Research Agency, personal communication). In the southeastern U.S., logging techniques and some market constraints dictate that *P. gigantea* stump treatments will likely be performed manually, a few days after the harvesting equipment has completed a thinning. For this reason, it is important that *P. gigantea* product designed for use in the southeastern U.S. can effectively control Heterobasidion Root Disease even if it is applied 3-7 days after a thinning.

Our long term goal is to develop a *P. gigantea* biocontrol product adapted to the specific environmental conditions and harvesting practices common in the

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

southeastern U.S. Towards that end, we identified several *P. gigantea* isolates from this region that were capable of limiting *H. irregulare* colonization of loblolly pine in the laboratory. One of the top performers in these experiments was chosen for further testing in thinned pine plantations. In addition to assessing how well this isolate controlled *Heterobasidion* root disease in the field, the experiment was designed to determine if delayed application of *P. gigantea* impaired its function as a biocontrol agent.

Loblolly pine stumps were inoculated with *H. irregulare* and treated with *P. gigantea* 0, 3 or 7 days later. The treatments were applied to 100 stumps in a blocked design at each of 6 different sites. Three to four months after the stumps were treated, disks were cut from each stump and the amount of *H. irregulare* colonization in each was recorded after 7 days incubation in the lab. The results for all disks, across all sites, were analyzed with a linear mixed-effects model, with treatment included as a fixed effect while block and site were considered random effects. The Day 0 and Day 3 *P. gigantea* treatments both significantly reduced *H. irregulare* colonization relative to the no-*P. gigantea* control treatment at all sites except for one, which had very low *H. irregulare* colonization in its control stumps. The Day 7 *P. gigantea* treatments were inconsistent, having a significant effect on *H. irregulare* colonization at only half of the sites. These results suggest that *P. gigantea* stump treatments can be used to control Heterobasidion root disease in the southeastern U.S., even if application of *P. gigantea* occurs 3 days after a thinning, thus enabling its safe application once harvesting machinery has left the forest.

Penicillium adametzii as a possible biological control agent against *Armillaria* and *Heterobasidion*

H. Kwaśna, L. Szwajkowska-Michałek, P. Łakomy, J. Perkowski

Poznań University of Life Sciences, Forest Pathology Department, Ul. Wojska Polskiego 71 c, 60-625 Poznań, Poland.
Corresponding author e-mail address: kwasna@up.poznan.pl

Abstract. *Penicillium adametzii* seems to be confined to central Europe; to the soil habitat, usually of living trees. It is one of the most commonly occurring fungi under coniferous (*Pinus sylvestris*) and deciduous (*Betula pendula*, *Larix decidua*, *Prunus serotina*, *Quercus robur, Q. petraea*) tree stands, in fallow soils and in barren post-agricultural soils in Poland. It also colonizes the surface of roots. The presence of coniferous wood eliminates the fungus. In paired culture, *P. adametzii* inhibited the growth of *Heterobasidion annosum* and formed extremely wide inhibition zone. Chloroform extracts from its cultures inhibited growth of *Armillaria* and *Heterobasidion in vitro.* The effect seemed to be strain specific and depended on metabolite concentration. *Armillaria gallica*, *A. ostoyae, H. abietinum* and *H. parviporum* were the most responsive. Mycelium of *P. adametzii* and the chloroform extract applied in water improved the health and stimulated growth of 2-year-old *P. sylvestris* plants inoculated or not inoculated with *Armillaria,* and inhibited necrosis caused by *H. annosum.* Higher concentrations of metabolites increased the effect. *Penicillium adametzii* metabolites were analysed. Twenty fractions from the *F. adametzii* chloroform extract were separated by TLC. Only four fractions inhibited growth of *Armillaria* and four different fractions inhibited growth of *H. abietinum* or *H. annosum in vitro*. None of the separate compounds inhibited growth of *H. parviflorum.* Carboxylic acids, glycosides and flavonoids were detected from mass spectrum analyses of the chloroform extract. This is the first report on interactions between *Armillaria, Heterobasidion, P. sylvestris* and *P. adametzii*.

Penicillium adametzii Zaleski was isolated for the first time from soil under conifers, near Poznań (52.46693N, 16.92549E) in Poland by Zaleski (Zaleski, 1927). The fungus seems to be confined to central Europe; to the soil habitat, usually of living trees. It is one of the most commonly occurring fungi under coniferous (*Pinus sylvestris*) and deciduous (*Betula pendula*, *Larix decidua*, *Prunus serotina*, *Quercus robur, Q. petraea*) tree stands, on the surface of tree roots, in fallow soils and in barren post-agricultural soils in Poland (Kwaśna, 1995), (Tab. 1). The presence of dead coniferous wood usually eliminates *P. adametzii* from soil (Sierota and Kwaśna, 1999; Kwaśna, 2000), (Tab. 2).

The common occurrence and biochemical properties of *P. adametzii* raised interests and hopes for possible application of the fungus in biological control of root pathogens. The aim of this study was to evaluate effects of *P. adametzii* mycelium and metabolites on growth of five species of *Armillaria* and three species of *Heterobasidion, in vitro*, and health of young *P. sylvestris* plants inoculated with *Armillaria* and *Heterobasidion.*

The paper presents the first observations on physiological and chemical relationships among *P. adametzii, Armillaria*, *Heterobasidion* and *P. sylvestris*.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Matherials and Methods

Two isolates of each of five *Armillaria* species (*A. borealis, A. cepistipes, A. gallica, A. mellea* and *A. ostoyae*), four isolates of each of three *Heterobasidion* species (*H. abietinum, H. annosum* and *H. parviporum*) and three isolates of *P. adametzii* isolated in Poland in 1997-2001 were used.

Studies on the (i) interaction between *H. annosum* and *P. adametzii*, *in vitro,* (ii) chemical nature of the chloroform extract of *P. adametzii* analysed with thin layer chromatography (TLC) and mass spectrometry (MS), (iii) effects of mycelium, filtrates, and chloroform and ethyl acetate extracts from *P. adametzii* cultures, and of individual fractions from the chloroform extract on growth of *Armillaria* and *Heterobasidion in vitro* and on the health of *P. sylvestris* inoculated with *Armillaria* or *Heterobasidion* were carried out.

Results and Discussion

In paired culture with *H. annosum* on 2% PDA, after 10 days *P. adametzii* inhibited the growth of *H. annosum* and formed extremely wide inhibition zones. The latter surrounded the *H. annosum* colony, which grew in the form of a narrow strip 4 cm long and 1 cm wide. Ultrastructural studies of *Heterobasidion* mycelium from colonies inhibited by *P. adametzii* did not reveal any damage of hyphae, disruption of internal structure or organization.

The chloroform extracts from *P. adametzii* culture filtrate and from *P. adametzii* grown on rice, inhibited growth of *Armillaria* and *Heterobasidion* on 1.5-2% MEA. The effect was strain-specific and increased with higher concentration of extract. Extract from *P. adametzii* KF 79 was the most effective. On average, particularly if used in higher concentration, it reduced dry weight of *Armillaria* colony mycelium to 24.5-40.5%, and diameter of *Heterobasidion* colony to 31.9-56.4% of the untreated control (significant at $P< 0.001$, $P< 0.05$). *Armillaria gallica*, *A. ostoyae, H. abietinum* and *H. parviporum* were most responsive, while *A. mellea* was less sensitive. The ethyl acetate extract from *P. adametzii* filtrate did not affect *Armillaria* or *Heterobasidion* growth on 1.5-2% MEA.

Twenty fractions from the *F. adametzii* chloroform extract were separated by TLC. Only four (VI, IX, XV, XVIII), out of 20 compounds inhibited *Armillaria* growth. Only four (III, VIII, XIV, XVIII) out of 20 compounds inhibited growth of *H. abietinum* or *H. annosum* after 4 days of incubation. None of the compounds inhibited growth of *H. parviporum* or of any *Heterobasidion* spp. after 10 days of incubation.

Mycelium of *P. adametzii* and the chloroform extract applied in water improved the health and stimulated growth of 2-year-old *P. sylvestris* plants inoculated or not inoculated with *Armillaria* (Fig. 1). Most stimulation was observed with higher concentrations of the extract and the effect was greater in

plants inoculated with *A. ostoyae* than with *A. gallica*. Culture filtrate of *P. adametzii* KF 79 applied in water decreased length of necrotic lesions on stems of *P. sylvestris* saplings inoculated with two *H. annosum* isolates after 4 months of treatment (Tab. 3). An inhibitory effect was observed only when plants were treated with 50 ml of filtrate, once a week, starting at the time of inoculation. Higher concentrations of metabolites increased the effect. *Penicillium adametzii* culture filtrate did not injure the non-inoculated *P. sylvestris* saplings**.**

Table 1. Occurrence of *Penicillium adametzii* in Poland

Habitat	Frequency (%) in fungal community	
Soil		
2-year-old *Pinus sylvestris* stumps		0-9 (-27)
10-year-old *Pinus sylvestris*		20.6-65.3
30-50 -year-old *Pinus sylvestris*		5-24
30-50 -year-old *Quercus robur*		3-10 (- 33)
Fallow soil (formerly agricultural soil)		0-7
Rhizosphere soil		
30-50 -year-old *Quercus robur*		0.4-16 (- 35)
Roots		
6-year-old *Betula pendula*		6.9
6-year-old *Fagus sylvatica*		1.5
6-year-old *Larix decidua*		10.1
6-year-old *Prunus serotina*		13.5
6-year-old *Quercus petrea*		20.0
30-50 -year-old *Quercus robur*	0.1-3	More rarely in roots >5 mm diam.
30-50 -year-old *Pinus sylvestris*	0-9	

Table 2. Effect of presence of *Pinus sylvestris* wood on *Penicillium adametzii* occurrence in soil

Removed from the plot	Thick branches removed, thin branched left on the surface of plot	Thick and thin branches split and left on the surface of plot
	Number of *P. adametzii* isolates	
3328	422	102
Frequency (%)		
67.0	15.1	4.3

Table 3. Effect of culture filtrate of *Penicillium adametzii* KF 79 applied in water on size of necrosis on 2-year-old *Pinus sylvestris* plants inoculated with *Heterobasidion annosum*

Experiment*	Decrease in length of necrotic lesions compared to control (%)			
	H. annosum isolate			
	P 20311	P 20316	P 95092	P 98315
A	+70**	-24	-2.8	-2.5
B	+29.2	-36**	-13.9	-27.5**
C	+29.2	-2	+13.9	-12.5

* Plants were treated with 50 ml of *P. adametzii* culture filtrate, once a week, starting 1 month before inoculation (experiment A), at the time of inoculation (experiment B) or 1 month after inoculation (experiment C).

** Statistically significant at $P< 0.05$.

Carboxylic acids, *glycosides* and flavonoids were detected from mass spectrum analyses of the chloroform extract. All compounds detected have antiviral, antibacterial and antifungal activities. Properties result from: (i) inhibition of synthesis, (ii) inhibition of DNA gyrase and other enzyme activity, (iii) production of extracellular enzymes, pigments and hormones, (iv) antioxidation, (v) modification of the structural and functional properties of the fungal cell membrane, (vi) modification of energy metabolism, (vii) stimulation of spore germination, (viii) hyphal growth and differentiation, (ix) regulation of mycorrhization, (x) induction of plant resistance, (xi) induction of signals, (xii) protection against UV.

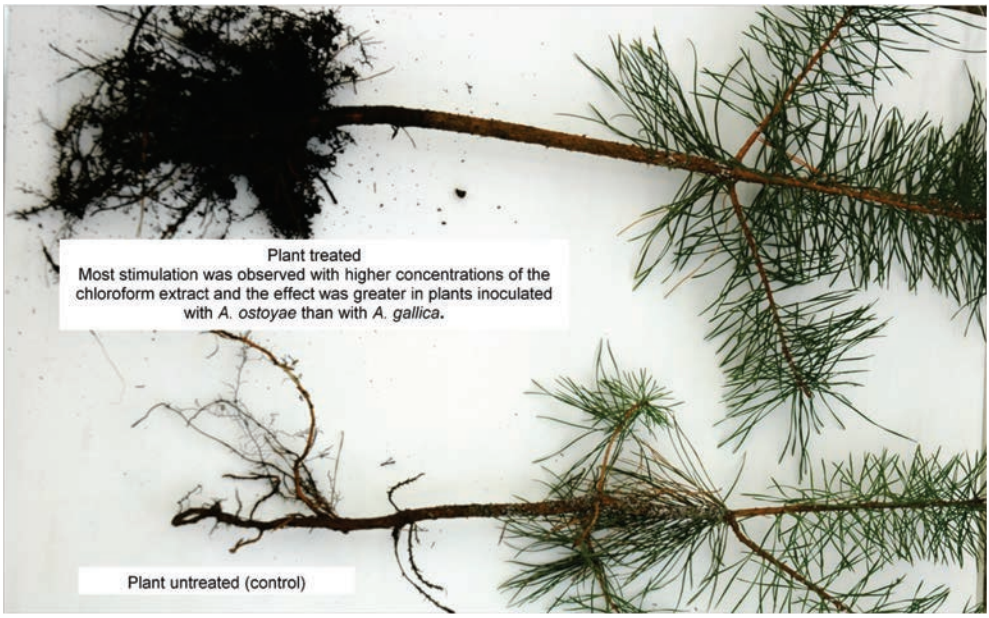


Figure 1. Stimulation of growth of *Pinus sylvestris* plants inoculated with *Armillaria*.

References

Kwaśna H. 1995. Fungal communities in soil beneath Scots pine and their stumps. Effect of fungi on *Heterobasidion annosum* and *Armillaria ostoyae* growth. *Acta Mycologica* 30: 193-205.

Kwaśna H., Sierota Z. and Bateman G.L. 2000. Fungal communities in fallow soil before and after ammending with pine sawdust. *Applied Soil Ecology* 14: 177-182.

Sierota Z. and Kwaśna H. 1999. Ocena mikologiczna zmian zachodzących w glebie gruntu porolnego po dodaniu trocin. Sylwan 4: 57-66.

Zaleski K.M. 1927. Über die in Polen gefundenen arten der gruppe Penicillium Link. I, II and III Teil. Bull. Acad. Polon. Sci., Math. et Nat. Sér B: 507-509.

Heterobasidion species in Turkey - occurrence, pathogenicity and control

H.T. Doğmuş-Lehtijärvi, A.G. Aday, F. Oskay, A. Lehtijärvi

Süleyman Demirel University, Faculty of Forestry, 32260 Isparta, Turkey.
Corresponding author e-mail address: tugbadogmus@sdu.edu.tr

Abstract. In this brief review, we summarize the current knowledge about the occurrence, pathogenicity and control of *Heterobasidion* species in Turkey. So far two different *Heterobasidion* species have been found in Turkey. *Heterobasidion abietinum* is the dominant species on *A. equi-trojani*, *A. bornmülleriana*, *A. nordmanniana* in the northern part of the country and on *Abies cilicica* in the Mediterranean region. The distribution of *H. annosum* s.s. has not been investigated thoroughly but there are sporadic records on *A. nordmanniana*, *A. cilicica* and *Pinus nigra*. The known distribution covers roughly the coastal zone of forests surrounding the dry Central Anatolian plateau. In inoculation experiments, *H. abietinum* was pathogenic on *A. bornmülleriana*, *A. nordmanniana*, *P. brutia* and *Cedrus libani*, while *H. annosum* s.s. was pathogenic on *P. brutia*, *P. nigra*, *P. sylvestris*. In *Abies* forests the proportion of trees infected by *Heterobasidion* spp. ranged from about 10 to 30%. The initial results of stump treatments indicate that efficacies of both urea and borax treatments would be satisfactory for control of stump infections.

In this brief review, we summarize the current knowledge about the occurrence, pathogenicity and control of *Heterobasidion* species in Turkey.

The first record of *Heterobasidion annosum* (Fr.) Bref. s.l. from Turkey dates back in 1932 when Pilát found the fungus on *Abies bornmülleriana* Mattf. in the Ilgaz Mountains north of Ankara. Until recently mainly sporadic, floristic records of *H. annosum* s.l. were published while the ecology and pathogenicity of the species was paid little attention. The first efforts to identify the distribution and hosts of *Heterobasidion* species using the modern species concept were conducted between 2004 and 2005 (Doğmuş-Lehtijärvi *et al.*, 2006; Doğmuş-Lehtijärvi *et al.*, 2007). So far two different *Heterobasidion* species have been found in Turkey (Fig. 1.). The distribution area of *H. abietinum* follows the distribution of Turkish *Abies* taxa. In the northern part of the country *H. abietinum* (Fr.) Niemelä & Korhonen is the dominant species on *A. equi-trojani* (Asch. & Sint. ex Boiss) Mattf. in the Aegean part of Balıkesir province, on *A. bornmülleriana* in Bolu province and on *A. nordmanniana* Spach in Ordu, Giresun, Gümüşhane, Rize and Artvin provinces (Doğmuş-Lehtijärvi *et al.*, 2006; Doğmuş-Lehtijärvi *et al.*, 2007). In southern Turkey records of *H. abietinum* cover the distribution area of *A. cilicica*, which extends from Kahramanmaraş to Antalya, approximately 450 km in east-west direction. The distribution of *H. annosum* s.s. has not been investigated thoroughly but there are sporadic records on *A. nordmanniana* and *Pinus nigra* Arn. ssp. *pallasiana* (Lamb.) Holmboe (Doğmuş-Lehtijärvi *et al.*, 2007, *unpublished*). Most probably the distribution is similar to that of *H. abietinum* and covers roughly the coastal zone of forests surrounding the dry Central Anatolian plateau.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

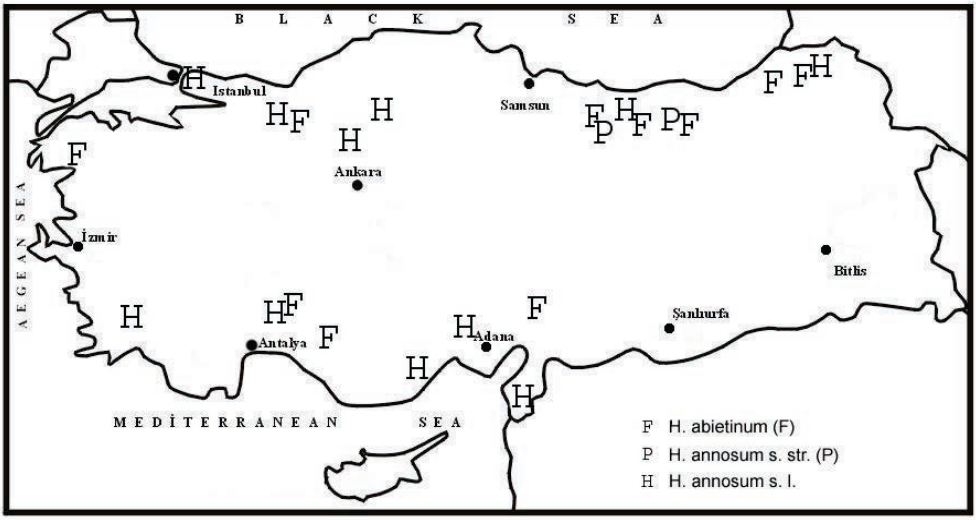


Figure 1. Distribution map of *Heterobasidion* species in Turkey.

As the distribution and host data is almost exclusively based on basidiocarps collected from stumps the role and significance of the two species as pathogens in Turkish forests has remained unclear. However, the results of recent and ongoing work indicate that at least *H. abietinum* can cause significant damage. In inoculation experiments on conifer seedlings, *H. abietinum* was pathogenic on *A. bornmülleriana*, *A. nordmanniana*, *P. brutia* and *Cedrus libani* A. Rich, while *H. annosum* s.s. was pathogenic on *P. brutia*, *P. nigra*, *P. sylvestris*, *C. libani* and *Juniperus excelsa* Bieb (Lehtijärvi *et al.*, 2009; Lehtijärvi *et al.,* 2011). In spring inoculations *C. libani* was the most susceptible and *Juniperus excelsa* the most resistant of the tested tree species (Lehtijärvi *et al.,* 2011). Although artificial wounding and placement of inoculum to the depth of vascular cambium is frequently used to measure pathogen virulence or host resistance, any resistance mechanism in the bark are bypassed by the method. Therefore the situation in forest may be different. For example, there is no information about incidence of *Heterobasidion* infections in *C. libani* forests in Turkey, indicating that natural infections are rare or, alternatively, have remained undetected due to their nature. So far all efforts to investigate disease incidence on living trees have focused on *Abies* species. On *A. bornmülleriana* in Bolu province, disease incidence on freshly cut stumps in a mixed 75-year-old stand and in a pure 120-year-old stand was 19 and 28%, respectively (Doğmuş-Lehtijärvi *et al.*, 2008). Some 200 km apart, in Kastamonu province, 33% of 80 to 120-year-old trees were infected (*unpublished*). Disease incidence on *A. cilicica* in pure stands was 11-32%, in a rocky, degraded stand 4% and in a stand admixed with *P. nigra* 12% (6, *unpublished*). This

accumulating evidence indicates that *H. abietinum* causes significant damage on living trees in Turkish fir forests and requires control.

From a practical point of view, a suitable control agent to be used in Turkish forests would be non-toxic, easy to handle, cheap and would not require lots of water as a solvent. So far 30% aqueous urea solution, borax powder, and spore suspensions of *Phlebiopsis gigantea* (Fr.) Jülich and *Trichoderma harzianum* Rifai have been tested in field experiments. The efficacies of the borax, urea and *T. harzianum* treatments on *A. cilicica* were approximately 90, 80-90, and 61-66%, respectively (Lehtijärvi *et al.,* 2011). The efficacies were somewhat lower on *A. bornmülleriana*, i.e. 60, 70 and 48%, respectively (*unpublished*). The *P. gigantea* treatment was tested only on *A. cilicica* stumps which it protected with an efficacy of 47-49% (Lehtijärvi *et al.,* 2011). These initial results indicate that efficacies of both urea and borax treatments would be satisfactory for control of stump infections. The treatments with the biological control agents, in contrast, were less effective and should be improved, e.g. by screening for suitable isolates.

References

Doğmuş-Lehtijärvi H.T., Lehtijärvi A., Korhonen K. 2006. *Heterobasidion abietinum* on *Abies* species in western Turkey. *Forest Pathology* 36: 280-286.

Doğmuş-Lehtijärvi H.T., Lehtijärvi A., Korhonen K. 2007. *Heterobasidion* on *Abies nordmanniana* in north-eastern Turkey. *Forest Pathology* 37: 387-390.

Doğmuş-Lehtijärvi H.T.,Lehtijärvi A.,Oskay F., Aday A.G., Karadeniz M. 2008. *Annosum* kök ve alt gövde çürüklüğünün *Abies bornmülleriana* ve *Abies cilicica* meşcerelerinde yoğunluğunun belirlenmesi. *Artvin Çoruh Universtity, Faculty of Forestry Journal* 9: 111-120 (in Turkish, English abstract).

Lehtijärvi A., Aday G., Doğmuş-Lehtijärvi H.T. 2009. Turkish *Heterobasidion abietinum* is pathogenic to inoculated *Abies nordmanniana* ssp. *Nordmanniana* and ssp. *Bornmülleriana*. *Forest Pathology* 39: 200-209.

Lehtijärvi A., Aday G., Doğmuş-Lehtijärvi H.T. 2011. *Cedrus libani*: the most susceptible Turkish conifer species to local *Heterobasidion* isolates in spring inoculations. *Forest Pathology* 41: 1-6.

Lehtijärvi A., Doğmuş-Lehtijärvi H.T., Aday A.G., Oskay F. 2011. The efficacy of selected biological and chemical control agents against *Heterobasidion abietinum* on *Abies cilicica*. *Forest Pathology*. 41: 470-476.

New ways of assessing *H. annosum* root inoculum

J.E. Pratt[1] and L. Wang[2]

[1]*Cross House, Mountain Cross, EH46 7DF, Scotland, UK.*
[2]*Southern Swedish Forest Research Centre, SLU, Box 49 230 53 Alnarp, Sweden.*
Corresponding author e-mail address: k.m.pratt@btinternet.com

Abstract. Collection of data on the morphology of conifer root systems is increasing and makes it possible to review the way we think about the dynamics of root inoculum in the *H. annosum* disease cycle. This in turn offers us the chance to improve our selection of options with which to manage inoculum *per se*. In addition to data collected in other forest research disciplines, the ubiquitous availability of large single-grip harvesting machines throughout Europe and their ability to extract whole trees (including their root systems) provides a means to study the relationship between root infection and disease symptom expression in a manner hitherto not economical.

Potential inoculum: the mass approach

Conifer roots colonised by *H. annosum* provide the main locus for disease spread within a stand, through root contact. Such roots may be attached to stumps or live trees, and the amount of disease within them can be expected to expand or contract over time. Clearly, the mass of diseased roots, and of roots that are susceptible to disease, provides a measure of the potential of the disease to cause losses among residual trees. Measurements of stump morphology, needed by other research disciplines (tree stability, carbon sequestration etc.) can provide data on root system mass (Kg, green) and on the surface area of land occupied by stumps and roots, and these data are usually related to the size of the tree removed from the stump. This is commonly expressed as diameter at breast height, shortened to dbh. The ubiquitous use of yield models which specify the numbers and sizes of trees to be felled at intervals throughout the life of a stand, makes it possible to predict changes in the mass of potential root inoculum over time.

Take, for example, crops of Sitka spruce in the UK, where data have been collected over the past 40 years or so to improve management of stands at risk from wind-blow. In this work stumps from trees whose over-turning moment was measured were then cleaned of soil, measured and weighed.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

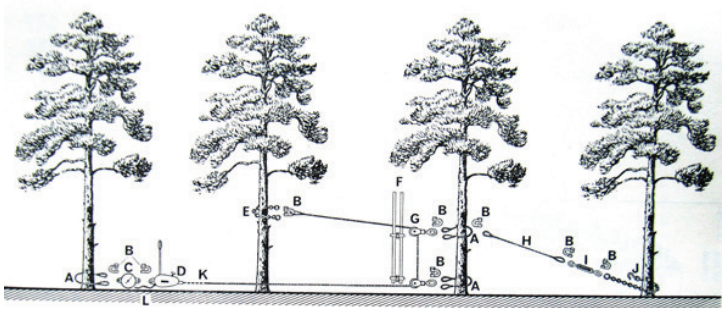


Figure 1. Traditional tree-pulling equipment designed to measure the over-turning moment on standing trees.

Root-plate mass (Kg) and root- plate surface area (m^2) were found to be positively correlated to dbh, on a range of mineral soils types[1,2] ([1](Kg = $0.0458^{2.5293}$ dbh [r^2 0.712]); [2](m^2= $0.293^{-3.05}$ dbh [r^2 0.501]) respectively, N =300, dbh range 10 –60cm).

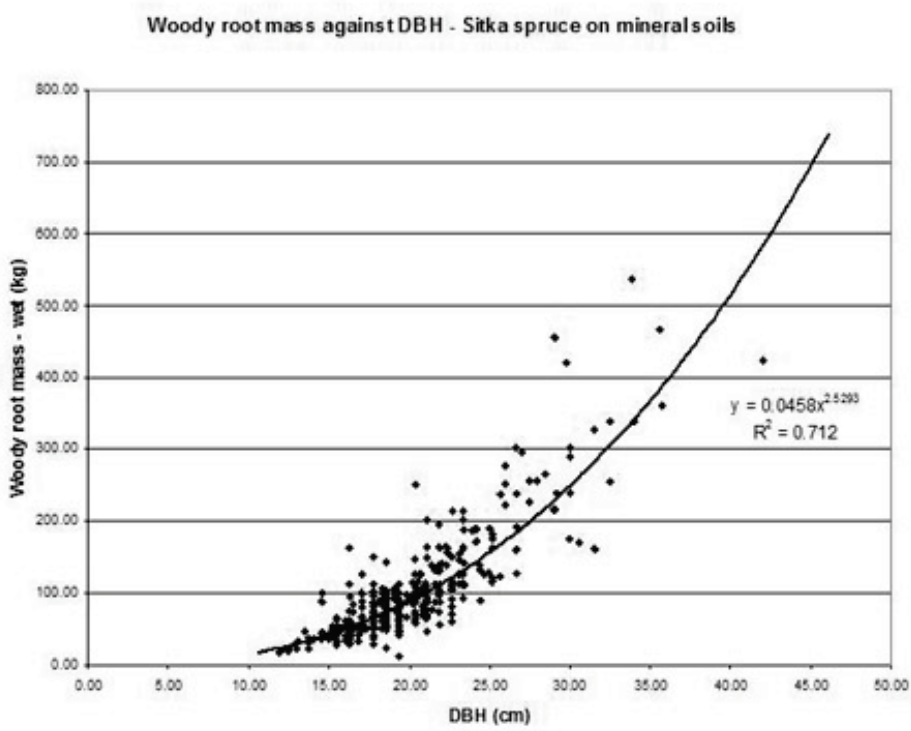


Figure 2. Relationship between root mass and tree diameter: Sitka spruce on mineral soils.

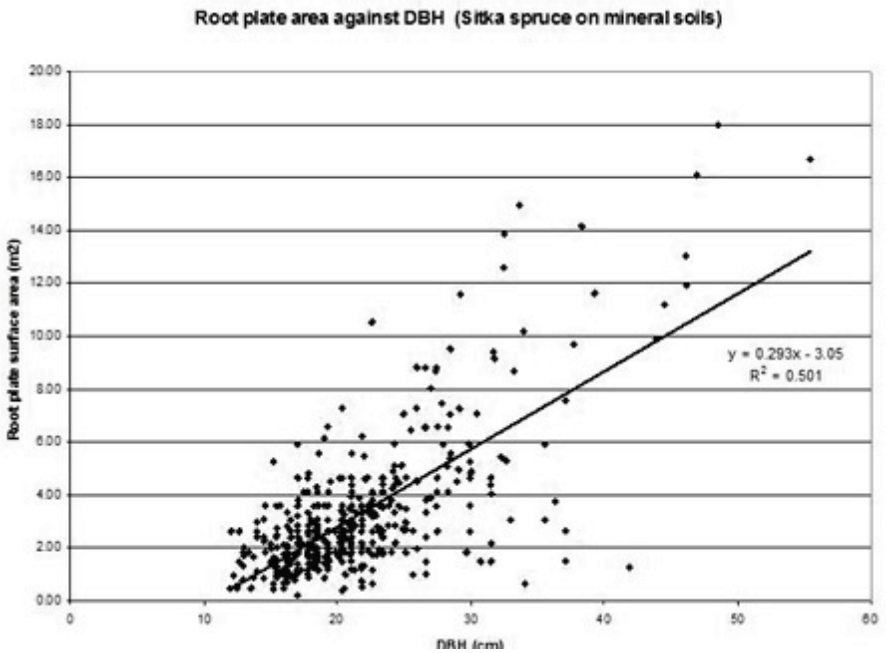


Figure 3. Relationship between root plate area and tree size; Sitka spruce on mineral soils.

Substituting these values within a standard yield model that optimises stand management in the UK, it is possible to estimate the mass (green weight) and surface area of stumps created at each of six thinnings and at clear fell throughout the life of the stand (Tab. 1).

Table 1. Estimated mass and surface area of woody roots in a UK stand of Sitka spruce over a full rotation.

Age (yrs)	Trees felled		Root plates		
	per ha	mean dbh (cm)	Total mass Kg per ha	Total area m^2 per ha	Area as % of stand
21	1,175	12	35	547	5.5
26	493	15	21	663	6.6
31	243	19	21	611	6.1
36	145	23	20	534	5.3
41	102	26	17	465	4.65
51 (fell)	334	38	164	2,700	27

What new insight do these data reveal? They show, for example, that in a crop subject to standard management in the UK, stumps created in the first thinning occupy a wider surface area than hitherto realised, and they have a mass greater than at any other thinning. These stumps are individually small (and therefore

rapidly colonised down to fine roots) and are in contact with more trees than those created later in the crop's life, and perhaps explains earlier observations by one of us (Pratt, unpublished) that more disease seemed to be present in those crops which followed an earlier crop felled when stumps were small but numerous than in crops with fewer (but larger) stumps from the previous rotation.

These data also allow us to partition root mass among stumps and trees throughout the life of the crop. Although roots occupy a smaller area than we might imagine, over a rotation as a whole, woody roots (>1 cm diam.) might have colonised some 60% of available space (Tab. 2).

Table 2. Cumulative areas occupied by woody roots from stumps and trees over a full rotation in the crop modelled in Tab. 1.

Age	Cumulative Area (%) colonised by:		
	Stump roots	Tree roots	uncolonised
21	5.5	15.2	79.3
26	12.1	23.8	64.1
31	18.2	26.0	55.8
36	23.6	28.8	47.6
41	28.2	28.9	42.9
46	32.5	27.5	30.0

Measuring roots is one thing: estimating the mass of viable inoculum is another. In this context, viable inoculum is woody root material that has potential to initiate disease in healthy host tissue via root contact. It can arise by vegetative growth of the pathogen through stump and root tissues following the infection of fresh stump tops by basidiospores or from existing infections on the root systems of trees and stumps by root contact. Inoculum levels rise and fall with recruitment of new sources and depletion of old ones. At any point in time, the amount of inoculum will depend on a host of factors, which include:

- pathogen species;
- stump and tree species and age;
- stump size;
- soil type;
- ambient and soil temperatures;
- source of infection;
- success of intervention.

A factor that in Britain most determines disease transmission, namely soil, is probably also key to influencing both the build-up and the survival of inoculum as well, and as we show in another paper in these proceedings (Pratt and Greig, this vol.), the pathogen can survive in ideal soil for more than a decade, even in small root fragments. Clearly, more data need to be collected on all these factors if this

approach is to be pursued. The data on root morphology of Sitka spruce in Britain were collected largely at the behest of silviculturists, at considerable cost and over many years. The sites on which measurements were made reflect silvicultural needs, and not those of pathologists who clearly need to be able to collect their own data from sites that are relevant to *H. annosum*. Below, we report a preliminary trial to test the feasibility of using harvesting machines to extract whole trees (including their root systems) from soil, and describe simple means of measuring roots.

Root extraction with harvesters

A wheeled John Deere (1,407E) tree harvester was used to extract three live 40-yr-old Scots pine (30 cm DBH, 20 m total ht.) from a dry sandy loam in England (50°25'0" N, 0°44'0" E) in November 2010. The operator used the knuckle boom to push the trees 45° from the vertical, from which position they were lifted from the soil. The trees were violently shaken to remove soil. The whole trees were carried to a place for assessment, and the stem was cut from the stump and laid nearby. It took 10 minutes to harvest three trees.

Excavating the whole tree allows the morphology and health of the root system to be recorded along with measurements of incremental stem growth (radial, linear and volumetric) and the status of the crown (needle retention, length and frequency). In addition, such work increases the available data-base on root form of species on different soils. This system depends for its success on the nature of the soil, the size of the trees, the availability of suitable harvesters and the competence of the harvester operators. There are clearly many soils (e.g. soils dominated by clay, or with induration) where the resistance to overturning is so great that excavation would result in multiple breakage of roots. However, on the light sandy soils which are typical of high-hazard sites for *H. annosum*, the system is feasible.

Conclusions

There are many sites where stumps and roots can now be excavated mechanically without specialised machinery, so that the relationship between root inoculum and disease expression in individual trees can be assessed. Collection of such data over time can increase the awareness of the importance of measuring inoculum per se, including the ability to estimate its bulk.

Acknowledgements

We would particularly like to thank the staff of the UK Forestry Commission's Thetford Chase for their help and generosity in organising the field work, and to Dr Bruce Nicol, Forest Research Edinburgh, for allowing us to quote the stump data.

The registration of *Phlebiopsis gigantea* in the USA: the process

J.E. Pratt[1], S. Covert[2], M. Cram[3], M. Niemi[4]

[1]*Cross House, Mountain Cross, EH46 7DF Scotland, UK.*
[2]*Warnell School of Forestry & Natural Resources, Univ. of Georgia, Athens, GA 30602,USA.*
[3]*USDA Forest Service, 320 Green St., Athens, GA 30602,USA.*
[4]*Verdera Oy, P.O. Box 5, FI-02271 Espoo, Finland.*
Corresponding author e-mail address: k.m.pratt@btinternet.com

Abstract. Because it occurred naturally and was not classed as a pesticide, *Phlebiopsis gigantea* (*Pg*) was approved for control of *Heterobasidion annosum* (*Ha*) in the southern pine forests of the USA in 1976. In 1988, the FIFRA law changed and *Pg* lost its exemption. A multi-national project to make the fungus available again for the control of *Ha* in the USA began in 2003. We describe the data needed to support regulatory approval, data collection to date, and potential use of *Pg* in southern pine forests, against a background of volatile timber and land prices and changes in the attitude of timber owners to the disease.

Registration

A 1976 request by the US Forest Service to use *Peniophora gigantea* (*Pg*) as a stump treatment elicited the following reply from the Environmental Protection Agency (EPA):

> *Since the wood rotting fungus (*Pg*) is a naturally occurring organism in southern pine forests, cultures of the organism would not be considered a pesticide as defined in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)...Therefore, the wood rotting fungus when used as an inoculum on freshly cut stumps would not require registration. We wish you success in developing this potentially valuable forest practice.*

In 1995, however, continued use of *Pg* was declared contrary to FIFRA. Future registration and labelling now required the submission of documents and data hitherto considered irrelevant. A multi-national project to register *Pg* began in 2003. We report here on data collected to support this effort and highlight some of the economic factors influencing future investment in this control programme.

Data requirements

Under current (2010) legislation, all Microbial Pest Control Agents (MCPAs) used in the USA must be registered with the EPA to ensure that they comply with FIFRA; i.e., the pesticide, when used in accordance with widespread and commonly recognized practice, must not cause (or significantly increase the risk

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

of) unreasonable adverse effects on humans or the environment. Data are required on:

- Product analysis;
- Toxicology;
- Residue;
- Ecological effects and environmental expression.

The data required by the EPA are broadly equivalent to those used for registration of *Pg* in the EU (Directive 91/414/EEC, in June 2011 replaced by Regulation (EC) 1107/2009). Therefore, to save costs, we asked the EPA to accept toxicology and environmental data derived from Europe. In general, this request has been granted, but with some clear provisos.

First, a US product would have to use North American isolates of *Pg*. Thus, local *P. gigantea* isolates were collected throughout the southern states and evaluated in a screening programme at UGA in 2006-2008 (Higgins *et al.,* 2011). Second, we had to document that the *Phlebiopsis* species to be used in the US is the same as the species in Europe. Although there is genetic differentiation between European and North American populations of *Pg* (Vainio and Hantula, 2000), strains from the two populations are equally interfertile, suggesting that they belong to the same species (Grillo *et al.,* 2005).

EPA also needs to know that the fungus occupies roughly the same ecological niche in the USA as in Europe, before accepting the considerable body of environmental data on the fungus from Europe. It has also been necessary to demonstrate that the mode of action of the fungus when used as a pesticide is similar in both continents. This work is well advanced.

The European trials that established the toxicology profile of *Pg* were conducted in accordance with European (OECD) protocols, and may or may not be acceptable to the US authorities since there is no common standard for assessing toxicity between the two continents. This remains an unresolved issue.

Another issue that remains unresolved is the number of isolates to register. In certain situations it would be valuable to have access to multiple registered strains. Approval on the species level would allow this. However, EPA's experience with other fungal MCPAs, which sometimes produce mycotoxins, makes them reluctant to approve more than one isolate, without a full suite of toxicology and environmental data for each strain. *Pg* does not produce mycotoxins nor is it to be used in food production, thusproviding such data whenever a new isolate of *Pg* is to be used would not be reasonable. The same pragmatic approach as that taken by the European Commission, to approve several isolates based on data for one, would resolve this issue.

Although evidence of efficacy is not required by the EPA, it is needed in some states and a prudent registrant will ensure that his product is efficacious when used in accordance with label directions and commonly accepted pest control practices.

Thus, a series of efficacy studies have been undertaken in pine stands in Georgia (Brown *et al.,* 2011).

In summary, a framework for the necessary data is emerging.

Market considerations

Applying for approval of a pesticide is a commercial decision, that depends on the perception of its benefits by those who would use it. Within the five states that make up the southeastern US, some 15.4 million ha of pine forests are managed for profit in an industry worth some $ 2.5 billion annually. A significant proportion of this forest is on sandy soils at high-risk for *Ha*. Although the potential for huge losses to this important national resource from *Ha* were sufficient to justify a multi-million dollar federal research programme in the 1960's and 1970's, today's interest among forest owners in *Ha* control is variable. Borate stump treatment, although available in the USA, is rarely used: the present volatility of the market for both timber products and for forest land discourages what is perceived to be a long-term investment in stump treatment. Furthermore, the impact of *Ha* is frequently underestimated, because ofa lack of visible evidence. Trees affected by root disease are preferentially attacked by bark beetles, and these are typically credited with being the primary cause of death. *Ha*-infected trees also suffer growth reductions which are difficult to see. *Pg* was (and remains) the only effective remedial stump treatment for affected stands.

Pg could also act as a useful national tool in carbon sequestering. The Southern forest is estimated to sequester 100 million metric tons of carbon dioxide per year, or a third of the U.S. forests' capacity to absorb carbon dioxide (Jose, 2007). Losses to *Ha* over 15 years could equal timber production from more than 2 m ha, based on the NIDRM (USD, http://www.fs.fed.us/foresthealth/technology/nidrm.shtml) models for this disease. Diseased stands are likely be clear cut, resulting in a temporary reduction in carbon sequestering while they are regenerated, or a total loss if they are converted to non-forest habitat. The use of *Pg* to minimise this loss needs to be reviewed.

The cost of registering a pesticide in the USA and bringing it to market is another obstacle. Society demands ever higher standards for pesticides, and strict safety assessments are required for relatively small-use biological control agents that are native microorganisms and known to be benign. The collection and collation of a dossier to support an application for approval of an MCPA can run into hundreds of thousands of dollars, to which has to be added EPA's cost of scrutinising the submission, which is not inconsiderable.

Discussion

Obtaining bio-pesticide approval for *Pg* in the USA depends on scientific researchto evaluate the environmental risks of the proposed control measure. The

irony is that the escalating costs of this research reflect society's growing aversion to pesticides at precisely the time when the value of forest products is falling and forest owners are growing more tolerant to disease losses. This makes it more difficult to create a market for *Pg* biocontrol.In summary, therefore, gaining approval of *Pg* is a more complicated, expensive and uncertain task than it was in the 1970's, and today there is no clear commercial incentive to proceed with applying for its approval in the USA. However, fashions change and the success of the treatment in Europe may help to boost interest in this beneficial organism.

References

Brown J.E., Cram M.M., Covert S.F., 2011. Evaluating *Phlebiopsis gigantea* as a biocontrol agent for *Heterobasidion* root disease in the southeastern United States. (In preparation).

Grillo R., Hantula J. Korhonen K., 2005. Interfertility between North American and European strains of *Phlebiopsis gigantea*. *Forest Pathology* 35: 173-182.

Higgins B., Brown J.E., Covert S.F., 2011. Screening *Phlebiopsis gigantea* isolates from the southeastern U.S. for control of *Heterobasidion* root disease. (In preparation).

Jose S., 2007. Carbon sequestration and intensive silviculture: the southern U.S. experience. Paper presented to the American Geophysical Union, Fall Meeting 2007 (abstract #B53A-03). Online at: adsabs.harvard.edu/abs/2006AGUSM.B53A.03J (accessed April 11, 2011).

USD – United States Department of Agriculture, Forest Service URL: http://www.fs.fed.us/foresthealth/technology/nidrm.shtml.

Vainio E.J. and Hantula J., 2000. Genetic differentiation between European and North American populations of *Phlebiopsis gigantea*. *Mycologia* 92: 436-446.

Impact and Benefits from control of *Heterobasidion parviporum* in Norway spruce forest in Serbia

N. Keča and L. Keča

Faculty of Forestry, University of Belgrade, Kneza Viseslava 1, 11030 Belgrade, Serbia.
Corresponding author: e-mail address: nenad.keca@sfb.bg.ac.rs

Abstract. Root decay fungi are among greatest treat to the coniferous forests of Northern Hemisphere. Earlier studies in spruce forests in Serbia showed that central decay can extend up to 15 m. Forest management data show that between 15-40% of trees in Norway spruce stands can be infected with *Heterobasidion* spp. Stumps were treated with Rotstop® and Borax™. Protection efficiency and succession of microorganisms on spruce stumps were controlled after 3, 9, 12 months. It is estimated that the minimal loss amount is between 875-1,093 €/ha, and the protection costs are 0.02 €/tree for Borax and 0.04 €/tree for Rotstop, i.e. 1.36 €/ha. Succession of antagonistic (*Penicillium* spp., *Trichoderma* spp., *Aspergillus* spp., etc.) and decaying fungi (*Fomitopsis pinicola*, *Resinicum bicolor*, *Stereum* spp., *Gloeophyllum* spp., etc.) on stumps treated with Rotstop and Borax was observed too.

Introduction

Root and butt rot caused by *Heterobasidion annosum s.l.* is, in economic terms, the most important disease of conifers in the forests of northern temperate regions (Woodward *et al.*, 1998). Newly cut stumps provide entrance for infection of basidiospores. In the middle of 20th century Rishbet (Rishbet, 1959) proved that Borax™ ($Na_2B_8O_{13}$ $4H_2O$) is very efficient in protection of fresh stumps from infection with *Heterobasidion* spp. spores.

However, freshly cut stumps contain tissues that are food base for many microorganisms. Since these tissues continue to live, for a while, after tree is cut they are good material for growth of fungus like *Heterobasidion* spp., but also for *Phlebiopsis gigantea* (Fr.) Jülich, *Resinicum bicolor* (Alb. & Schw.: Fr.) Parm., etc. Observation that *Phlebiopsis gigantean* can colonize stumps from thinning, but also kill and replace *H. annosum* opened possibility for use of this fungus for Annosum disease control (Rishbet, 1963).

Until now, Rotstop and Borax are only products registered for control of infection of *Heterobasidion* species. None of them are registered for use in Serbia. Macro-experiment was established to check efficiency of these two products in control of root rot caused by *Heterobasidion* in spruce forests in Serbia.

Materials and Methods

Two products Rotstop® (Verdera Oi, Finland) and Borax (Borax Europe Ltd) were tested. Both products are used according to the manufacturer instructions.

Control of efficiency was performed on stumps from regular selective cutting in Norway spruce forest (*Piceetum excelsae serbicum luzuletosum*), on a brown

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

podzolic soil. Experiment was set in three repetitions and control was treated with distillate water. Analyses of efficiency have been performed after 3 and 9 months and 1 year after the treatments.

Experimental forest is on slope (6-10°C). Altitude of 1,470-1,530 m a.s.l. High selective spruce forest is well preserved. According to the last forest inventory survey stand density is 493 trees ha^{-1}, volume is estimated on 409.5 m^3 ha^{-1}. Average diameter is 29 cm and height 20.5 m.

Results

Studied compartments had loss and damages from both *Heterobasidion* and *Armillaria* species. Percentage of infected stumps ranged from 16-33% (Tab. 1). Other data about number of treated stumps, treated stump surface and other are presented in Tab. 1.

Table 1. Overview of cut and treated trees regarding compartments and treatments

	Compartment 1		**Compartment 2**		**Control**	**Average**
	Borax	Rotstop	Borax	Rotstop	Water	
Total number of stumps	423	658	192	206	71	
Number of healthy stumps	356	599	129	145	53	
Number of infected stumps	67	159	63	61	18	
Percentage of infected (%)	16	24	33	30	25	26
Number of stumps infect. with *Heterobasidion*	13	38	6	10	1	
Percentage of infected *Heterobasidion*	3	6	3	5	1	4
Number of stumps with *Armillaria*	54	121	57	51	17	
Percentage of infected *Armillaria*	13	18	30	25	24	22
Area of healthy stumps (m^2)	38,12	79,82	41,25	35,20	12,92	
Area of diseased stumps	10,67	16,10	4,60	6,29	0,97	
Percentage of infected in ration with total stand tree area	28	20	11	18	7	17
Area of infected with *Heterobasidion*	2,40	8,05	2,30	3,15	0,48	
Area of infected with *Armillaria*	8,27	29,97	21,47	16,19	7,81	
Area of stumps decayed with *Heterobasidion*	0,21	1,46	0,26	0,46	0,07	
Area of stumps decayed with *Armillaria*	2,12	6,36	5,82	2,97	3,06	
Percentage of area decayed with *Heterobasidion*	9	18	11	15	14	13
Percentage of area decayed with *Armillaria*	26	21	27	18	39	26

After three months it was observed that both products prevented infections with *Heterobasidion* spores. Only 3% and 5% of checked stumps had infections with *Heterobasidion.*

After three months, most of the surface of stumps, protected with Borax, was without of saprophytic and parasitic fungi. Only small scattered colonies of *Ophiostoma* spp., *Penicillium* spp., *Trichoderma viride* were present on stump surface. These species were present could be found at the depth of 5 cm, below stump surface.

Rotstop, in fact fungus *Phlebiopsis gigantea* successfully colonized stump surface. Average colonized stump area, after three months, was 19% (Fig. 1). First fungus colonizes softwood and after that, spreads into the hardwood. Colonization is much slower in the central part of stump. Other fungi are not observed during disk analyses. *P. gigantean* was spread at least 5 cm below the stump surface (Fig. 2).

Twelve months after treatment with Borax different rotting fungi (*Fomitopsis pinicola*, *Resinicum bicolor*, *Stereum* spp., *Gloeophyllum* spp.) were observed on stump surface.

Surface of stumps, treated with Rotstop were from 20-91% with *Phlebiopsis gigantea* (Fig. 1). Inside the stumps mycelia was spreading 50 or even more centimetres in depth. Area colonized with fungus at the depth of 15 cm from the top of stump was ~48%. Destruction of wood of stumps was very intense, and noticeable by bare eye.

It is estimated that the minimal loss amount is between 875-1,093 €/ha, and the protection costs are 0.02 €/tree for Borax and 0.04 €/tree Rotstop®, i.e. 1.36 €/ha.

Discussion

According to the observed facts we can conclude that combination of appropriate protection and good management practice, infection of root rot *Heterobasidion annosum* can be reduced to minimum.

Management process should be set to reduce infection of basidiospores with avoiding of cuttings from middle of August until end of October (Keča, unpublished).

In all stands where cutting is performed stumps should be treated. We should stress that better results for forest ecosystems were noticed for biofungicide - Rotstop.

Further studies should take in consideration establishment of new experiments that will test new products like Rotstop®S (Rönnenberg *et al.*, 2006), but also it would be useful to test efficiency of isolates from Serbia.

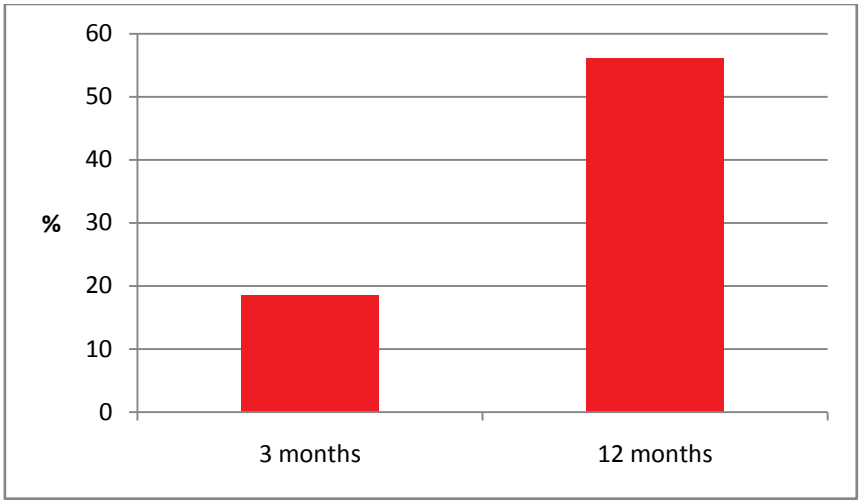


Figure 1. Percentage of colonized stump surface, treated with Rotstop.

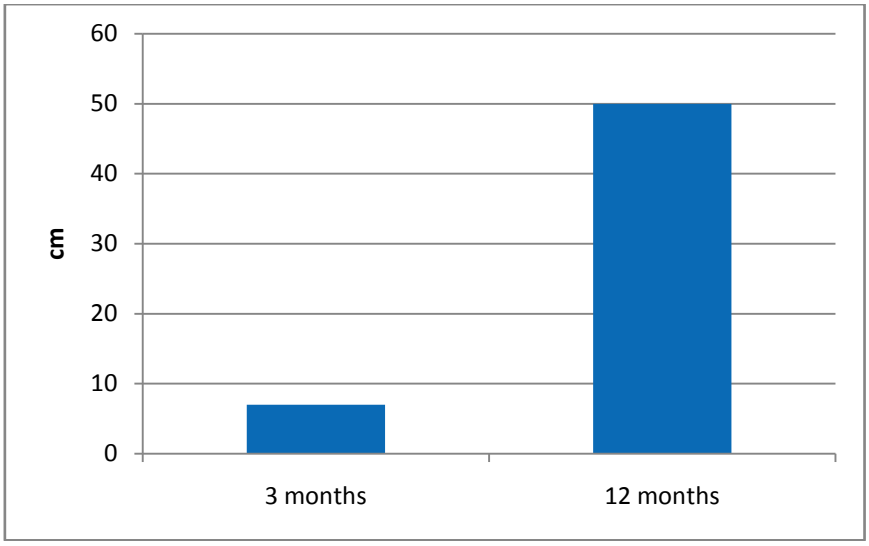


Figure 2. Colonization with *Phlebiopsis gigantea* inside stumps after 3 and 12 months

Aknowledgements

This research was supported by grant from the Ministry of Education and Science of the Republic of Serbia. We would like to thank to the projects TR 37008 and TR 31041. We would like to thank Verdera Oi for donation of Rotstop for this experiment.

References

Korhonen K., 2003. Stimulated stump treatment experiments for monitoring the efficacy of *Phlebiopsis gigantea* against *Heterobasidion* infection. In: *Root and Butt Rots of Forest Trees* (Proc .10th Int. Conf. on Root and Butt Rots, Quebec City, 16 - 22 Sep. 2001). Ed by Laflamme, G.; Bussieres, G. Sainte-Foy Quebec, Canada: Laurentian Forestry Centre, Inforamtio Report LAU-X-126, pp. 206-210.

Rishbet J., 1959. Stump protection against *Fomes annosus* II. Treatment with substances other than creosote. Annals of Applied Biology 47: 529-541.

Rishbet J., 1963. Stump protection against *Fomes annosus* III. Inoculation with *Peniophora gigantea*. Annals of Applied Biology 52: 63-77.

Rönnenberg J., Sidorov E., Ptrylaite E., 2006: Efficacy of different concentrations of Rotstop® and Rotstop®S and coverage of Rotstop®S against *Heterobasidion* spp. spore infections on Norway spruce stumps. *Forest Pathology* 36: 422-433.

Woodward S., Stenlid J., Karjalainen R., Hüttermann A., 1998. *Heterobasidion annosum* Biology, Ecology, Impact and Control, CAB International, Wallingford, UK.

Survey on *Heterobasidion* species and perspectives of butt rot control in Germany

B. Metzler[1], G. Langer[2], P. Heydeck[3], F. Peters[1], J. Scham[4], A. Renfer[1], E. Langer[5]

[1]*Forestry Research Institute of Baden-Wuerttemberg (FVA), Wonnhaldestr. 4, D- 79100, Freiburg/Br, Germany.*
[2]*Forestry Research Institute of North-West Germany (NW-FVA), Grätzelstraße 2, D-D-37079, Göttingen, Germany.*
[3]*Eberswalde Forestry Competence Center (LFE), Eberswalde, Germany.*
[4]*Forest Administrative District Sigmaringe, Germany.*
[5]*University of Kassel, D-34132 Kassel, Germany.*
Corresponding author e-mail address: berthold.metzler@forst.bwl.de

Abstract. In southern Germany the most important forest tree species economically is Norway spruce (*Picea abies*). Its value is influenced significantly by attacks of *Heterobasidion parviporum*. In contrast, Scots pine (*Pinus sylvestris*) is the prevailing conifer in north and northeastern Germany, which is attacked by *H. annosum* s. str., especially after thinnings in afforestations on former arable land and on lignite mine sites. This pathogenic species affects also in some cases underplantings of Douglas fir (*Pseudotsuga menziesii*) in pine stands. Silver fir (*Abies alba*), however, is known to be void of butt rot problems within its natural distribution in southern Germany. However, *H. abietinum* could be detected here for the first time. *Heterobasidion*-strains isolated from coniferous forests in Germany are characterized by means of PCR methods in order to verify the distribution of the respective species. In order to reduce economic losses due to butt rot in susceptible forest stands, conidial suspensions of *Phlebiopsis gigantea* have increasingly been used for stump treatment in mechanized wood harvesting since 2002. Urea may rather be used as an alternative in small scale forestry during motor manual thinnings. Efficacy and consequences are discussed.

Introduction

The 2nd National Forest Inventory (www.bundeswaldinventur.de) shows that 57.6% (i.e. more than 6 Mio ha) of the German forest area are covered by coniferous forests which can potentially be affected by *Heterobasidion* root and butt rot. Regarding the latter, this paper concentrates on four topics with relevant problems a) the southwestern Norway spruce forests b) the northern and north eastern Scots Pine forests c) threat of Douglas fir butt rot after pine d) *H. abietinum* in the regional context of the south-western silver fir forests.

More than 50 *Heterobasidion* strains from the above mentioned regions were identified to species level by molecular methods. Polymorphism of the ribosomal IGS1 region (Kasuga and Mitchelson, 2000) was used to differentiate *H. annosum* from both *H. parviporum* and *H. abietinum.* In some cases sequences of other portions of DNA regions were used. *H. abietinum* was distinguished by differences in the sequence of the elongation factor 1-α (Johannesson and Stenlid, 2003) as published in NCBI.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Southwestern Norway Spruce forests

Besides some incidental occurrence of *Armillaria ostoyae*, butt rot problems arise mainly from *H. parviporum* and nearly all strains of this species are isolated from living Norway spruce.

Severe damage is concentrated in stands of former arable land on calcareous soil. For example annual economical losses caused by butt rot in Norway spruce aspects were calculated for the county of Sigmaringen/Swabian Alb at approx. 1.2 Mio. € (44 €/ha). In a stump treatment experiment with the antagonist *Phlebiopsis gigantea* (as product ROTSTOP) comprising 5 pairs of plots including 182 stumps 80% reduction of infected stumps was recorded after 6 to 12 months (Metzler *et al.*, 2005). A similar ratio of benefit had been recorded in the remaining stand 12 years after experimental stump treatment with a chemical and by the examination of 350 trees (Metzler and Kublin, 2003). Better efficacy was found in stump treatment trials with urea, which can easily be used by small scale forest owners. Generally it is assumed that losses caused by butt rot can be reduced by 50% when applying meaningful stump treatment. Costs and benefit ratio is expected to be 1/3.5.

The northern and north eastern Scots pine forests

Scots pine (*Pinus sylvestris*) is the prevailing conifer in northern and northeastern Germany, which is also the main distribution area of *H. annosum* s.str. Recent estimations in Lower Saxony show more than 100,000 ha of infested forest stands. Afforestations on former arable land and on lignite mine recultivation sites in southern Brandenburg are prone to early mortality due to *H. annosum* s.str. The pH value of such sites is shown to be typically higher than 6.0. A few years after the first thinnings typical but rott mortality gaps appear. Stump treatment experiments with two different strains of *P. gigantea* show a very good establishment of the antagonist (Heydeck *et al.*, 2010).

Threat of Douglas fir butt rot after pine

Although Douglas fir and beech are not very susceptible to root and butt rot, there are some cases where they become lethally infected by *H. annosum s.s.* in underplantings in pine stands. Older Douglas fir trees may be diseased by butt rot on former pine forests. These are emerging problems in Lower Saxony but also in some cases in the sandy soils of the upper Rhine valley in the southwest.

H. abietinum in the regional context of silver fir forests

Forest of *A. alba* occurring in the south of Germany are void of butt rot problems. However, this is the first record of *H. abietinum* strains identified by PCR in Germany. It was found a) parasitic causing butt and stem rot in *A. alba* trees of unknown provenance planted outside of the natural distribution area of silver fir b) parasitic in a garden plant of *Chamaecyparis lawsoniana*, c)

saprophytic in timber of *A. alba and P. abies* under water storage and d) saprophytic in a rotting stump of *A. Alba*

Prospect

There is some variety of ecological niches in German forest used/occupied by the three European *Heterobasidion* species. From the economic point of view, the respective most spectacular butt rots are on soil types of high pH-value, esp. in pure stands of Norway spruce on calcareous soil in the south and of Scots pine on sandy soils and on recultivation sites in the north and north-east of Germany. Here stump treatment with antagonistic fungi or with urea seem to be feasible in order to reduce the economical losses. Respective experiments as well as some practical work are on the way.

References

Heydeck P., Knoche D., Dahms C., Rakel T, Bieler T., Sauermann J., Duhr M. 2010. Prophylaktische Maßnahmen zur Abwehr des Kiefern-Wurzelschwammes (*H. annosum*) in Erstaufforstungen auf Kippenstandorten im südlichen Brandenburg. *Arch. Forstw. Landsch.* 44: 107-115.

Johannesson H., Stenlid J. 2003. Molecular markers reveal genetic isolation and phylogeography of the S and F intersterility groups of the wood-decay fungus *Heterobasidion annosum*. *Molecular Phylogenetics and Evolution* 29: 94-101

Kasuga T., Mitchelson K.R. 2000. Intersterility group differentiation in *Heterobasidion annosum* using ribosomal IGS 1 region polymorphism. *Forest Pathology* 30: 329-344.

Metzler B., Kublin E. 2003. Langzeitwirkung von Stubbenbehandlungen auf das Stockfäulerisiko in Fichten-Erstaufforstungen. AFJZ 174: 81-84.

Metzler B., Thumm H., Scham J. 2005. Stubbenbehandlung vermindert das Stockfäulerisiko an Fichte. AFZ-Der Wald 60: 52-55.

Relations between the area of root rot diseases occurrence (*Heterobasidion annosum* and *Armillaria* spp.) and selected weather components in last 35 years in Poland

O. Mykhayliv[1] and M. Małecka[2]

[1] *National Forestry and Wood University of Ukraine, Lviv, Ukraine.*
[2] *Forest Research Institute, Department of Forest Protection, Sękocin Stary, Braci Leśnej 3, 05-090 Raszyn, Poland.*
Corresponding author e-mail address: m.malecka@ibles.waw.pl

Abstract. The data used for analyses comprised the occurrence of the root rot *Heterobasidion annosum* and *Armillaria* spp. in Poland within 1972-2007 period. The occurrence of these diseases was investigated in forest stands representing age categories up to - and over 20 years as well as different meteorological parameters (precipitations, air and soil temperature in the same period). The relationship between the damage area in an n-year and some weather components in years n-l (where l=0,1,2,3) were investigated with the use of multiple regression analysis following the SAS Enterprise Guide 4. It was demonstrated that meteorological components had decisive effect on disease development. For example, the occurrence of *Heterobasidion annosum* in all age categories of stands depended on air temperature in the previous year. In case of root rot *Armillaria* spp. in old stands, strong correlation with soil temperature in the previous year was found. It was noticed that amount of monthly precipitations had higher influence on threat caused by *Armillaria* spp. than by *Heterobasidion annosum*. Based on the results of this work some prognostic model algorithms were designed to forecast the occurrence of mentioned diseases in Poland.

Introduction

Root diseases are the most important group of diseases occurring in Polish stands, present on the area of about 250 thousand hectares, which represents over 60% of the incidence of all diseases of the forest. In 2010 the total occurrence of root rot diseases was recorded on 262 thousand hectares. In stands younger than 20, root rot diseases are observed on area about 10-13 thousand hectares, in older stands (over 20-year-old) occurrence of *Armillaria* (*sensu lato*) root rot was noticed on 100 thousands hectares, while *Heterobasidion annosum* root rot on 150 thousands hectares. (STF, 2011)

The aim of the study was to determine the relationships between the area of root rot diseases in Polish stands and selected weather components on the basis of the long-term data.

Materials and Methods

The data of root rot diseases occurrence in Poland in years 1975-2007 and meteorological data from the period 1972-2007 were used. Generally, the influence of precipitations and both, air and soil temperature has a complex character on

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

living organisms, so the analysis based on the method of multiple regression (following the SAS Enterprise Guide 4 statistical package, 2007) was chosen.

The equation presents below the linear model of multiple regression (Stanisz, 2007), based on the assessment of the significance of the impact of several independent variables X (in this case - meteorological parameters like temperature, precipitations) on dependent variable Y (in this case - area of disease occurrence).

$$\mathbf{Y= \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k}$$

where:
Y - dependent variable, X - independent variable, βi (i =1, 2,…,k) - parameters of model describing an influence of i - independent variable.

At the same time this model rejects these independent variables, which influence on dependent variable is statistically not significant or the least significant. The statistical analysis of multiple regression allowed to find these weather components, which are significantly correlated with root rot development and to create the most credible model which could be used to predict the scale of threat.

Results

Model of multiple regression for *Armillaria* spp. root rot occurrence in stands over 20 years of age

$$\mathbf{Y= 220{,}7 + 0{,}51\Sigma P_{n-1}(III) + 1{,}1\Sigma P_{n-1}(V) + 0{,}6\Sigma P_{n-1}(VI) + 0{,}6\Sigma P_{n-1}(IX) - 0{,}76\Sigma P_{n-1}(X) + 10{,}37\, Ts_{n-1}(XII) - 21{,}17 Ts_{n-1}(I) + 4{,}61 Ts_{n-1}(II) + 5{,}64 Ts_{n-1}(IV) + 9{,}39 Ts_{n-1}(V) + 12{,}61 Ts_{n-1}(IX) - 17{,}55 Ts_{n-1}(X) + 9{,}77 Ts_{n-1}(XI) - 3{,}1 Sd_{n-1}}$$

where:

ΣP - monthly total precipitation, Ts - monthly mean soil temperature, Sd - annual mean depth of snow cover, (X) - month, $_{n-1}$ - number of years before disease occurrence

The obtained model indicates that the largest impact on threat caused to forests by *Armillaria* root rot had: the sum of precipitations in March, May, June, September and October as well as monthly mean soil temperature of eight months (specified in brackets) and snow cover in previous year. The strongest correlation relationships expressed by high value of coefficient of regression with the level of significance less than 0.0001 was found between root rot area and total precipitation in October and soil temperature in February, November and December in the previous year (Tab. 1).

Table 1. Results of multiple regression for *Armillaria* spp. root rot occurrence

Adjusted coefficient of determination		**R^2 = 0.82**	
***P*-value**		***P*< 0.0001**	
Weather components		***r* - standarized coefficient of regression**	***P* - level of significance of particular weather component**
Precipitation	ΣP_{n-1}(III)	0.24	0.0513
	ΣP_{n-1}(V)	0.44	0.0008
	ΣP_{n-1}(VI)	0.43	0.0143
	ΣP_{n-1}(IX)	0.42	0.0016
	ΣP_{n-1}(X)	-0.72	<0.0001
Soil temperature	Ts_{n-1}(XII)	-0.65	<0.0001
	Ts_{n-1}(I)	0.32	0.0121
	Ts_{n-1}(II)	-0.73	<0.0001
	Ts_{n-1}(IV)	0.19	0.0941
	Ts_{n-1}(V)	0.26	0.0598
	Ts_{n-1}(IX)	0.49	0.0001
	Ts_{n-1}(X)	0.54	0.0004
	Ts_{n-1}(XI)	-0.73	<0.0001
Snow cover	Sd_{n-1}	0.38	0.0124

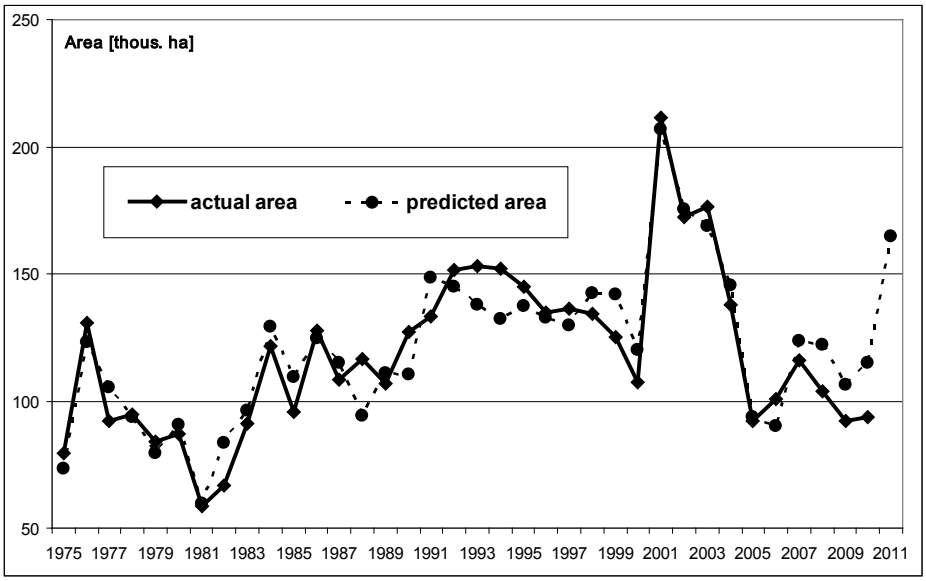


Figure 1. Actual and predicted (basing on model) area of *Armillaria* spp. root rot occurrence in stands over 20 years of age in Poland.

Figure 1 presents the actual and predicted area (based on previously showed model) of *Armillaria* spp. root rot occurrence in younger stands. The course of two lines describing the actual (red line) and predicted area (green one) is convergent, the obtained high value of determination coefficient (R^2= 0.82) with significance level less than 0.0001 indicates the good quality of matching.

Model of multiple regression for *Heterobasidion annosum* root rot occurrence in stands over 20 years of age

$$\mathbf{Y= -448,53 - 9,50T_{n-1}(XII) + 19,09T_{n-1}(IV) + 20,09T_{n-1}(VII) + 0,52\Sigma P_{n-1}(VII) + 0,70\Sigma P_{n-1}(IX)}$$

where:

T - monthly mean air temperature, ΣP - monthly total precipitation, (X) - month, $_{n-1}$ - number of years before disease occurrence

In case of *Heterobasidion annosum* root rot the model presents that the greatest importance in the disease development showed: the air temperature in April, July and December, as well as the precipitation in July and November, both factors in previous year.

Table 2. Results of multiple regression for *Heterobasidion annosum* root rot occurrence

Adjusted coefficient of determination		**R^2= 0.73**	
***P*-value**		***P*< 0.0001**	
Weather components		***r* - standarized coefficient of regression**	***P* - level of significance of particular weather component**
Air temperature	T_{n-1}(XII)	-0.39	0.0004
	T_{n-1}(IV)	0.54	<0.0001
	T_{n-1}(VII)	0.69	<0.0001
Precipitation	ΣP_{n-1}(VII)	0.38	0.0008
	ΣP_{n-1}(IX)	0.31	0.0028

The strongest correlation relationships expressed by a high value of coefficient of regression with level of significance less than 0.0001 was found between *Heterobasidion annosum* root rot area and monthly mean air temperature of April and July in the previous year (Tab. 2).

The credibility of this model is quite high (Fig. 2). The obtained value of determination coefficient (R^2= 0.73) with significance level less than 0.0001 shows a significant correlation relation and indicates that the impact of the analyzed climatic factors is quite strong.

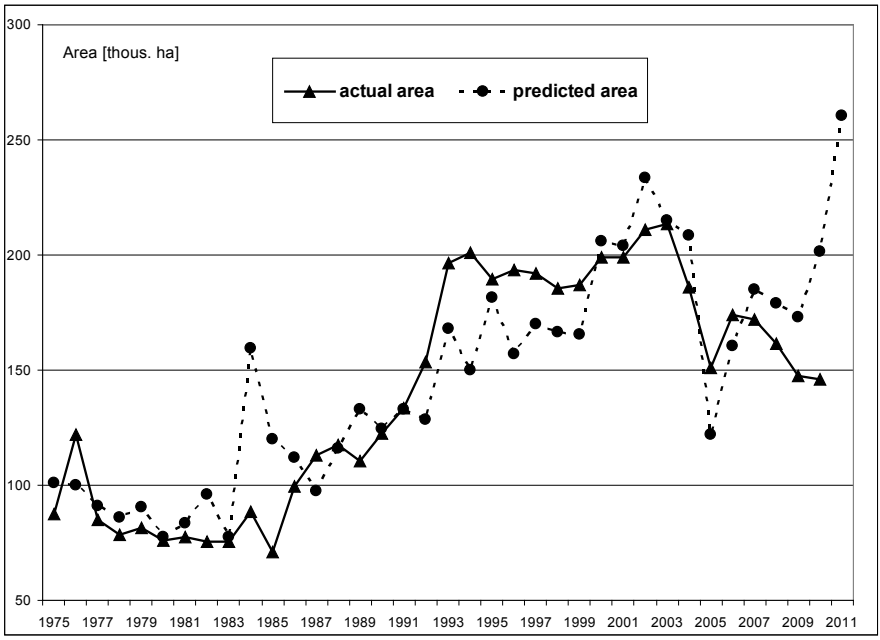


Figure 2. Actual and predicted (basing on model) area of *Heterobasidion annosum* root rot occurrence in stands over 20 years of age in Poland

Final Remarks

The relationships between development of some pathogens and weather conditions were described in some articles (Fedorow, 1984; Korhonen and Stenlid, 1998; Mykhayliv and Sierota, 2010). Presented analysis confirmed the strong correlation between temperature and precipitation anomalies and the increasing area of root rot diseases. The differences between the actual and forecasted surface of diseases occurrence, where deviations (in plus or minus) developed in the range of 2-22% in the case of *Armillaria*, and 3-38% for *Heterobasidion annosum*, were found. The obtained results not correlated in some years (*H. annosum*: 1984, 1994) confirm that the development of roots diseases may also affect not only the meteorological factors, but also some factors initiating or predisposing disease phenomena, e.g. biotic (insects) and anthropogenic (industrial imission) disruption. These factors were not the subject of the analysis in the presented studies. Multiple regression analysis showed a significant impact of weather conditions on root rot incidence area, so the obtained models can be useful for developing short-term forecasts (for 1-3 years) and for practical application in forest protection.

Acknowledgements

Presented results are the part of investigations which are conducted within PROZA project: "Operational decision-making based on atmospheric conditions". This project is co-financed by the EU from the European Regional Development Fund in the framework of Innovative Economy program.

References

Fedorow N.I., 1984. Kornevyje gnili hvojnych porod. Moskva, Lesnaja promyslennost. (in Russian).

Korhonen K., Stenlid J., 1998. Biology of *Heterobasidion annosum*. In: Woodward S., Stenlid J., Karjalainen R., Hütterman A. (eds.). *Heterobasidion annosum* Biology, ecology, impact and control, CAB International, Wallingford, Oxon OX10 8DE, UK, pp. 43-70.

Mykhayliv O., Sierota Z., 2010. Threat caused to forests by the root rot *Heterobasidion annosum* (Fr.) Bref. in relation to soil temperature and precipitation. *Forest Research Papers*, 71: 51-59.

Short-term forecast (STF) of forest's pests and diseases occurrence in 2010 in Poland., 2011. FRI, Sekocin Stary (in Polish).

Stanisz A., 2007. Przystępny kurs statystyki z zastosowaniem STATISTICA PL na przykładach z medycyny. Tom 1, 2. Wyd. 3 StatSoft Polska, Kraków (in Polish).

Armillaria mellea, the causal agent of grapevine root rot, induces a set of defense genes in grapevine roots

M. Perazzoli[1], F. Bampi[1], S. Faccin[1], M. Moser[1], F. De Luca[1], A.M. Ciccotti[1], R. Velasco[1], C. Gessler[2], I. Pertot[1], C. Moser[1]

[1]*Genomics and Biology of Fruit Crops Department, IASMA Research and Innovation Centre, Fondazione Edmund Mach; Via E. Mach 1, 38010 San Michele all'Adige, (TN), Italy.*
[2]*Institute of Plant Science, ETH Zürich, Switzerland.*
Corresponding author e-mail address: claudio moser@iasma.it

Grapevine root rot, caused by *Armillaria mellea*, is a serious disease in some grape-growing regions. Young grapevines start to show symptoms of *Armillaria* root rot from the second year after inoculation, suggesting a certain degree of resistance in young roots. We used a suppression subtractive hybridization approach to study the molecular reaction of rooted cuttings during the first stages of *A. mellea* infection. 24 genes were up-regulated in the roots of the Kober 5BB (*V. berlandieri* × *V. riparia*) rootstock 24 h after *A. mellea* challenge. Real-time RT-PCR analysis confirmed the induction of genes encoding protease inhibitors, thaumatins, glutathione S-transferase, and aminocyclopropane carboxylate oxidase, as well as phase-change related, tumor-related and proline-rich proteins, and gene markers of the ethylene/jasmonate-signaling pathway. Gene modulation was generally stronger in Kober 5BB than in plants of the cultivar Pinot Noir, and *in vitro* inoculation induced higher modulation than greenhouse *Armillaria* treatments. The full-length coding sequences of seven of these genes were obtained, expressed as recombinant proteins and assayed for their activity against *A. mellea*. The grapevine homologue of the *Quercus* phase change-related protein inhibited the growth of *A. mellea* mycelia *in vitro*, suggesting that this protein may play an important role in the defense response against this fungus.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Distribution and impact of *Phellinus* root disease in the southern Interior of British Columbia - A first approximation of disease impacts for timber supply review.

M. Cleary[1] and R. Sturrock[2]

[1]*Department of Forest Mycology and Pathology, Swedish University Agricultural Sciences (SLU), Uppsala, Sweden.*
[2]*Canadian Forest Service-Pacific Forestry Centre, Victoria, British Columbia, Canada.*
Corresponding author e-mail address: michelle.cleary@telia.com

Abstract. *Phellinus sulphurascens* (syn. *P. weirii*), the cause of Laminated root disease, occurs across a variety of ecosystems in southern British Columbia (BC), Canada, where it causes mortality and growth loss in natural and planted stands of Douglas-fir. Though impacts on timber volume and growth have been demonstrated for the coastal region of BC, and in Washington and Oregon, U.S.A., much less is known about the distribution and impact of *Phellinus* root disease in BC's southern interior. Landscape-level surveys were conducted across 6 Timber Supply Areas (TSAs) using a network of Douglas-fir-leading growth and yield permanent sample plots. Interior wetbelt forests showed the highest impacts caused by both *Phellinus* and *Armillaria* root diseases however, these stands also have some of the highest volumes. Operational adjustment factors (OAF) are used by growth and yield models as input parameters to adjust (reduce) predicted yield to account for non-productive areas within stands, gaps, decay, and endemic pests and diseases. This work suggests an additional reduction of up to 20% in some ecosystems to account for existing and future managed Douglas-fir stands suffering volume losses due to root disease.

Phellinus sulphurascens (syn. *P. weirii*), occurs across a variety of ecosystems in southern British Columbia (BC), Canada, where it causes mortality and growth loss in natural and planted stands of Douglas-fir. Currently, its presence in mature forests is not being properly acknowledged in yield forecasts because of the lack of information on long-term impacts. Though impacts on timber volume and growth have been demonstrated for the coastal region of BC, and in Washington and Oregon, USA (Bloomberg and Reynolds 1985, Bloomberg and Wallis 1979) much less is known about the distribution and impact of Phellinus root disease (DRL) in BC's southern interior.

In timber supply planning, Operational adjustment factors (OAFs) are applied to potential yields generated by growth and yield (G&Y) models with inherent assumptions to make them reflect an operational environment, to net down potential yields as a result of, e.g. non productive areas and losses in timber productivity resulting from the presence of root disease. Landscape-level surveys were conducted across five Timber Supply Areas (TSAs) (Fig. 1) using a network of established Douglas-fir-leading G&Y permanent sample plots (PSPs) in two biogeoclimatic zones to determine the distribution and incidence of root disease. Inventory datasets were then used to determine volumes for trees in plots.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

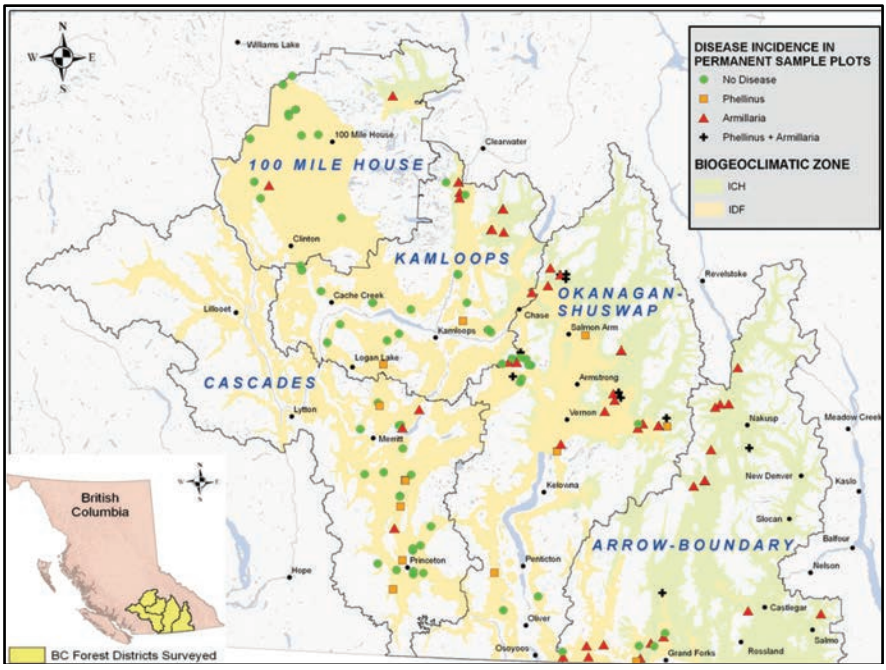


Figure 1. Map showing TSAs and PSPs surveyed in the southern interior BC

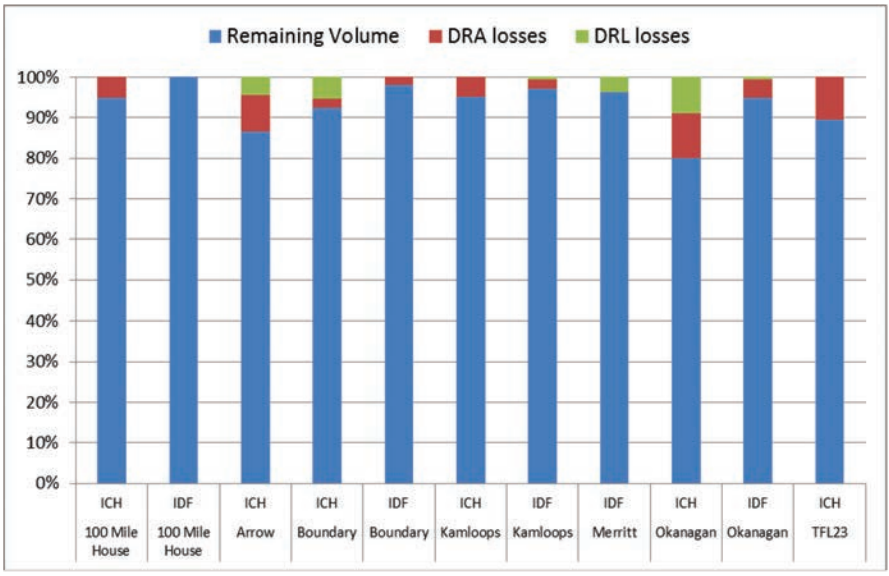


Figure 2. Whole tree volume losses (m^3 ha^{-1}) and remaining whole tree volume *for all PSPs* since the last G&Y re-measurements for DRA and DRL by TSA and biogeoclimatic zone.

Disease incidence varied across ecosystems and TSAs. Armillaria root disease (DRA) is distributed more widely and comprised a greater percent infection rate than DRL. Preliminary losses estimates in plots based only on trees tallied as alive in the previous re-measurement; (i.e. trees which have in since died from root disease or have become infected), suggest volume losses of up to 20% for some ecosystems (Fig. 2). Douglas-fir suffered the greatest losses from DRL and DRA root disease with up to 34.1% of the mature Douglas-fir tallied as dead or infected.

Results should enable forest managers to develop more effective disease management strategies that might reduce losses in timber volume and value. Further refinement of root disease OAFs into models are required.

References

Bloomberg W.J., Reynolds G., 1985. Growth loss and mortality in laminated roor rot infection centers in second-growth Douglas-fir on Vancouver Island. *Forest Science* 3: 497-508.

Bloomberg W.J., Wallis G.W., 1979. Comparison of indicator variables for estimating growth reduction associated with *Phellinus weirii* root rot in Douglas-fir plantations. *Canadian Journal Forest Research* 9: 76-81.

Inoculum of *Heterobasidion parviporum* on stump-harvested sites

T. Piri

Finnish Forest Research Institute, Vantaa Research Unit, PO Box 18 (Jokiniemenkuja 1), FI-01301 Vantaa, Finland.
Corresponding author e-mail address: tuula.piri@metla.fi

Abstract. Removal of infected stumps and large roots from Norway spruce sites infested by *Heterobasidion* root rot (*Heterobasidion parviporum*) eliminates most inoculum and reduces infections in subsequent stands. However, the extent to which leftover woody debris supports *Heterobasidion* and acts as a source of infection in the next tree generation is unknown. In order to understand more about the efficacy of stump removal as a means of controlling *Heterobasidion* root rot, the survival of mycelium in root fragments and its ability to infect Norway spruce seedlings were investigated under field conditions. Root fragments naturally infected by a known strain of *H. parviporum* were buried in forest soil just below the surface close to recently planted 2-year-old Norway spruce seedlings. In the majority (60%) of roots, *H. parviporum* perished after 12 months. However, mycelium survived in ca. 20% of roots after six years and 8% of roots with live mycelium exhibited small fruit bodies. We confirmed the vegetative spread of *H. parviporum* by identifying the same genotype in the buried root fragments and adjacent spruce seedlings. The first seedlings were infected after ca. 4.5 years. Although the experiment continues, results to date indicate that even small roots (e.g.,15 mm diameter) remaining on the harvested area may support infective inoculum for at least six years. In addition, the occurrence of fruit bodies on root residues might increase the risk of spore infection of spruce seedlings on stump-harvested sites.

Since 2001, tree stumps have been increasingly used as fuel-wood in Finland. Stumps are mainly harvested after clear felling of coniferous stands dominated by Norway spruce (*Picea abies*). In addition to fuel production, stump-harvesting aims to reduce the spread of root rot caused by *Heterobasidion annosum s.l.* to subsequent generations. While there is evidence to suggest that removal of infected stumps and large roots eliminates most inoculum and reduces the risk of future infections, the extent to which leftover woody debris harbors *Heterobasidion* and acts as a source of infection is unknown. The objective of this study was to evaluate the efficacy of stump removal as a means of controlling *Heterobasidion* root rot and investigate the role played by root residues in the persistence and transmission of this disease.

Survival of *Heterobasidion* mycelium in broken root pieces and its capability to infect nearby Norway spruce seedlings were investigated under field conditions. Root fragments naturally infected by a known strain of *Heterobasidion parviporum* were buried in forest soil just below the surface and close to recently planted 2-year-old Norway spruce seedlings. Root fragments were an average length of 20 cm, 1-20 cm in diameter, and varied from incipient decay to soft wood in an advanced state of decay.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

To date, results obtained from 12 experimental plots suggest that ca. 60% of inoculated roots were free of *H. parviporum* after 1 year. However, after six years mycelium survived in ca. 20% of roots and live mycelium was observed with small fruit bodies in 8%. Roots in an early stage of decay sustained *H. parviporum* longer than those that were more decomposed.

The first seedlings were infected ca. 4.5 yrs after the experiment began. In all, 4.6% of 174 spruce seedlings were infected with *H. parviporum*. However, disease prevalence varied greatly among experimental plots. On three plots, no infected seedlings were detected. On four plots, the frequency of infected seedlings was 2.8-20.8%. The highest frequency was on the oldest plot (established for 6.9 yrs) and lowest on the youngest plot (4.5 yrs). No correlation was found between the abundance of infective roots and the frequency of infected seedlings. One quarter of seedling infections were fatal. We confirmed the vegetative spread of *H. parviporum* by identifying the same genotype in root fragments and adjacent seedlings.

Results suggest that the size of the root fragment does not be a major factor affecting the persistence of *H. parviporum*; even in roots of 15 mm diameter, the fungus remained viable for at least six years and was able to infect vegetatively a nearby seedling. The observation of fruit bodies on root residues confirms the risk of spore infection on stump-harvested sites. Consequently, control of root rot by stump removing in heavily infested sites requires efforts and might not lead to the desirable result. The long-term field experiment continues and will eventually yield more information concerning stump removal and transmission of the disease to the next generation.

Precommercial thinning stumps of Norway spruce; the influence of stump height on spore infection by *Heterobasidion* spp. and the efficacy of stump treatment with *Phlebiopsis gigantea*

A. Gunulf, R. Mc Carthy, J. Rönnberg

Southern Swedish Forest Research Centre, SLU, P.O. Box 49, SE-230 53 Alnarp, Sweden.
Corresponding author e-mail address: anna.gunulf@slu.se

Abstract. The potential of a new precommercial thinning technique to influence infection by *Heterobasidion* spp. was studied. As an attempt to reduce the precommercial thinning costs and increase the timber quality a new technique is being developed in Sweden. Instead of cutting the precommercial thinning trees at a normal low height, 15-20 cm, the new technique suggests they are cut 71-132 cm above ground. To investigate if there were any differences in infection frequency from airborne *Heterobasidion* spp. spores on Norway spruce stumps depending on stump height (15 cm or 1 m), five Norway spruce dominated sites in southern Sweden were precommercially thinned. The effect of stump treatment of small Norway spruce stumps by *Phlebiopsis gigantea* on infection by *Heterobasidion* spp. was also investigated. In all 600 young Norway spruce trees, with diameters between 2-14 cm, were felled, 300 stumps were high and 300 low, half of each height category was stump treated. Two months after the trees were cut infection by *Heterobasidion* spp. was investigated. The height of the precommercial thinning stump did not affect the infection frequency for the treated nor for the untreated stumps. Treated stumps had a significantly lower infection frequency compared to untreated stumps, still 31% of the treated stumps were infected. Using binary regression we found that the probability of infection increased with increasing diameter of the stump for the untreated stumps (P= 0.016), this relationship did not exist for the treated stumps.

To reduce precommercial thinning (PCT) costs and increase timber quality a new technique, suggesting PCT tress to be cut 71-132 cm above ground instead of the normal 15-20 cm, has been developed. Differences of infection by *Heterobasidion* spp. on Norway spruce (*Picea abies*) stumps depending on stump height (15 cm or 1 m) and the effect from stump treatment, was investigated in five Norway spruce dominated sites established on former forested land in southern Sweden. On every site 60 high and 60 low stumps, with diameters 2-14 cm, were subjected to natural spore deposition. Half of the stumps of each height category were manually treated with *Phlebiopsis gigantea* (Rotstop®S). After two months stumps were sampled by cutting a 5 cm disc 1 cm below the stump surface. Presence and size of infection were registered on both sides of discs.

The height of the PCT stump did not affect infection frequency or relative infected area (Tab. 1). Treated stumps had a significantly lower infection frequency (P< 0.001) compared to untreated stumps (Tab. 1), still 31% of the treated stumps were infected. On infected stumps, the relative infected area was significantly smaller on treated stumps than on untreated (P= 0.008). Treated PCT stumps had a significantly higher efficacy in reducing infection frequency than the untreated

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

high cut (Tab. 1). Efficacy in reducing relative infected area (only infected stumps included) was highest for low treated stumps which differed significantly from high untreated stumps (Tab. 1). Probability of infection increased with increasing stump diameter for the untreated stumps (P= 0.016, binary regression), this relationship did not exist for the treated stumps.

The height of the PCT stump of Norway spruce does not have a substantial effect on early stages of *Heterobasidion* spp. infection, but the influence on secondary infection is unknown. Although stump treatment with *P. gigantea* reduced infection frequency as well as relative infected area, efficacy was low compared with studies dealing with the larger and older stumps from commercial thinnings (Berglund and Rönnberg, 2004; Thor and Stenlid, 2005). Consequently a study on the effect of size and age of stumps on control efficacy of *P. gigantea* treatment is of interest.

Table 1. The effect of precommercial thinning stump height and stump treatment on infection by *Heterobasidion* spp.

Stump type	Infection frequency (%)[y]	Relative infected area of infected stumps (%)[y]	Efficacy on infection frequency (%) (95% CI)[z]	Efficacy on relative infected area of infected stumps (%) (95% CI)[z]
Low Untreated	58.1 a	8.1 a	Not appl.	Not appl.
High Untreated	52.0 a	15.2 a	11.1 b (-2.5 ; 24.7)	-77.5 b (-143.6 ; -11.4)
Low Treated	34.0 b	4.0 b	44.8 a (31.2 ; 58.4)	43.5 a (-22.6 ; 109.6)
High Treated	28.7 b	4.3 b	60.0 a (42.4 ; 69.6)	35.8 ab (-30.3 ; 101.9)

Means within columns that do not share a letter are significantly different

[y]Statistics based on factorial design with sites as block

[z] CI, confidence interval. All confidence intervals that do not cover 0 are significantly different (P< 0,05) from the control i.e. the low untreated

References

Berglund M., Rönnberg J., 2004. Effectiveness of treatment of Norway spruce stumps with *Phlebiopsis gigantea* at different rates of coverage for the control of *Heterobasidion*. *Forest Pathology* 34: 233-243.

Thor M., Stenlid J., 2005. *Heterobasidion annosum* infection of *Picea abies* following manual or mechanized stump treatment. *Scandinavian Journal of Forest Research* 20: 154-164.

Biological control against *Heterobasidion annosum* root rot in coniferous stands in Hungary

A. Koltay[1], T. Lakatos[2], T. Tóth[2], Z. André[3]

[1]*Forest Research Institute, Department of Forest Protection, Hungary, 3232 Mátrafüred, Hegyalja u. 18. 18 3232, Hungary.*
[2]*Nemaform Research and Service Ltd., Hungary, 4320 Nagykálló, Jókai Mór u. 37. Hungary*
[3]*NEFAG Plc., Monor Forestry, Hungary, 2200 Monor Petőfi u. 17. Hungary, 3232 Mátrafüred, Hegyalja u. 18. Hungary.*
Corresponding author e-mail address: koltaya@erti.hu

In forest-ecosystems it is very important to apply selective and biological control technologies against pests and pathogens. Such method in forestry is the usage of antagonistic fungi or other micro-organisms. One of the most dangerous pathogens of conifers in Hungary is the *Heterobasidion annosum* root rot. Formerly Dr. Hubert Pagony applied successfully a biological control agent against it in Scots pine (*Pinus sylvestris*) forests. However, these research achievements fell into oblivion, although the pathogen is still present in our forests. In the past few years we re-developed this method, by the usage of *Phlebiopsis gigantea*, and rearranged it according to present-day requirements. We were able to produce an inoculatum, which is suitable for industrial-scale usage and manufacturing. Our experiments so far evidently claimed that this method can open new perspectives in root rot control in Scots pine and Austrian pine forests. However, the unsuccessful inoculation experiments in Norway spruce stands show that the technology in its current form is not suitable for the prevention of root rot in spruce forests.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Evaluation of presence of *Phlebiopsis gigantea* in Scots pine stumps after treatment with EU commercial preparations

A. Żółciak, M. Małecka, Z. Sierota, K. Sikora

Department of Forest Protection, Forest Research Institute Braci Leśnej 3, 05-090 Raszyn, Poland.
Corresponding author e-mail address: a.zolciak@ibles.waw.pl

Abstract. The study was conducted in northern and eastern Poland (20 forest districts). In spring and autumn 2007-2008 pine stumps were inoculated with four preparations of *P. gigantea*: Rotstop F, Rotstop S (Finland), PG Suspension (Great Britain) and Pg IBL (Poland). Presence of i) *P. gigantea* subcortical mycelium and fruit bodies on stumps and ii) *P. gigantea* mycelium in stumps sapwood (PCR analysis) was assessed one year after treatments. The effectiveness of these preparations was calculated both: as per cent of colonized stumps and as BTE index (Biological Treatment Efficacy = $\Sigma(a+b+c)/3N$, where a - number of stumps with subcortical mycelium, b -number of stumps with fruit body, c - number of stumps with sapwood decay, N - number of assessed stumps. BTE=1 means that inoculation was successful and all assessed stumps were characterized by mycelium and fruit bodies and sapwood decay. The results were analysed with the use of ANOVA and logistic regression analysis STATGRAHICS© Centurion XV. It was showed that spring 2008 was the best timing of application, generally for Finnish and English products, while Polish preparation gave the best results when applied in autumn 2007. The highest effectiveness measured by presence of mycelium revealed the isolates in Rotstop S and Rotstop F while the highest frequency of fruit bodies was found on stumps treated with PgIBL. Molecular analysis confirmed the identity of applied isolates of *P. gigantea.* Values of BTE index confirmed, that PG Suspension preparation is most effective if applied in spring 2008. Results of the logistic regression analysis showed that the occurrence frequency of PG Suspension subcortical mycelium on stumps was on the same level as Polish preparation. PG Suspension revealed lower activity (-25%), than PgIBL in case of fruit body frequency.

Introduction

Until 2010 three commercial products with *Phlebiopsis gigantea* (Fr.: Fr.) Jülich isolates were applied in the forest practice in Europe - Rotstop, PG Suspension and Pg IBL. The differences between them were following: i) manner of preparation, ii) form of application and iii) the effect in the forest (Pratt *et al.*, 2000).

According to Directive 91/414/EEC it has obliged to use preparations with registered active substances (in this case isolates of *P. gigantea*) only. It has resulted in registration 10 isolates of this fungus from Great Britain and 4 from Finland. It means that only preparations with these isolates can use against root rot in European forests.

The aim of presented study was assessment the degree of Scots pine stumps colonization by *P. gigantea* one year after treatment with EU commercial preparation with this fungus.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Materials and Methods

The experimental plots (39) were situated in 20 Forest Districts in about 40-years old Scots pine stands. In autumn 2007, 2008 and spring 2008 the thinning was performed and 11.7 thousand stumps were inoculated by manual treatment with three commercial *P. gigantea* preparations: Rotstop F (Finnish isolate No 21,434), Rotstop S (Swedish isolate No 00247), PG Suspension (English isolate No 390,101) and Pg IBL (not registered isolate from Poland) to compare.

Presence of i) *P. gigantea* subcortical mycelium and fruit bodies on stumps, and ii) *P. gigantea* mycelium in stumps sapwood (PCR analysis) was assessed one year after treatments. The effectiveness of these preparations was calculated both: as per cent of colonized stumps and as Biological Treatment Efficacy index (BTE index):

$$\mathbf{BTE = \Sigma(a+b+c)/3N}$$

where:
a - number of stumps with subcortical mycelium,
b - number of stumps with fruit body,
c - number of stumps with sapwood decay,
N - number of assessed stumps.

BTE = 1 means that inoculation was successful and all assessed stumps were characterized by mycelium and fruit bodies and sapwood decay. The results were analyzed with the use of ANOVA and logistic regression analysis STATGRAHICS© Centurion XV.

Results

It was showed that spring 2008 was the best timing of application (Polish preparation gave the best results when applied in autumn 2007) (Fig. 1). The highest effectiveness measured by presence of mycelium revealed the isolates in Rotstop S and Rotstop F while the highest frequency of fruit bodies was found on stumps treated with Pg IBL (Fig. 1, 2). Molecular analysis of mycelium isolated from stumps confirmed the identity with applied isolates of *P. gigantea.* Values of BTE index showed that PG Suspension preparation was most effective if applied in spring 2008 (Fig. 3).

As part of the logistic regression analysis the odds ratio (OR) was calculated; it shows how much more (OR>1) or less (OR<1) observations of the characteristics (mycelium, fruiting body) was observed in a variant of the experience (Tab. 1).

Results of this analysis showed that the incidence of PG Suspension subcortical mycelium on stumps was on the same level as Polish preparation (OR=1.02551) while Rotstop S and Rotstop F showed better activity than Pg IBL in case of subcortical mycelium {respectively +39% and +29% [value odds ratio (OR) 1.39

and 1.29 respectively]. Rotstop F and PG Suspension revealed lower activity than Pg IBL in case of fruit body frequency [respectively -58% and -25% (OR = 0.42 and 0.75)]. It was observed that the most advantageous time to produce mycelium was the autumn 2008 (OR value assigned to the remaining two periods less than 1), while for fruiting bodies - the spring time (value of the odds ratio for this period amounted to 2.40).

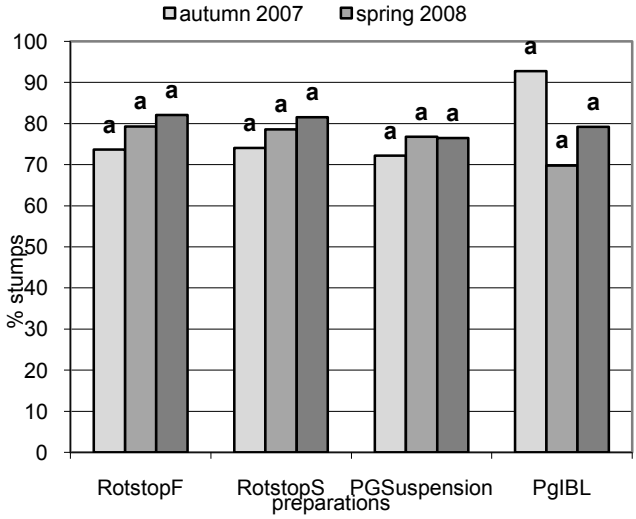


Figure 1. Comparison of stumps settlement by *P. gigantea* (autumn 2007, spring 2008 and autumn 2008; considering four preparations) for subcortical mycelium.

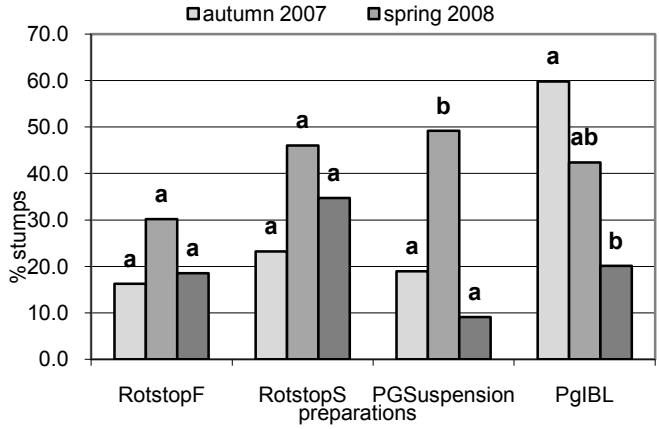


Figure 2. Comparison of stumps settlement by *P. gigantea* (autumn 2007, spring 2008 and autumn 2008; considering four preparations) for fruit body.

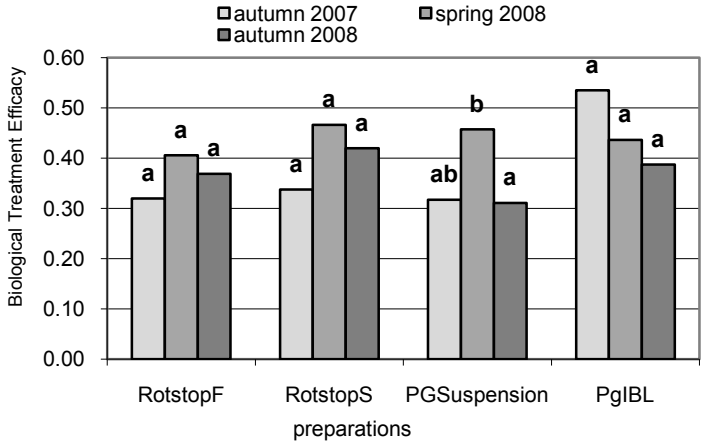


Figure 3. Comparison of stumps settlement by *P. gigantea* (autumn 2007, spring 2008 and autumn 2008; considering four preparations) for BTE index.

Table 1. Logistic regression analysis (program STATGRAPHICS™ Centurion) (reference level: PgIBL, autumn 2008).

Parameter of evaluation	Preparation				Date of treatment		
	Rotstop F	Rotstop S	PG Suspension	PgIBL	Autumn 2007	Spring 2008	Autumn 2008
Subcortical mycelium	1.29478 (+29%)	1.39195 (+39%)	1.02551	x	0.49219 (-51%)	0.63538 (-36%)	x
Fruit body	0.42243 (-58%)	0.92396	0.74707 (-25%)	x	0.94913	2.39998* (+140%)	x

Final Remarks

1. The assessment of the presence of mycelium in protected stumps compared with the results of both mycological analyses and logistic regression, points out to the Rotstop preparations as the most efficient in application to pine stumps in climatic circumstances of treated Scots pine stands in the period 2007-2009.
2. Rotstop F and S showed good results both for spring and autumn treatments, while PG Suspension - only for spring treatment.

Acknowledgements

We thank: dr Marina Niemi from Verdera (Finland) and dr Katherine Tubby from the Forestry Commission (Great Britain) for Finnish and English preparation of *P. gigantea*.

References

Pratt J.E., Niemi M., Sierota, Z.H., 2000. Comparison of three products based on *Phlebiopsis gigantea* for the control of *Heterobasidion annosum* in Europe. *Biocontrol Science and Technology* 10: 467-477.

Efficacy testing of Latvian *Phlebiopsis gigantea* strains

K. Kenigsvalde[1], K. Korhonen[2], T. Gaitnieks[1]

[1]*Latvian State Forest Research Institute „Silava", 111, Rigas Street, Salaspils, LV-2169, Latvia.*
[2]*Finnish Forest Research Institute, PO Box 18, FI-01301 Vantaa, Finland.*
Corresponding author e-mail address: kristine.kenigsvalde@inbox.lv

Abstract. Biological control agent Rotstop® was registered in Latvia in 2007 for stump treatment in thinnings of spruce and pine. From the year 2014 Rotstop will be used also in final cuttings. The *P. gigantea* strain in Rotstop preparation is of Finnish origin. In Latvia, it would be better to use native strains in order to diminish the effect of the Rotstop genotype on the genetic diversity of local *P. gigantea* populations. Since 2007 more than 100 strains of *P. gigantea* have been collected from different parts of Latvia. Variables like growth rate, antagonism against *Heterobasidion,* and production of oidia were tested in laboratory, and experiments in log pieces of spruce and pine were carried out to assess the growth rate and efficacy of different strains. For the determination of growth rate in wood, small pits were carved on the upper surface of a log piece and inoculated with different strains. With this method it was possible to analyse up to 15 strains in the same log piece. Growth rate in spruce wood was estimated for 59 strains. Three Latvian *P. gigantea* strains were selected that did not show significant differences in their properties as compared with Rotstop. Their efficacy against *Heterobasidion* was tested in stumps of spruce and pine.

Biological control agent Rotstop® was registered in Latvia in 2007 for stump treatment in thinnings of spruce and pine. From the year 2014 Rotstop will be used also in final cuttings. The *P. gigantea* strain in Rotstop preparation is of Finnish origin (Korhonen *et al.,* 1994). In Latvia, it would be better to use native strains in order to diminish the effect of the Rotstop genotype on the genetic diversity of local *P. gigantea* populations (Vainio *et al.,* 1994).

Since 2007 more than 100 strains of *P.gigantea* have been collected from different parts of Latvia. Variables like growth rate, antagonism against *Heterobasidion,* and production of oidia were tested in laboratory, and experiments in log pieces of spruce and pine were carried out to assess the growth rate and efficacy of different strains. For the determination of growth rate in wood, small pits were carved on the upper surface of a log piece and inoculated with different strains. With this method it was possible to analyse up to 15 strains in the same log piece. Growth rate in spruce wood was estimated for 59 strains. Three Latvian *P. gigantea* strains were selected that did not show significant differences in their properties as compared with Rotstop. Their efficacy against *Heterobasidion* was tested in stumps of spruce and pine.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

References

Korhonen K., Liponen K., Bendz M., Johansson M., Ryen I., Venn K., Seiskari P., Niemi M., 1994. Control of *Heterobasidion annosum* by stump treatment with Rotstop, a new commercial formulation of *Phlebiopsis gigantea*. - In: *Proceedings of Eighth IUFRO Conference on Root and Butt Rots. (*Johansson M., Stenlid J., eds.) Sweden/Finland. August 9-16, 1993. Swedish University of Agricultural Sciences, Uppsala, Sweden, pp. 675-685.

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

Efficacy of *Phlebiopsis gigantea* against *Heterobasidion spp.* on hybrid larch stumps *in situ*

L. Wang, J. Rönnberg, E. Ek

Southern Swedish Forest Research Center, SLU, P.O. Box 49, SE-23053, Alnarp, Sweden.
Corresponding author e-mail address: liying.wang@slu.se

Abstract. Stumps of hybrid larch (*Larix* × *eurolepis*) are not commonly treated with *Phlebiopsis gigantea* as is normal practice for Norway spruce in Sweden. In a study in southern Sweden the efficacy of *P. gigantea* treatment against *Heterobasidion* spp. was investigated. Woody spore traps of hybrid larch (HL), Norway spruce (NS) and Scots pine (SP) were used to detect ambient spores. Spore traps were exposed for 2-3 hours, and HL stumps were sampled after two months. The frequency, relative area and number of *Heterobasidion* infections were all smaller on *P. gigantea* treated than on the untreated HL stumps. Infections of both *H. annosum* and *H. parviporum* were found. SP trap discs had the highest infection frequency and the largest relative infected area, followed by NS and HL. *H. annosum* germinated more frequently on SP traps than NS or HL traps however, no such preference was shown for *H. parviporum*. The relative infected area of untreated HL stumps was positively correlated to HL spore traps but not to SP or NS traps. Treatment of HL stumps with *P. gigantea* was clearly effective against *Heterobasidion spp*. Hence it seems prudent to apply stump treatment when thinning HL stands to prevent primary infection from *Heterobasidion* spp.

Hybrid larch (*Larix* × *eurolepis* Henry.) is very susceptible to infection by *Heterobasidion spp.* (Rönnberg *et al.*, 1999; Vollbrecht and Stenlid, 1999). Freshly created stumps can be an entry point for the pathogen which can subsequently infect residual larch trees (Rönnberg, 2007). However, stumps of hybrid larch are not commonly treated with *Phlebiopsis gigantea* as is normal practice for Norway spruce (*Picea abies*) in Sweden. The objective of this study was to investigate the efficacy of *P. gigantea* in preventing the establishment of infection by *Heterobasidion spp.* on hybrid larch stumps *in situ*.

The experiment was conducted in five young (aged 9-13 years) hybrid larch stands in Skåne, southern Sweden. On each site, 20 stumps were treated with suspension of *P. gigantea* immediately after cutting, and 20 stumps untreated as control. Woody spore traps of hybrid larch, Norway spruce and Scots pine (*Pinus sylverstris*) were used to detect ambient spores. Spore traps were exposed for 2-3 hours, and hybrid larch stumps were sampled after two months.

The frequency, area and total number of *Heterobasidion* infections were all smaller on *P. gigantea* treated hybrid larch stumps than on the untreated stumps (Tab. 1). Infections of both *H. annosum* and *H. parviporum* were found. Scots pine trap discs had the highest infection frequency and the largest relative infected area, followed by Norway spruce and hybrid larch. *H. annosum* germinated more frequently on Scots pine traps than Norway spruce or hybrid larch traps. However, no such preference was shown for *H. parviporum* (Tab. 2). The relative infected

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

area of untreated hybrid larch stumps was positively correlated to hybrid larch spore traps but not to Scots pine or Norway spruce traps.

Treatment of hybrid larch stumps with *P. gigantea* was clearly effective against *Heterobasidion spp. in situ.* Hence it seems prudent to apply stump treatment when thinning hybrid larch, especially on former arable land. It is also recommended to treat hybrid larch stumps to prevent infection by *H. parviporum* in the remaining stand when mixed with Norway spruce or in the next rotation depending on tree species. The cost for performing stump treatment has been estimated to be beneficial in earlier studies (Rönnberg *et al.*, 2007). Infected areas on stumps correlated to the same species of spore traps but not the others. To be accurate, it is suggested to use the same species of spore traps as the stumps investigated to reflect the potential colonizing spores.

Table 1. Incidence, relative and total area of colonies, total and average number of colonies, average area of colonies and control efficacy on Phlebiopsis gigantea treated and control stumps.

	Incidence (%)	**Relative Area (%)**	**Total Infected Area (cm^2)**	**Total Nr. colonies**	**Ave. Nr. colonies**	**Ave. Area (cm^2)**	**Control efficacy (%)**
P.gigantea	10.1 a	0.4 a	1.0 a	2.4 a	1.4 a	0.6 a	84.0 (77.3-90.7)
Control	32.2 b	2.8 b	6.7 b	7.6 b	1.5 a	1.3 b	--
	P= 0.037	*P*= 0.003	*P*= 0.008	*P*= 0.001		*P*= 0.04	
General linear model was used to test the difference between treatment							

Table 2. Frequency of infection, relative infected area, total area of infection and average size of colony of *H. annosum* s. s. (*H. a.)* and *H. parviporum* (*H. p.*) on different species of spore traps*.

Species	**Freq. of Infection (%)**	**Relative Inf. Area (%)**	**Total Inf. Area (cm^2)**			**Ave. Size Colony (cm^2)**		
			H. a.	*H. p.*	total	*H. a.*	*H. p.*	total
Scots pine	98 a	9.6 a	54.1 a	36.1 a	93.2 a	3.2 a	2.5 a	2.8 a
Norway spruce	79 b	3.3 b	11.8 b	11.9 a	24.2 b	1.6 b	0.9 a	1.4 b
hybrid larch	63 b	3.7 b	12.9 b	14.4 a	27.3 b	1.2 b	1.3 a	1.3 b

* Four sites included in the analysis

References

Rönnberg J., Vollbrecht G., Thomsen I.M., 1999. Incidence of butt Rot in a tree species experiment in northern Denmark. *Scandinavian Journal of Forest Research* 14: 234-239.

Rönnberg J., Mårtensson S., Berglund M., 2007. Incidence of *Heterobasidion* root and butt rot in first rotation *Larix* stands and justification for stump treatment. In: Proceedings 12th International Conference on Root and Butt Rots. Garbelotto M., Gonthier P. (eds.). Berkeley, California - Medford, Oregon, USA, pp. 86-89.

Vollbrecht G., Stenlid J., 1999. Transfer of the P-type of *Heterobasidion annosum* from old growth stumps of *Picea abies* to *Picea abies* and *Larix* × *eurolepis*. *European Journal of Forest Pathology* 29: 153-159.

Session 7

New Reports, Diagnostics and Research Applications of Diagnostic Methods

Invasive alien pathogens: new reports

A. Santini and L. Ghelardini

Istituto per la Protezione delle Piante (IPP-CNR), via Madonna del Piano 10, 50019 Sesto Fiorentino Firenze, Italy.
Corresponding author e-mail address: a.santini@ipp.cnr.it

Abstract. Emerging infectious forest plant diseases (caused by fungi, viruses, bacteria and similar organisms) cause significant losses to forest economy and ecology by decreasing the yield and quality of timber and causing losses in biodiversity at species or population level. Main factors able to cause the occurrence of an emerging infective disease are linked to the introduction of alien species into a new environment. For this reason, in the frame of the EU FORTHREATS and ISEFOR projects a comprehensive database on invasive forest fungal pathogens, reported from 20 European countries, has been compiled. Aim of this work was to organize information relative to invasive alien forest pathogens in order to set up a reliable strategy to control the effects of old and new introductions into European territory. Data obtained were able to give a realistic picture of the present situation, of the temporal trends of arrival and of the main factors conditioning the arrivals and the spread of new forest pathogens in Europe.

Introduction

Invasive alien species (IAS) are a significant component of human-caused global environmental changes (Vitousek et al., 1997) and they are responsible for dramatic deleterious effects on biodiversity and large economic costs (Westphal *et al.*, 2008). Introduced species are commonly known as alien species, (also named as "exotic", "non-indigenous", or "non native"). The International Union for Conservation of Nature (IUCN) defines as alien any species that was introduced and became established in a natural or semi-natural ecosystem, is agent of change, and threatens native biological diversity.

During the last decades, the research on invasive species has dramatically increased (Callaway *et al.*, 2006). Considerable evidence indicates a recent and rapid growth in the number and impact of IAS (Mooney and Hobbs, 2000). Trade and economic development increase the number of invasions by alien species. Vilà and Pujadas (2001), for example, found that countries that are more effectively tied into the global trading system tend to have more IAS. Invasion by alien species is positively linked to the development of terrestrial transport networks, migration rates, number of tourists in the country, and trade in commodities (Dalmazzone, 2000). The constant increases in the speed of transportation further favors IAS: the time period during which IAS propagules have to survive the restrictive conditions of transport is significantly shortened, possibly leading to an increase of the inoculum.

Emerging infectious diseases (EIDs) of forest plants (caused by fungi, oomycetes, viruses, bacteria and similar organisms) greatly damage forest economy and ecology by decreasing the yield and quality of timber, causing losses

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

to biodiversity at species or population level, and reducing ecosystem services as a whole.

In ordinary and undisturbed conditions, only a few disease agents are able to cause appreciable damage to the tree component of an ecosystem. The infection is effective and the disease may spread only if the tree is susceptible exactly when environmental conditions are favorable to the development of the pathogen (Dodd *et al*., 2008; Ghelardini and Santini, 2009). Therefore, the relative importance of a particular disease on a tree species is a dynamic state characterized by continuous change. In some cases, an important disease may become unseen when new measures are applied to forest management. In other cases, an unimportant disease may reemerge as damaging as a consequence of changes in forest practices, and still in other cases a completely new disease may emerge as important in a new geographic area (Bandyopadhyay and Frederiksen, 1999).

Emerging infectious diseases of plants challenge plant protection both at the local and global levels. During the last 100 years, the natural barriers such as oceans and mountains, which had always restricted the distribution of the world's biota, have been circumvented as a consequence of human activities, especially international travel and trade, so that alien species are nowadays invading new continents at increasing rate, with substantial disturbance to forest ecosystems and severe socio-economic impact (Liebhold *et al*., 1995).

In forest ecosystems, the main factors responsible for the emergence of new infective diseases are: (1) Arrival of a new pathogen, (2) movement of new virulent strains, including the emergence of a new aggressive strain in an area where the pathogen existed, (3) introduction of new and efficient vectors of a pathogen, (4) immediate and critical alterations of the local climate, and (5) changes in the use of forest species (Bandyopadhyay and Frederiksen, 1999).

Among the factors cited in the literature as drivers of plant EIDs, introductions (56%) and weather conditions (25%) are the most important, followed by farming techniques, changes in the population of vectors, recombination and habitat disturbances (Anderson et al., 2004). In general, the agents of plant EIDs belong more frequently to the taxonomic group of viruses, that account for the 47% of the total, followed by fungi (30%), bacteria (16%), phytoplasma (4%), and nematodes. A special case is represented by forest plants, whose principal EIDs have been historically caused by fungi introduced from a different ecosystem (Anderson *et al.*, 2004).

In the frame of the European Projects FORTHREATS and ISEFOR, a comprehensive database of the forest pathogens invasive in 20 European countries since the beginning of the Nineteenth Century (the century when forest pathology is considered to be born thanks to the studies by Robert Hartig) was compiled. The aim of this work was to gather and organize the information relative to invasive alien forest pathogens in order to set up a reliable strategy to control the effects of old and new introductions into the European territory.

Results and Conclusions

Ascomycetes were the most frequent class of introduced invasive forest pathogens, and their arrivals increased from the 1980s to the 2000s. Chromista were the second most frequent class, whose introduction rate was constant until 1990 but registered afterwards a booming increment. Expanding European pathogens were the main threats until the1940s; then North America and Asia were the main source of alien pathogens. An important recent phenomenon is the increasing appearance of new pathogens originated by natural hybridisation of resident and introduced species. All introductions occurred unintentionally, chiefly by trade of living plant material. Amenity trees are the most affected by alien fungi, followed by forest trees and nursery material. The introduced pathogens agents of canker above all, followed by foliar diseases and, especially in recent times, root rots. Root rots are increasing since the beginning of the 1990s.

Many of the introduced forest pathogens are able to cause lethal diseases. However none of the host species was lead to extinction by invasive alien pathogens, although many hosts suffered heavy numeric reduction or phenotypic modifications. Even if the link between invasions by alien pathogens and extinction of native hosts is widely accepted by scientists and conservationists, invasion as a cause of extinction is in most cases supported only by anecdotic or speculative data based upon limited observation (Gurevitch and Padilla, 2004). Hence, as a general conclusion, alien pathogens are not responsible for widespread extinction or severe genetic depauperation, unless their effect is combined with other disturbances. Nevertheless, alien pathogens can cause the extinction of small and marginal populations that may be precious reserves of genetic diversity. The threat by alien pathogens should therefore be intended at germplasm rather than at species or genus level. For this reason, these results should urge forest managers, ecologists and conservationists to fasten their attention on small marginal or fragmented populations, and to set up specific strategies of in situ and ex situ conservation.

Acknowledgements

The research has received funding from the European Union Sixth and Seventh Framework Programme (FP6 and FP7) under grant agreements 044436 FORTHREATS and 245268 ISEFOR, respectively.

References

Anderson P.K., Cunningham A.A., Patel N.G., Morales F.J., Epstein P.R. and Daszak P., 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *TRENDS in Ecology and Evolution* 19: 535-544.

Bandyopadhyay R., Frederiksen R.A., 1999. Contemporary Global Movement of Emerging Plant Diseases. *Annals of the New York Academy of Sciences* 894: 28-36.

Callaway R.M., Miao S.L., Guo Q., 2006. Are trans-Pacific invasions the new wave? *Biological Invasions* 8: 1435-1437.

Dalmazzone S., 2000. Economic factors affecting vulnerability to biological invasions. In: C. Perrings, M. Williamson and S. Dalmazzone (Eds.), The Economics of Biological Invasions, Edward Elgar, Cheltenham, UK, pp. 17-30.

Dodd R.S., Hüberli D., Mayer W., Harnik T.Y., Afzal-Rafii Z., Garbelotto M., 2008. Evidence for the role of synchronicity between host phenology and pathogen activity in the distribution of sudden oak death canker disease. *New Phytologist* 179: 505-514

Ghelardini L., Santini A., 2009. Avoidance by early flushing: a new perspective on Dutch elm disease research. *iForest* 2: 143-153 [online: 2009-07-30] http:// www.sisef.it/iforest/show.php?id=508

Gurevitch J., Padilla D.K., 2004. Are invasive species a major cause of extinctions? *TRENDS in Ecology and Evolution* 19: 470-474.

Liebhold A.M., Macdonald W.L., Bergdahl D., Mastro V.C., 1995. Invasion by exotic forest pests-a threat to forest ecosystems. *Forest Science* 41: 1-49.

Mooney H.A., Hobbs R.J., 2000. Invasive Species in a Changing World. Island Press, Washington, DC.

Vilà M., Pujadas J., 2001. Socio-Economic Parameters Influencing Plant Invasions in Europe and North Africa. In: J.A. McNeely (Ed.), The Great Reshuffling: Human Dimensions of Alien Invasive Species. IUCN, Gland, Switzerland, pp. 75-78.

Vitousek P.M., D'Antonio C.M., Loope L.L., Rejmanek M., Westbrooks R., 1997. Introduced species: a significant component of human caused global change. *New Zealand Journal of Ecology* 21:1-16.

Westphal M.I., Browne M., MacKinnon K., Noble I., 2008. The link between international trade and the global distribution of invasive alien species. *Biological Invasions* 10: 391-398.

Distribution, host preference and pathogenicity of *Armillaria* species on conifers in Japan

E. Hasegawa[1], Y. Ota[2], T. Hattori[1], N. Sahashi[2], T. Kikuchi[2]

[1]*Kansai Research Center, Forestry and Forest Products Research Institute (FFPRI), Kyoto 612-0855, Japan.*
[2]*FFPRI, Tsukuba, Ibaraki 305-8687, Japan.*
Corresponding author e-mail address: haseg@ffpri.affrc.go.jp

Abstract. Distribution, host preference and pathogenicity of Japanese *Armillaria* species on conifers were investigated on the basis of field collections of 65 isolates. We identified seven *Armillaria* species from 19 conifer species using mating tests and sequences of the translation elongation factor-1α gene. *Armillaria mellea* (Vahl: Fr.) P. Kumm., *Armillaria ostoyae* (Romagn.) Herink (=*Armillaria solidipes* Peck), *Armillaria cepistipes* Velen. and *Armillaria sinapina* Bérubé & Dessur. were frequently collected, whereas *Armillaria nabsnona* T.J. Volk & Burds., *Armillaria tabescens* (Scop.) Emel and a biological species Nagasawa's E were rare. Host conifer species included the major Japanese plantation conifers: *Cryptomeria japonica* (L. f.) D. Don, *Chamaecyparis obtusa* (Siebold & Zucc.) Siebold & Zucc. ex Endl. and members of *Pinus*, *Larix*, *Abies*, and *Picea*. On the basis of host condition when the isolates were collected, *A. mellea*, *A. ostoyae*, *A. cepistipes* and *A. tabescens* were considered as moderate to aggressive pathogens of conifers in Japan.

Introduction

In Japan, there are 16 genera, 37 species of indigenous conifers. Some of them and a few introduced conifers are utilized for plantation. Forest plantations cover 10,000,000 ha and they are mostly conifers. Although damages by Armillaria root disease on conifers have been frequently reported, there have been very few reports on ecology of *Armillaria* species on conifers in Japan. The present study was conducted to reveal distribution, host preference and pathogenicity of Japanese *Armillaria* species on conifers on the basis of collection records of the isolates.

Materials and Methods

Armillaria isolates were collected based on information on the occurrence of Armillaria root disease provided by local researchers and by surveys in apparently healthy forest. Isolates studied included stock cultures in the Laboratory of Forest Plants and Forest Health in the Department of Forest Science, the University of Tokyo, and the Forest Pathology Laboratory and Microbial Ecology Laboratory in FFPRI. The biological species of each isolate was determined using mating tests with tester strains of the eight Japanese *Armillaria* species (Ota *et al.,* 2009), and analysis of sequence data of the translation elongation factor-1αgene (EF-1α gene) (Hasegawa *et al.,* 2010). The sequences were deposited in the DNA Data Bank of Japan (DDBJ).

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Kira's Warmth Index (WI) of collection sites of *Armillaria* isolates were calculated to show the thermal preference for each *Armillaria* species in Japan. WI is defined as the annual sum of positive differences between monthly mean temperature and +5°C (Kira, 1949; Kira, 1991). WI was calculated using 30-year monthly mean temperatures for the period 1971 to 2000 for each 1 km × 1 km grid of land surface on the Japanese islands (Japan Meteorological Agency, 2002). WI is based on the idea that the sequence of forest formations follows a thermal gradient under sufficiently moist climates (Kira, 1991). WI represents the total amount of heat available for the growth of plants. Following correlations between WI and forest types have been reported: 180 to 240 for subtropical evergreen forest, 85 to 180 for warm-temperate evergreen forest, 45 to 85 for cool-temperate deciduous forest and 15 to 45 for subarctic / subalpine coniferous forest (Kira, 1991).

Isolate pathogenicities were categorized into classes developed by the previous authors (Gregory, 1989; Guillaumin *et al.,* 1993; Keča *et al.,* 2009), with some modifications (Tab. 2).

Results and Discussion

In total 65 isolates of *Armillaria* were collected and seven species were identified. Results of mating tests and sequence analysis of the EF-1α gene were consistent with each other. However, some of the results of mating test were ambiguous whereas those of the EF-1α gene analysis were distinct. *Armillaria mellea* (Vahl: Fr.) P. Kumm., *Armillaria ostoyae* (Romagn.) Herink, *Armillaria cepistipes* Velen. and *Armillaria sinapina* Bérubé & Dessur. were frequently collected and regarded as representative species on conifers in Japan. *Armillaria nabsnona* T.J. Volk & Burds., *Armillaria tabescens* (Scop.) Emel and a biological species Nagasawa's E were rare (Fig. 1). WI values of the collection sites indicated that among the four frequently collected species, *A. sinapina* was collected from the cooler places, followed by *A. ostoyae* and *A. cepistipes*, and *A. mellea* from the warmer places (Fig. 1).Host conifer ranged 19 species, including the Japanese conifers important for timber production: *Cryptomeria japonica* (L. f.) D. Don, *Chamaecyparis obtusa* (Siebold & Zucc.) Siebold & Zucc. ex Endl. and members of *Pinus*, *Larix*, *Abies*, and *Picea* (Tab. 1). *Armillaria ostoyae* and *A. cepistipes* were isolated from various host species and *A. sinapina* was collected from *Abies*, *Picea* and *Larix* that distribute in high latitude or altitude. *Armillaria mellea* was frequently collected from *C. obtusa* (Tab. 1). Considering WI of both fungi and host conifers (Fig. 2), *A. ostoyae*, *A. cepistipes* and *A. sinapina* utilized conifers that grow in their thermal ranges. However, *A. mellea* was collected almost selectively from *C. obtusa* although other major plantation conifers also distribute in its range (Tab. 1, Fig. 2). This result suggests that *A. mellea* prefers *C. obtusa* as its host. Collection records of *A. mellea*, *A. ostoyae*, *A. cepistipes* and *A. tabescens* included the pathogenicity class (a) and (a') that correspond to primary pathogen

(Tab. 2). Therefore, these four species were considered as moderate to aggressive pathogens of conifers in Japan.

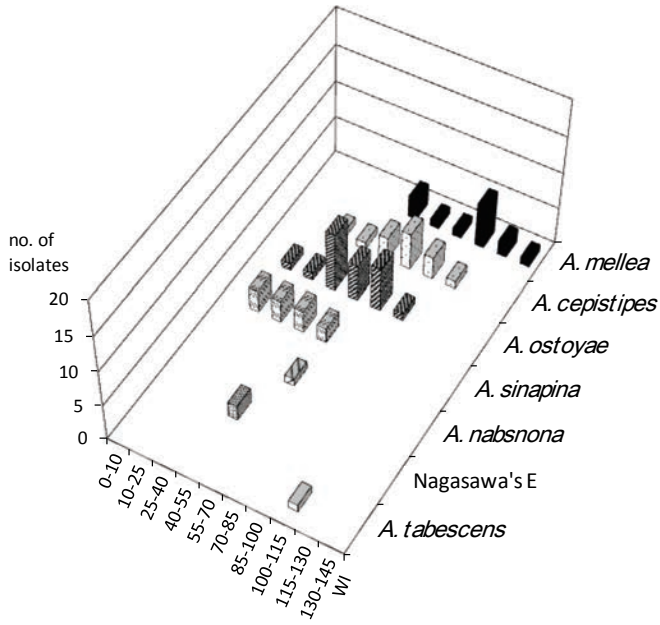


Figure 1. WI of the collection sites of *Armillaria* species isolated from conifers.

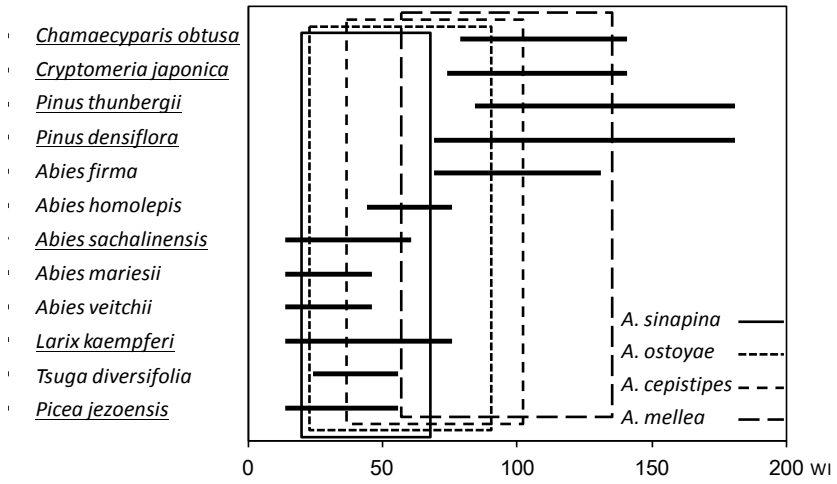


Figure 2. WI of the distribution areas of Japanese conifers and the collection sites of four *Armillaria* species. Horizontal bars indicate WI of the distribution areas of conifers (Kira, 1949 with modification), and squares indicate WI of the collection sites of *Armillaria* species. Underlined conifers are the important species for timber production.

Table 1. The host/substrate conifer species of *Armillaria* isolates

Conifer species	***Armillaria* species, no. of isolates**						
	A. mellea	*A. ostoyae*	*A. cepistipes*	*A. sinapina*	*A. nabsnona*	Nagasawa's E	*A. tabescens*
Cupressaceae							
Chamaecyparis obtusa	11	3	1				
Cryptomeria japonica			6		1		
Pinaceae							
Abies firma		1	1				
Abies homolepis		1					
Abies mariesii			1	1			
Abies sachalinensis		6					
Abies veitchii				7			
Cedrus deodara							1
Larix kaempferi		2		2		1	
Picea abies		1	1			1	
Picea glehnii		1					
Picea jezoensis		1		1			
Picea koyamae			1				
Picea polita			2				
Pinus densiflora		4					
Pinus palustris	1						
Pinus sylvestris			1				
Pinus thunbergii	1						
Tsuga diversifolia		1					
not identified	1	1					
Total	14	22	14	11	1	2	1

Table 2. Occurrens of *Armillaria* isolates classed according to condition of host

Species	**Category, no. of isolates**						
	a	a'	b	c	d	NR[a]	total
A. mellea	3	7	0	0	2	2	14
A. ostoyae	1	3	0	1	5	12	22
A. cepistipes	0	2	1	0	9	2	14
A. sinapina	0	0	0	1	9	1	11
A. nabsnona	0	0	0	0	1	0	1
Nagasawa's E	0	0	0	0	2	0	2
A. tabescens	0	1	0	0	0	0	1

a Isolates from mycelium or a fruit body in/on a living tree showing no obvious factor predisposing to infection.
a' Isolates from mycelium or a fruit body in/on a tree that had been killed in the previous year.
b Isolates from mycelium or a fruit body in/on a living tree stressed by some factor other than *Armillaria*.
c Isolates from mycelium or a fruit body in/on decayed heart wood of a living tree showing no symptoms.
d Isolates from mycelium, rhizomorph, or a fruit body on a tree or a stump that had been killed more than one year ago, on a wind-thrown tree or on woody debris.
[a]no record.

Acknowledgments

We are grateful to Taizo Hogetsu for helpful suggestions and Eiji Nagasawa for donation of tester strains. We also thank Asuka Shichiri for providing us with technical assistance and many people who gave us samples and useful information regarding the collection of isolates.

References

Gregory S.C., 1989. *Armillaria* species in northern Britain. *Plant Pathology* 38: 93-97.

Guillaumin J.-J., Mohammed C., Anselmi N., Courtecuisse R., Gregory S.C., Holdenrieder O., Intini M., Lung B., Marxmüller H., Morrison D., Rishbeth J., Termorshuizen A.J., Tirró A., van Dam B., 1993. Geographical distribution and ecology of the *Armillaria* species in Western Europe. *European Journal Forest Pathology* 23: 321-341.

Hasegawa E., Ota Y., Hattori T., Kikuchi T., 2010. Sequence-based identification of Japanese *Armillaria* species using the elongation factor-1 alpha gene. *Mycologia* 102: 898-910.

Japan Meteorological Agency, 2002. Mesh Climatic Data 2000. Japan Meteorological Agency, Tokyo (CD-ROM).

Keča N., Karadžić D., Woodward S., 2009. Ecology of Armillaria species in managed forests and plantations in Serbia. *Forest Patholology* 39: 217-231.

Kira T., 1949. Forest Zones of Japan. *Forestry Commentary Series* 17. Ringyo Gijutsu Kyokai, Tokyo, p 41 (in Japanese).

Kira T., 1991. Forest ecosystems of East and Southeast Asia in a global perspective. *Ecological Research* 6: 185-200.

Ota Y., Sotome K., Hasegawa E., 2009. Seven Armillaria species identified from Hokkaido Island, northern Japan. *Mycoscience* 50: 442-447.

Initial studies on the characterisation of *Rigidoporus lignosus* isolates from rubber tree plantations in Nigeria (West Africa)

A.O. Oghenekaro[1], V.I. Omorusi[2] G.A. Evueh[2], F.O. Asiegbu[1]

[1]*Department of Forest Sciences, University of Helsinki, P.O Box 27, FI-00014, Helsinki, Finland*
[2]*Plant Protection Division, Rubber Research Institute of Nigeria, P.M.B 1049, Benin City, Nigeria*
Corresponding author e-mail address: abbot.oghenekaro@helsinki.fi

Rigidoporus lignosus, the causative agent of white root rot disease is the most destructive pathogen of rubber trees in Nigeria. A survey of the geographical range of the disease in forest regions in Nigeria was conducted in order to ascertain the extent of the damage it causes. Identification of the disease is however difficult because the above ground symptoms often look much like other pathogenic polypores. Rapid identification of the pathogen is necessary for early diagnosis. For specific detection of the pathogen, a combination of morphological and sequencing of the internal transcribed spacer (ITS) regions are being evaluated. This would help to confirm the distinctiveness of *Rigidoporus lignosus* as compared to other related species from diverse ecological and geographical sources. Rapid detection methods would facilitate development of appropriate control measures in rubber tree plantations.

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Basidiomycetes associated with wood decay in urban trees in Valencia (Spain)

A. Pérez-Sierra , M. León, O. Martínez, V. De Luca, J. Armengol

Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia.
Corresponding author e-mail address: aperesi@eaf.upv.es

Abstract. Wood decay caused by basidiomycete fungi is one of the most important problems in gardens and urban trees. Decay decreases the mechanical strength of the tree and can increase the risk of a stem collapsing, especially in situations of stress from wind, storms and snow. The identification of these basidiomycete species is one of the critical aspects when evaluating the stability and safety of trees growing in urban environments. The objectives of this work were to determine the presence of decay fungi in urban and garden trees in the city of Valencia in Spain, to identify them and to help the authorities to identify potential tree hazards. For this purpose, since 2007 surveys were carried out in urban trees and gardens. Trees were examined for the presence of wood decay fungi, trees were referenced and samples of basidiomycetes were taken for identification in the laboratory. Basidiomata were identified based on morphological features and isolation was made on MEAS. In order to confirm the identification, the ITS region of rDNA of all isolates obtained was amplified and sequenced with primers ITS1-ITS4F. Sequences were compared with sequences deposited in GenBank. Cultures were preserved in the fungal culture collection maintained at the Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia (Spain). A total of 150 samples were processed from 30 different tree species and 16 different species of basidiomycetes were identified. New host-fungus associations were recorded and the results revealed that some tree-wood decay fungus association supposed a high risk of tree failure as a high percentage of tree failure occurred during the course of the study.

Wood decay caused by basidiomycete fungi is one of the most important problems in gardens and urban trees. Decay decreases the mechanical strength of the tree and can increase the risk of a stem collapsing, especially in situations of stress from wind, storms and snow. The identification of these basidiomycete species is one of the critical aspects when evaluating the stability and safety of trees growing in urban environments (Lonsdale, 1999). The objectives of this work were to determine the presence of decay fungi in urban and garden trees in the city of Valencia in Spain, to identify them and to help the authorities to recognize potential tree hazards. For this purpose, since 2007 surveys were carried out in urban trees and gardens. Trees were examined for the presence of wood decay fungi, trees were referenced and samples of basidiomycetes were taken for identification in the laboratory. Isolation from basidiomata were made directly onto malt extract agar (MEA) amended with 0.5% of streptomycin sulphate. Plates were incubated at 25°C for 4-5 days in the dark and hyphal tips were transferred to potato dextrose agar (PDA) for later identification. Basidiomata were identified based on morphological features. Genomic DNA was isolated from two week old pure cultures grown on PDA at 25°C in the dark. In order to confirm the identification, the ITS region of rDNA of all isolates obtained was amplified with

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

primers ITS1F-ITS4B (Gardes and Bruns, 1993). PCR products were purified with the High Pure PCR Product Purification Kit and sequenced in both directions by the DNA Sequencing Service of the Universidad Politécnica de Valencia- CSIC (Spain). Sequences were edited using the Sequencher™ software and consensus sequences were aligned using the CLUSTAL W program. The sequences were subjected to an NCBI BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/) to identify the closest related sequences. Cultures were preserved in the fungal culture collection maintained at the Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia (Spain). A total of 150 samples were processed from 30 different tree species and 16 different species of basidiomycetes were identified. New host-fungus associations were recorded and the results revealed that some tree-wood decay fungus association supposed a high risk of tree failure as a high percentage of tree failure occurred during the course of the study.

References

Gardes M., Bruns T.D., 1993. ITS primers with enhanced specificity for Basidiomycetes - application to identification of mycorrhizae and rust. *Molecular Ecology* 2: 113-118.

Lonsdale D., 1999. The principles of tree hazard assessment and management. Research for amenity trees nº 7. *Forestry Commission*, Great Britain.

Effect of *Heterobasidion annosum s.l.* root and butt rots on the stability of Norway spruce: an uprooting test

L. Giordano, G. Lione, G. Nicolotti, P. Gonthier

University of Torino, Department of Exploitation and Protection of the Agricultural and Forestry Resources (DI.VA.P.R.A.), Plant Pathology, Via L. da Vinci 44, I-10095 Grugliasco (TO), Italy
Corresponding author e-mail address: paolo.gonthier@unito.it

Abstract. In the Alps, many forests play a significant role in protecting human infrastructures against natural hazards, such as avalanches, rock falls and debris flow. *Heterobasidion annosum sensu lato* (*s.l.*) is deemed to predispose trees to uprooting and wind throws. However, very little is known on the extent to which such assumption is true. In this work we assessed the effect of *H. annosum s.l.* root and butt rots on the stability of Norway spruce [*Picea abies* (L.) H. Karst.] through an uprooting test with wire rope winch. The study was conducted on 120 trees in a subalpine stand (Aymavilles - Aosta Valley; western Italian Alps). Before uprooting, all trees were sampled at the root collar by drilling them with a 4 mm diameter, 43 cm long bit. *H. annosum s.l.* was detected from wood chips by using a DNA-based technique. Infected trees resulted in a resistance to uprooting in comparison to uninfected trees reduced by 20% and 33% in trees with DBH (diameter at breast height) > and < 21.3 cm (average diameter of sampled trees), respectively. The reduction in resistance to uprooting of infected trees with respect to uninfected ones was always significant ($P < 0.05$).

In the Alps, most forests play a significant role in protecting human infrastructures against natural hazards, such as avalanches, rock falls and debris flow. An adequate understanding of the disturbances that these forests may undergo is a prerequisite for the successful maintenance of the protection function. Mechanical stability of trees was linked to forest structure, tree/stand features and site characteristics such as tree species, tree height and diameter, crown area, rooting depth and width, stand density, soil type, and topography (Peltola, 2006). Root and butt rot fungi can also reduce the stability of standing trees and predispose them to uprooting and windthrows as they affect the structural integrity of roots. However, very little is known on the extent to which such reduction occurs.

In this work we assessed the effect of *Heterobasidion annosum* (Fr.) Bref. *sensu lato* (*s.l.*) root and butt rots on the stability of Norway spruce [*Picea abies* (L.) H. Karst.] through an uprooting test.

The study was conducted in a subalpine stand (Aymavilles - Aosta Valley, western Italian Alps; elevation 1700 - 1900 m a.s.l.).

Before uprooting test, Norway spruce trees were sampled at the root collar by drilling them with a 4 mm diameter, 43 cm long bit (Guglielmo *et al.,* 2010). In order to discriminate between *H. annosum s.l.*-affected trees and uninfected trees, fungal DNA was extracted from wood frass (Nicolotti *et al.*, 2009) and typed

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

through a PCR-based assay by using universal and taxon-specific primers (HET8 and ITS 3) (Bahnweg *et al.*, 2002). PCR was performed in a 25 µl volume containing 1× PCR buffer, 0.2 mmol l^{-1} of dNTPs mix, 0.5 µmol l^{-1} of each primer, 0.5 mg ml^{-1} of Bovine Serum Albumin, 0.025 U μl^{-1} of Taq polymerase (Promega, Madison, WI, USA) and 6.25 µl of a wood DNA extract. Amplification reaction was conducted using a initial denaturation at 94°C for 3 min, followed by 35 cycles with each cycle consisting of a denaturation at 94°C for 45 s, an annealing at 60°C for 45 s and an extension at 72°C for 45 s, and one final cycle with a 72°C extension for 10 min. Amplicons were visualized on a gel containing 1% of high resolution MetaPhor (Lonza, Rockland, ME, USA) and 1% of standard agarose (Applichem GmbH, Darmstadt, Germany). On the whole 56 trees were affected by *H. annosums s.l.;* another 64 trees were uninfected and were used as controls in the uprooting test.

The uprooting test was performed by using a wire rope winch and a forest tractor. During the experiment, the force applied was recorded at 4 s intervals by using a dynamometer. The maximum value of uprooting force was also annotated for each tree.

For calculation of bending moment (Kg • m) anchorage point of wire on the pull-tree stem and the angle of wire (relative to horizontal by using a hald-held clinometer) were recorded. For every tree, the diameter at breast height (DBH) and total height were also measured.

After uprooting, infected trees were cut in transversal sections every 50 cm and cross-sections were visually analyzed to assess the stage of decay. Trees were assigned to one of the following groups based on the stage of decay: 1) discoloured, no structural decay, 2) advanced decay, visible changes in wood structure and 3) hollowed, a hollow had formed in the main stem. On the whole 16%, 70% and 14% of infected trees showed discolouration, advanced decay and a hollow, respectively.

Statistical analyses of data were performed with PASW Statistics 18 (PASW Statistics 18, 2009).

Heterobasidion annosum s.l.-affected trees were characterized by a mean DBH significantly higher than uninfected trees ($P< 0.009$; Mann-Whitney U test). Therefore, to evaluate the role of *H. annosum s.l.* on mechanical stability of trees independently of DBH, trees were divided into two homogeneous classes on the basis of average DBH (21.3 cm).

Infected trees resulted in a resistance to uprooting in comparison to uninfected trees reduced by 33% and 20% in trees with DBH $<$ and $\geq$ 21.3 cm, respectively. The reduction in resistance to uprooting of infected trees with respect to uninfected ones was always significant ($P< 0.05$, Mann-Whitney U test) (Fig. 1). Our results indicate that the presence of *H. annosum s.l.* butt rots significantly account for tree stability. No differences were detected in terms of bending moment in the

comparison among trees characterized by different stages of decay ($P > 0.05$ - Kruskal-Wallis test).

Further investigations will be necessary to develop mechanicistic models aimed at predicting the critical wind speeds for tree uprooting and to understand how these critical wind speeds change with the properties of the trees within the stand.

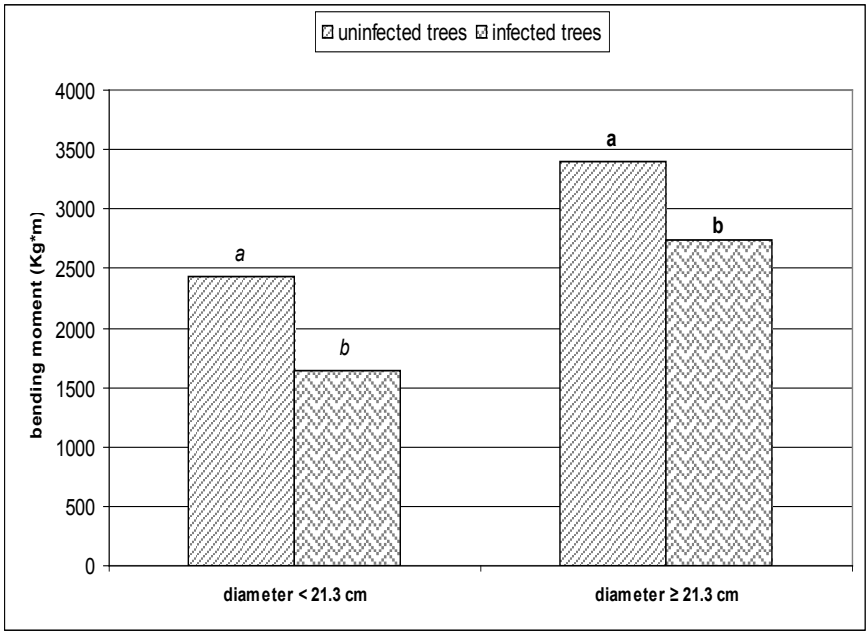


Figure 1. Bending moment of trees infected and uninfected by *Heterobasidion annosum s.l.* in two diameter classes. Values of columns with different letters differ significantly ($P < 0.05$).

Acknowledgements

This research was supported by European Commision, Regione Piemonte and Regione Autonoma Valle d'Aosta - Cooperazione transfrontaliera Alcotra 2007-2013: "Foreste di protezione: tecniche gestionali e innovazione nelle Alpi occidentali" (Proj. N. 32).

References

Bahnweg G., Möller E.M., Anegg S., Langebartels C., Wienhaus O., Sandermann H.jr., 2002. Detection of *Heterobasidion annosum* s. l. [(Fr.) Bref.] in Norway Spruce by Polymerase Chain Reaction. *Journal of Phytopathology* 150: 382-389.

Guglielmo F., Gonthier P., Garbelotto M., Nicolotti G., 2010. Optimization of sampling procedures for DNA-based diagnosis of wood decay fungi in standing trees. *Letters in Applied Microbiology* 51: 90-97. DOI: 10.1111/j.1472-765X.2010.02860.x

Nicolotti G., Gonthier P., Guglielmo F., Garbelotto M., 2009. A biomolecular method for the detection of wood decay fungi: a focus on tree stability assessment. *Arboric Urban For* 35: 14-19.

PASW Statistics 18, 2009 Software version 18.0.0, SPSS Inc., Chicago, Illinois.

Peltola H.M., 2006. Mechanical stability of trees under static loads. *American Journal of Botany* 93: 1501-1511. DOI: 10.3732/ajb.93.10.1501.

Incidence of root and butt rots is largely underestimated when assessment is based upon signs of the disease agent

L. Giordano, F. Guglielmo, G. Nicolotti, P. Gonthier

University of Torino, Department of Exploitation and Protection of the Agricultural and Forestry Resources (DI.VA.P.R.A.), Plant Pathology, Via L. da Vinci 44, I-10095 Grugliasco (TO), Italy;
Corresponding author e-mail address: paolo.gonthier@unito.it

Abstract. In this work we compared the frequency of root and butt rots assessed through visual inspection of signs of the causal agents (fruiting bodies or rhizomorphs) and the actual frequency of agents as determined by PCR-based diagnosis from internal wood tissues. In the fall of 2010, a total of 738 trees were inspected for the presence of signs of root and butt rot agents and sampled at the root collar through a drill-based technique. Trees were located in 3 forest sites and in 8 urban sites and tree species included: *Picea abies* (L.) H. Karst., *Larix decidua* L., *Pinus uncinata* Mill., *Aesculus hippocastanum* L., *Acer* spp. and *Platanus* spp. Samples were analyzed through multiplex-PCR techniques. Although in urban and forest sites 16.7% and 49.3% of trees were infected, respectively, only 9% and 7.5% of infected trees displayed fruiting bodies or rhizomorphs. This last frequency was variable depending on fungal species. These results suggest that diagnosis based on visual inspection overlooks more than 90% of root and butt rot affected trees.

The loss of wood mechanical strength caused by root and butt rot fungi can predispose trees to windthrows or limb failures. In urban landscapes, root and butt rot diseases are often associated with hazardous situations, resulting in significant damage of property and/or tragic injuries. In forest ecosystems, wood decay fungi may reduce the protection against avalanches, rock falls and debris flow, and this is particularly true in protection forests.

Current diagnostic methods to detect root and butt rot fungi are mainly based on visual analysis of macro- and micro-morphological features of fruiting bodies emerging from trees (Mattheck and Breloer, 1992). In the absence of such external signs, rapid and sensitive diagnostic methods, such as multiplex-PCRs, may be helpful in detecting these hazardous fungi (Nicolotti *et al.*, 2009).

The goal of this study was to compare the frequency of root and butt rot fungi assessed through visual inspection of signs (fruiting bodies or rhizomorphs) and the actual frequency of agents as determined by PCR-based diagnosis from internal wood tissues.

In the Fall of 2010, a total of 738 trees were inspected for the presence of signs of root and butt rot agents and sampled at the root collar through a drill-based technique (Guglielmo *et al.,* 2010). Trees were located in 8 urban sites of the city of Turin and in 3 forest sites (Aosta Valley and Piedmont). Tree species included:

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Aesculus hippocastanum L., *Acer* spp., *Platanus* spp., *Larix decidua* L., *Picea abies* (L.) H. Karst. and *Pinus uncinata* Mill.

Fungal DNA was extracted from wood frass and analyzed through two multiplex-PCR-based approaches. The first one was recently summarized by Nicolotti *et al.,* 2009, and was used for urban samples. The second one was specifically developed in this study and was used for forest samples to detect *Armillaria* spp. and *Heterobasidion* spp., two of the most widespread root and butt rot fungi in protection forest of western Italian Alps (Giordano *et al.*, 2010).

Although in urban and forest sites 16.7% and 49.3% of trees were infected, only 9% and 7.5% of these infected trees displayed fruiting bodies or rhizomorphs. This last frequency was variable depending on fungal species (Tab. 1). Both in forest and urban sites, signs of the most frequent fungal taxa detected through multiplex PCRs (i.e., *Armillaria* spp. and *Heterobasidion* spp., respectively), were rarely observed.

These results suggest that diagnosis based on visual inspection of signs overlooks more than 90% of root and butt rot affected trees. PCR-based diagnosis allowed detecting and identifying decay fungi at an incipient stage of colonization and may be appropriate for phytosanitary surveys.

Table 1. Frequency of infected trees detected in urban and forest sites thought PCR-based diagnosis and visual inspection of fruiting bodies.

Fungal taxa in urban sites	PCR-based diagnosis (n.)	Visual inspection (n.)
Armillaria spp.	36	0
Inonotus/Phellinus spp.	18	1
Perenniporia fraxinea	10	3
Ganoderma spp.	7	2
Kretzschmaria deusta	7	1
Total infected trees	78	7

Fungal taxa in forest sites	PCR-based diagnosis (n.)	Visual inspection (n.)
Heterobasidion spp.	86	0
Armillaria spp.	25	1
Armillaria/Heterobasidion spp.	22	9
Total infected trees	133	10

Acknowledgements

This research was supported by European Commision, Regione Piemonte and Regione Autonoma Valle d'Aosta - Cooperazione transfrontaliera Alcotra 2007-2013: "Foreste di protezione: tecniche gestionali e innovazione nelle Alpi occidentali" (Proj. N. 32) and by the City of Turin.

References

Giordano L., Nicolotti G., Gonthier P., 2010. Presence and abundance of root rot, butt Rot and stem rot fungi in protection forests of the western alps. Journal of Plant Pathology 92: S4.84

Guglielmo F., Gonthier P., Garbelotto M., Nicolotti G., 2010. Optimization of sampling procedures for DNA-based diagnosis of wood decay fungi in standing trees. Letters in Applied Microbiology 51: 90-97. DOI: 10.1111/j.1472-765X.2010.02860.x

Mattheck C., Breloer H., 1992. Tree monitoring with VTA - visual tree assessment. *Baumkontrollen mit VTA visual tree assessment* 41: 777-784.

Nicolotti G., Gonthier P., Guglielmo F., Garbelotto M., 2009. A biomolecular method for the detection of wood decay fungi: a focus on tree stability assessment. *Arboriculture & Urban Forestry* 35: 14-19.

The incidence of *Heterobasidion annosum* sensu stricto on young *Alnus incana*

P. Łakomy

Poznan University of Life Sciences, Department of Forest Pathology
Corresponding author e-mail: plakomy@up.poznan.pl

Abstract. Two Scots pine plantations, infested by both *Armillaria ostoyae and Heterobasidion annosum s.s.* were monitored through several years. Both stands were growing as a II generation on the post arable land. In 1995 all dead trees with root systems were removed from several mortality gaps. In addition alive trees without disease symptoms were also dig out from 3 m wide rings around the gaps. In these areas different tree species were planted - *Alnus incana*, *Tilia cordata* and *Betula pendula.* During the first few years *Tilia cordata* and most of *Betula pendula* died, because of drought or deer damages. Only alders survived. First symptoms of *Heterobasidion* infection of *Alnus incana* were found thirteen years after planting. Fifteen years after planting the infection were common. In 2010 and 2011 only single alders survived in the gaps. There is another example including *Fagus sylvatica* of *H. annosum s.s.* common infection of a young generation of broadleaved trees species in Poland.

Heterobasidion annosum s.s. is the most important pathogen especially in Scots pine stands growing on the post agricultural soil in Poland. The question is what species should be used for altering Scots pine in totally destroyed stands by pathogen. In some cases, the routine species for this purpose is *Fagus sylvatica* L. , but might be also commonly infected (Łakomy and Cieślak, 2008).

The aim of this study was to alter pine in gaps by different tree species suitable for the site.

The experiment was establish in Scots pine stands (pedzole soil, pH 3.65 – 3.90) infested by *Heterobasidion annosum s.s.* in west part of Poland 52°97'N, 17°06'E). Stands were growing as a second generation on the post agriculture land. The monitoring of root rot disease have been conducted since 1992. Both *Heterobasidion annosum s.s.* and *Armillaria ostoyae* were recognized as a reasons of dying pines. But the *Heterobasidion* was the major factor of death - 90% of cases. In 1995 the 13 disease centres were localized on 1 ha area of one stand. These gaps cover 12% of stand. In second stand the seven gaps were found in area 0.5 ha. (cover 10%). In 1996 killed trees were excavated and in addition healthy-looking pines from about 6 m ring around each gap (3 rows) were removed. In 1997 new trees were planted. *Alnus incana* (L.) Moench (260), *Tilia cordata* Mill. (260), *Betula pendula* Roth (150), *Pinus sylvestris* L. (160) were used. And each tree species were improved in three gaps. All together 1,000 trees were planted.

After one year all new plants were in good health condition and well grew. The most problem appeared with lime and pine, which were totally damaged by deer. In this area high pressure of red, roe and fallow deer populations have occurred. After three years only birch and alder grew well. In addition the very dry seasons in this

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

region caused the death of birch and pines. This situation have not changed until 2005, when in Autumn the first *A. incana* infected by *Heteroibasidion* was found (Fig. 1). During following years the infection of other alders were observed. Isolation of pathogen from alder roots and somatic compatibility test with these isolates, that had been found in 1996 showed the existence of the same genets in gaps. In 2011 all trees of pine birch and lime were totally disappeared. Alder survived in two gaps, but in one two alive trees and in second seven were found, but growing in the border zone. Most of the alder planted in the border zone were also infected by *H. annosum s.s.* In addition the transfer of pathogen among alder roots was found.

The mortality of young alder was confirmed only on the base of infection experiment buy had not been found in natural environment (Yde-Anderson 1970; Wagn 1987). This is the second example in Poland of *Heterobasidion annosum s.s.* infection of young broadleaved trees. After beech the grey alder have not survived in area severely infested by *Heterobasidion.*

Figure 1. *Heterobasidion annosum s.s.* sporocarps on the root collar of *Alnus incana*

References

Łakomy P., Cieślak R., 2008. Early infection of Fagus sylvatica by *Heterobasidion annosum sensu stricto*. *Forest Pathology* 38: 314-319.

Yde-Andersen A., 1970. *Fomes annosus* in conifer stands of first and second rotation. In: Hodges C.S., Rishbeth J. Yde-Andersen A. (eds.). *Proceedings of the Third Conference on* Fomes annosus, Aarhus, Denmark, July-August 1968. Southeastern Forest Experiment Station, Forest Service, USDA Asheville, North Carolina, pp 137-148.

Wagn O., 1987: Infection experiment with *Fomes annosus* (Fr.) Cooke in shelter trees II. Final report. *Tidsskrift for Planteavl* 91: 173-181. (In Danish with English summary).

Notes on the genus *Buchwaldoboletus,* Basidiomycetes fungi related with forest trees

D. Migliorini[1,2] and A. Santini[2]

[1]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Piazzale delle Cascine 28, 50144, Firenze, Italy.*
[2]*Istituto per la Protezione delle Piante, CNR, via Madonna del Piano 10, 1-50019 Sesto Fiorentino, Firenze, Italy.*
Corresponding author e-mail: nowanda2@gmail.com

Abstract. The large part of research on saprophytic fungi in forests focus on their role in dead wood degradation. *Buchwaldoboletus* seems to be the only genus of *Boletaceae* reported as a saprophytic although data are mainly based on field descriptions. Samples of *Buchwaldoboletus* from different origin were characterized in respect to physiological aspects and DNA sequences were compared with related taxa.

Currently described as saprophytes and lignivorous, members of the genus *Buchwaldoboletus* belonging to the Boletaceae family, received up to now little attention as regard their ecology.

In literature most of work has been dedicated to morphology description finalized to species differentiation (Galli, 1998; Munoz, 2005). Recent systematic works divide the genus in several taxa, among them *B. lignicola* and *B. sphaerocephalus* are two rare species related with European forest trees. *B. lignicola* is localized on mountain sites, associated generally to *Larix* (Miller), *Pinus* L., *Pseudotsuga menziesii* (Franco), and *B. sphaerocephalus* produces basidiomata preferably during autumn and winter in Mediterranean forest in dry sites, on old stumps of *Pinus* spp., mostly *P. pinea* L., *P. pinaster* (Aiton), *P. halepensis* (Miller) (Galli, 1998; Munoz, 2005).

The number of tree species associated to *B. lignicola* include also other trees. The fungus has been found on *Pinus silvestris* L., *Picea abies* (Karsten), *Larix decidua* (Miller), *Sequoiadendron* (Bucholz) (Szczepka, 1981), *Pinus strobus* L. (Snell and Dick, 1970). Basidiomata have been collected *at the base of the trunk of Prunus avium* L. trees (Szczepka, 1981; Alessio, 1985) in a forest dominated by *Quercus* spp.

As regard fungal ecology information are quite scarce. It has been supposed a symbiotic relationship with other fungi when basidiocarps of *B. lignicola*, have been found associated to *Phaeolus schweinitzii* (Fr.) Pat. a pathogen of conifers (Szczepka, 1981).

Preliminary results of our investigations work on *B. lignicola* showed, after DNA comparison of amplification products obtained with ITS1 and ITS4 primers, that the fungus is genetically close to the genus *ChaIciporus*. Morphologically *B.*

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

lignicola has a peculiar characteristic, apparently is the only species of family *Boletaceae* producing clamps connections.

On ecological point o view *B. lignicola* should be probably classified as saprophyte. It was able to degrade *Pinus silvestris* fragments of wood in artificial conditions, but enable to kill *P. silvestris* seedlings after pathogenicity tests.

References

Alessio C.L., 1985. *Boletus* Dill. Ex L. (Fungi Europaei Vol.2), Libreria editrice Biella Giovanna. Saronno, Varese, Italia.

Galli R., 1998. I Boleti 1° ed. Edinatura s.r.l. Milano, Italia.

Muñoz J.A., 2005. Fungi Europaei, *Boletus s.I.* Edizioni Candusso. Alassio, Savona, Italia.

Migliorini D., 2012. Caratterizzazione morfo-fisiologia ed analisi di alcuni aspetti ecologici di *Buchwaldoboletus lignicola. Micologia Italiana* (in press).

Snell W.H., Dick E.A. 1970. The Boleti of Northeastern North America. S.H. Service Agency, Inc., New York, NY.

Szczepka M., 1981. Buchwaldoboletus lignicola (Kallenb.) Pil. in Poland. *Fragmenta Floristica et Geobotanica* 27: 265-274.

Index by Author

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

Z

XIII Conference "Root and Butt Rot of Forest Trees" IUFRO Working Party 7.02.01

List of participants

Gülden ADAY
Süleyman Demirel University, Faculty of Forestry
32263 Isparta, TR
guldenaday@orman.sdu.edu.tr

Natalija ARHIPOVA
Dept. of Forest Mycology and Pathology, SLU
PO Box 7026, SE-75007, Uppsala, SE
natalija.arhipova@slu.se

Fred ASIEGBU
University of Helsinki, Dep. of Forest Sciences,
Latokartanonkaari 7, Helsinki, FI - 00014, FI
Fred.Asiegbu@Helsinki.fi

Liz BEAL
Royal Horticultural Society
Wisley Gardens, Woking, UK
ejy157@hotmail.com

Paolo CAPRETTI
Department of Agricultural, Food and Environmental Science
Piazzale delle Cascine, 28 - 50144 - Firenze, IT
paolo.capretti@unifi.it

Maria Teresa CECCHERINI
Department of Agricultural, Food and Environmental Science
Piazzale delle Cascine, 18 - 50144 – Firenze, IT
mariateresa.ceccherini@unifi.it

Hongxin CHEN
Dept. of Forest Sciences,
P.O. Box 27 (Latokartanonkaari 7) University of Helsinki, FI
hongxin.chen@helsinki.fi

Michelle CLEARY
BC Ministry of Natural Resource Operations
441 Columbia Street, Kamloops BC V2C 2T3, CA
Michelle.Cleary@gov.bc.ca

Cecilia COMPARINI
Department of Agricultural, Food and Environmental Science
Piazzale delle Cascine, 28 - 50144- Firenze, IT
cecilia.comparini@unifi.it

Sarah COVERT
Dept. of Forest Protection
Athens, GA 30602, US
covert@uga.edu

Mike CRUICKSHANK
Natural Resources Canada, Canadian Forest Service
506 W. Burnside Rd. Victoria, BC, CA
Mike.Cruickshank@NRCan-RNCan.gc.ca

Kerstin DALMAN
Dept. Forest Mycology and Pathology, SLU
PO Box 7026, SE-750 07 Uppsala, SE
Kerstin.dalman@slu.se

Tugba DOGMUS LEHTIJARVI
Süleyman Demirel University, Faculty of Forestry
32260 Isparta, TR
tugbadogmus@sdu.edu.tr

Cyril DUTECH
Inra, UMR 1202 BIOGECO Equipe de Pathologie Forestière
Domaine de Pierroton 69 route d'Arcachon,
33612 Cestas cedex, FR
cdutech@bordeaux.inra.fr

Lori ECKHARDT
Auburn University, Forest Health Dynamics Lab
3301 School of Forestry and Wildlife Sciences,
Auburn, AL 36830, AL
eckhalg@auburn.edu

Robert L. EDMONDS
University of Washington, School of Forest Resources
Box 352100, Seattle, WA, USA 98195, US
bobe@u.washington.edu

Carl Gunnar FOSSDAL
Norwegian Forest and Landscape Institute
Høgskoleveien 8, NO -1432 Ås Norway, NO
foc@skogoglandskap.no

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)

Gabriella FRIGIMELICA
Dip. Scienze agrarie e ambientali – Patologia vegetale
Via delle Scienze, 208 33100 Udine, IT
frigimelica@hotmail.com

Matteo GARBELOTTO
Dept. of ESPM, UC Berkeley,
54 Mulford Hall, Berkeley, CA, 94720 - US
matteog@berkeley.edu

Luana GIORDANO
University of Torino - Dept. DIVAPRA
Via L. da Vinci 44, Grugliasco, IT
luana.giordano@unito.it

Paolo GONTHIER
University of Torino - Dept. DIVAPRA
Via L. da Vinci 44, Grugliasco, IT
paolo.gonthier@unito.it

Elena GOTTARDINI
Sustainable Agro-ecosystems and Bioresources Department, IASMA Research and Innovation Centre, Fondazione Edmund Mach,
Via E. Mach 1, 38010
San Michele all'Adige, (Trento), IT
elena.gottardini@iasma.it

Anna GUNULF
Swedish University of Agricultural Sciences –
Dept. of Southern Swedish Forest Research Centre
SLU - P.O. Box 49 - SE-230 53 Alnarp, SE
Anna.Gunulf@ess.slu.se

Eri HASEGAWA
Kansai Research Center,
FFPRI - Forestry and Forest Products Research Institute
Kyoto 612-0855, JP
haseg@ffpri.affrc.go.jp

Renate HEINZELMANN
Swiss Federal Research Instit. WSL,
Zuercherstr. 111, 8903 Birmensdorf, CH

Beatrice HENRICOT
Royal Horticultural Society
Wisley Gardens, Woking, UK
beatricehenricot@rhs.org.uk

Ari M. HIETALA
Norwegian Forest and Landscape Institute
P.b. 115 1431 Aas, NO
hia@skogoglandskap.no

Ottmar HOLDENRIEDER
Institut F. Integrative Biologie - CHN G 66 –
Universitätstrasse 16 -8092 Zürich, CH
ottmar.holdenrieder@env.ethz.ch

Lioba HOLDENRIEDER PAUL
Institut f. Integrative Biologie - CHN G 66
Universitätstrasse 16 -8092 Zürich, CH

Eugenia ITURRITXA
Neiker tecnalia Granja Modelo de Arkaute.
Apartado 46, Vitoria-Gasteiz 01080, ES
eiturritxa@neiker.net

Emad JABER
Forest Sciences Dept.
University of Helsinki
Viikki P.O. Box 27 (Latokartanonkaari 7)
FI-00014 Helsinki, FI
jaber@mappi.helsinki.fi

Nenad KECA
Faculty of Forestry University of Belgrade
Kneza Viseslava 1, 11030 Belgrade, Serbia, RS
nenad.keca@sfb.bg.ac.rs

Kristīne KENIGSVALDE
Latvian State Forest Research Institute “Silava”
111 Rigas str, Salaspils, Latvia, LV-2169,
kristine.kenigsvalde@inbox.lv

Susanna KERIO
Dept. of Forest Sciences, University of Helsinki,
P.O. BOX 27, FIN-00014, FI
susanna.kerio@helsinki.fi

András KOLTAY
Forest Research Institute
3232 Mátrafüred, Hegyalja u. 18, HU
koltaya@erti.hu

Hanna KWAŚNA
Poznań University of Life Sciences,
Forest Pathology Dept.
Ul. Wojska Polskiego 71 c, 60-625 Poznań, PL
kwasna@up.poznan.pl

Nicola LA PORTA
IASMA Research and Innovation Centre,
Fondazione Edmund Mach
Sustainable Agro-ecosystems and Bioresources Department
Via E. Mach 1, 38010
San Michele all'Adige, (Trento), IT
nicola.laporta@fmac.it

Gaston LAFLAMME
Canadian Forest Service
1055 rue du P.E.P.S., C.P. 10380, succ.
Sainte-Foy, Québec, Qc, Canada G1V 4C7, CA
Gaston.Laflamme@RNCan-NRCan.gc.ca

Piotr ŁAKOMY
Dept. of Forest Pathology,
Poznan University of Life Sciences
Wojska Polskiego 71c, 60-625 Poznań, PO
plakomy@up.poznan.pl

Asko LEHTIJARVI
Süleyman Demirel University, Faculty of Forestry
32261 Isparta, TR
askolehtijarvi@sdu.edu.tr

Elisa LOCANDRO
Department of Agricultural, Food and
Environmental Science
Piazzale delle Cascine, 28 - 50144- Firenze, IT
eli.loca@libero.it

Francesco LORETO
Institute of Plant Protection - CNR
Via Madonna del Piano 10, - 50019
Sesto Fiorentino, Firenze, IT
f.loreto@ipp.cnr.it

Nicola LUCHI
Institute of Plant Protection - CNR,
Via Madonna del Piano 10, 50019
Sesto Fiorentino, Firenze, IT
n.luchi@ipp.cnr.it

Monika MALECKA
Instytut Badawczy Leśnictwa, Zaklad Ochrony
Lasu
Forest Research Institute, Dept. of Forest
Protection
Sêkocin Stary, ul. Braci Lesnej 3 - 05-090
Raszyn, PO
M.Malecka@ibles.waw.pl

MaŁgorzata MAŃKA
Dept. of Forest Pathology,
Poznan University of Life Sciences
Wojska Polskiego 71c, 60-625 Poznań, PO
mmanka@up.poznan.pl

Giorgio MARESI
Fondazione Edmund Mach – Istituto di S.Michele
all'Adige (IASMA) – Service Centre
Via E. Mach 1, 38010
San Michele all'Adige, (Trento), IT
giorgio.maresi@iasma.it

Johan MEFFERT
Plant Protection Service
6700 HC Wageningen, The Netherlands, NL
j.p.meffert@minlnv.nl

Nebai MESANZA
Neiker tecnalia,Granja Modelo de Arkaute.
Apartado 46, Vitoria-Gasteiz 01080, ES
nmesanza@neiker.net

Berthold METZLER
Forest Research Institute Baden-Wuerttemberg
Wonnhaldestr. 4, D- 79100 Freiburg/Br. DE
Berthold.metzler@forst.bwl.de

Anthony MGBEAHURUIKE
Dept. of Forest Sciences University of Helsinki
Box 27, FI-00014, Helsinki, FI
Anthony.Mgbeahruike@helsinki.fi

Marco MICHELOZZI
Institute of Plant Genetics - CNR
Via Madonna del Piano 10,
50019 Sesto Fiorentino, Firenze, IT
marco.michelozzi@igv.cnr.it

Duccio MIGLIORINI
Department of Agricultural, Food and
Environmental Science
Piazzale delle Cascine, 28 - 50144- Firenze, IT
nowanda2@gmail.com

Salvatore MORICCA
Department of Agricultural, Food and
Environmental Science
Piazzale delle Cascine, 28 - 50144- Firenze, IT
salvatore.moricca@unifi.it

Emma MOTTA
Plant Pathology Research Centre
Via C. G. Bertero, 22 - 00156 – ROMA, IT
emma.motta@entecra.it

Laura MUGNAI
Department of Agricultural, Food and
Environmental Science
Piazzale delle Cascine, 28 – 50144 – Firenze, IT
laura.mugnai@unifi.it

Michael MÜLLER
Finnish Forest Research Institute
PL 18, 01301 Vantaa, FI
Michael.mueller@metla.fi

Marina NIEMI
VERDERA OY, Kurjenkellontie 5 B,
FI 02270 Espoo, FI
mniemi@lallemand.com

Dina NITIŠA
Latvian State Forest Research Institute “Silava”
111 Rigas str, Salaspils, Latvia, LV-2169,
nitisa.dina@inbox.lv

Abbot OGHENEKARO
Dept. of Forest Sciences, University of Helsinki, FI
P.O Box 62 (Viikinkaari 11), FI00014, FI
abbot.oghenekaro@helsinki.fi

Jonàs OLIVA
Dept. Forest Mycology and Pathology
Box 7026, 750 07 Uppsala, SE
jonas.oliva@slu.se

Yuko OTA
Forestry and Forest Products Research Institute,
Matsunosato, Tsukuba, Ibaraki, JP
yuota@affrc.go.jp

Elena PAOLETTI
Institute of Plant Protection - CNR
Via Madonna del Piano 10,
50019 Sesto Fiorentino, Firenze, IT
e.paoloetti@ipp.cnr.it

Ana PEREZ SIERRA
Grupo de Investigación en Hongos Fitopatógenos,
Instituto Agroforestal Mediterráneo
Universidad Politécnica de Valencia,
Camino de Vera s/n, 46022 Valencia, ES
aperesi@eaf.upv.es

Giacomo PIETRAMELLARA
Department of Agricultural, Food and
Environmental Science
Piazzale delle Cascine, 18 - 50144 Firenze ITA
giacomo.pietramellara@unifi.it

Tuula PIRI
Finnish Forest Research Institute
Jokiniemenkuja 1, FI-01301 Vantaa, Finland
tuula.piri@metla.fi

Jim PRATT
Cross House, Mountain Cross, West Linton,
Peeblesshire, EH46 7DF, Scotland UK
k.m.pratt@btinternet.com

Simone PROSPERO
Swiss Federal Research Institute WSL,
Zuercherstrasse 111, 8903 Birmensdorf,
Switzerland
simone.prospero@wsl.ch

Alessandro RAGAZZI
Department of Agricultural, Food and
Environmental Science
Piazzale delle Cascine, 28 - 50144- Firenze, IT
alessandro.ragazzi@unifi.it

Jonas RÖNNBERG
SLU, Southern Swedish Forest Research Centre
SLU, P.O. Box 49, SE-23053 Alnarp, Sweden
Jonas.ronnberg@slu.se

Gaurav SABLOK
Sustainable Agro-ecosystems and Bioresources
Department, IASMA Research and Innovation
Centre, Fondazione Edmund Mach
Via E. Mach 1, 38010
San Michele all'Adige, (Trento), IT
sablokg@gmail.com

Alberto SANTINI
Institute of Plant Protection - CNR
Via Madonna del Piano 10, - 50019
Sesto Fiorentino, Firenze, IT
a.santini@ipp.cnr.it

Aniello SCALA
Department of Agricultural, Food and
Environmental Science
Piazzale delle Cascine, 28 - 50144- Firenze, IT
aniello.scala@unifi.it

Petr SEDLÁK
Mendel University in Brno
Zemedelska 3, 613 00 Brno, CZ
petrsedlak@klikni.cz

György SIPOS,
WSL Swiss Federal Research Institute
Zürcherstrasse 111, Birmensdorf, Switzerland, CH
gyoergy.sipos@wsl.ch

Jan STENLID
Dept Forest Mycology and Pathology, SLU
PO Box 7026, SE-750 07 Uppsala, SE
Jan.stenlid@slu.se

Hui SUN
Dept. of Forest Sciences, University of Helsinki
P. O. Box 27, 00014, Latokartanonkaari 7,
Helsinki, Finland
hui.sun@helsinki.fi

Giuseppe SURICO
Department of Agricultural, Food and
Environmental Science
Piazzale delle Cascine, 28 - 50144- Firenze, IT
giuseppe.surico@unifi.it

Wojciech SZEWCZYK
Poznań University of Life Sciences
Wojska Polskiego 71c, 60-625 Poznań, PL
wszew@up.poznan.pl

Michal TOMSOVSKY
Mendel University
Zemedelska 3, 613 00 Brno, CZ
michal.tomsovsky@mendelu.cz

Rimvys VASAITIS
Dept Forest Mycology and Pathology, SLU
PO Box 7026, SE-750 07 Uppsala, SE
Rimvys.Vasaitis@slu.se

LiYing WANG
POB 18 (Jokiniemenkuja 1), SE
Liying.wang@ess.slu.se

Steve WOODWARD
University of Aberdeen,
Institute of Biological and Environmental Sciences,
Cruickshank Building 2.24, St. Machar Drive,
Aberdeen, UK
s.woodward@abdn.ac.uk

Astra ZAĻUMA
Latvian State Forest Research Institute Silava",
111 Rigas Street, Salaspils, LV-2169, Latvia, LV
astra_z@inbox.lv

ANNA ŻÓŁCIAK
Forest Research Institute, Dept. of Forest
Protection
Sêkocin Stary, ul. Braci Lesnej 3 - 05-090
Raszyn, PO
a.zolciak@ibles.waw.pl

The Montesclaros Declaration

Prepared by a group of more than 70 forest pathologists (representing 17 nations) that attended an international IUFRO (International Union of Forest Research Organizations http://www.iufro.org/) meeting held at the Montesclaros Monastery in Cantabria, Spain during May 23th – 27th, 2011.

As scientists studying diseases of forest trees, we recognize that the international trade of plant material is increasing the risks to forest health worldwide. The evidence for this view is based on the recent, unprecedented rise in numbers of alien pathogens and pests emerging in natural and planted forest ecosystems in all parts of the globe. We thus propose a phasing out of all trade in plants and plant products determined to be of high risk to forested ecosystems but low overall economic benefit.

We regard all international trade in containerized ornamental plant seedlings and trees intended as plants for instant landscape planting as low benefit in terms of overall economy but high risk to forest health. For instance, production of seedlings in low cost localities for outplanting in different and distant environments provides only a marginal net economic benefit to the whole area, but provides an efficient pathway for pathogen and pest dispersal. In addition, international trade in other plant materials (e.g., wood packaging, wood chips, etc.) should be scrutinized and more strictly regulated.

The complete document was available at URL:
http://www.iufro.org/science/divisions/division-7/70000/publications/montesclaros-declaration/

Individuals who wish to express their endorsement can send an email with contact information (address, etc) to:
noliveplants@gmail.com

P. Capretti, C. Comparini, M. Garbelotto, N. La Porta, A. Santini (edited by), *XIII Conference Root and Butt Rot of Forest Trees IUFRO Working Party 7.02.01,* ISBN 978-88-6655-352-6 (print), ISBN 978-88-6655-353-3 (online)