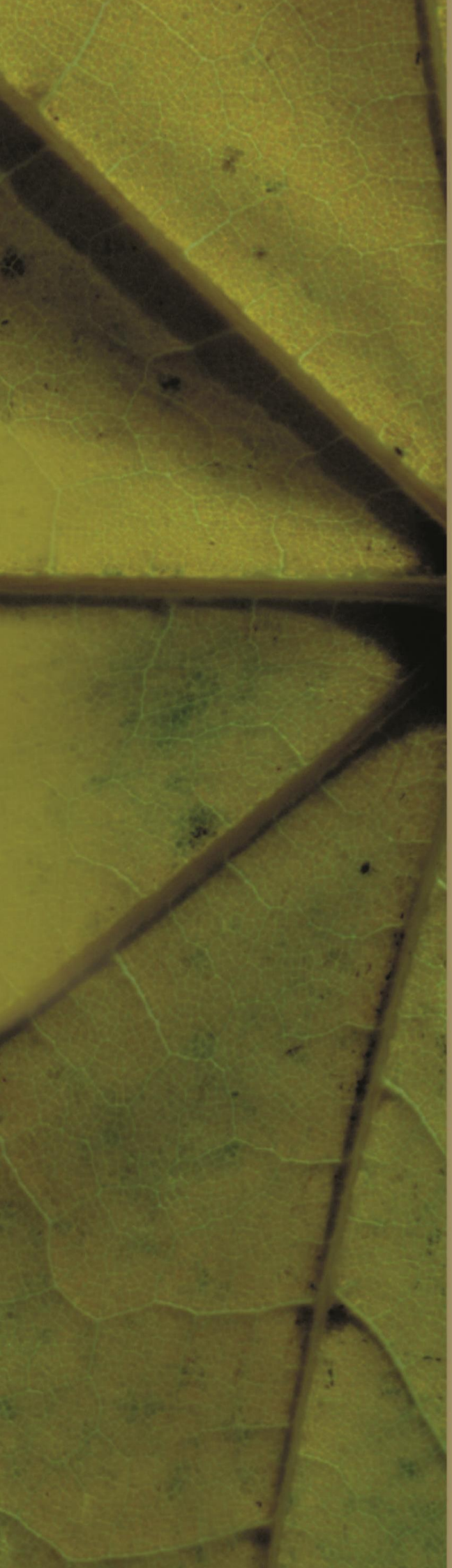


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An International Journal in Forest, Wood and Environmental Sciences

Special Edition
2007

Proceedings
of the Conference of
IUFRO Working Party 7.02.02
21–26 May 2007
Sopron, Hungary

Foliage, Shoot and Stem Diseases of Forest Trees

Guest editor:
Ilona Szabó

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International Conference on Foliage, Shoot and Stem Diseases

IUFRO Working Party 7.02.02

21-26 May 2007 Sopron, Hungary



Preface

The IUFRO Working Party (WP) 7.02.02 on Foliage, Shoot and Stem Diseases organizes a meeting once every three to five years. The 2007 meeting was held in Sopron, Hungary. Our local organizer was Dr. Ilona Szabó from the Faculty of Forestry, University of West Hungary, assisted by several collaborators. We had the privilege of meeting the dean of the Faculty of Forestry, Dr. András Náhlik, who welcomed the participants at the opening. Being Canadian, Gaston Laflamme, the coordinator of the WG, took the opportunity at the opening of the meeting to underline the 50th anniversary of the Sopron Forestry School exodus, in 1957, to the Faculty of Forestry at the University of British Columbia, Vancouver, Canada. This unique migration was composed of 14 faculty members and 200 students. What was supposed to be a short stay ended up being the final destination.. In June 2007, several of these people met in Vancouver to celebrate this special anniversary.

Based on comments from the participants, our 2007 meeting was a very successful one. The fact that presenters each had 30 minutes for their talks left enough time for animated discussion after each presentation. We thank the attendees for their active participation; they made this scientific meeting, including the poster session, very lively. We thank the local organizers and also the Faculty of Forestry of the University of West Hungary for their help with the smooth running of the conference. The conference was held in the downtown area, so we had the opportunity to visit this historic city with our hosts, and we also had the chance to taste local food and wine. We thank the forestry management units of Balatonfüred, Devecser and Síkvidék for hosting the field demonstrations. The scientific excursions covered different subjects such as needle diseases of conifers, including Dothistroma needle cast, Swiss needle cast, Rhabdocline needle cast, and Sphaeropsis shoot blights. We also visited experimental sites dealing with chestnut blight and its biological control.

One of our objectives in holding the meeting in Sopron was to attract more scientists from Eastern Europe. Our strategy did work in part; we had participants from the Czech Republic, Croatia, Serbia and Estonia. In short, we had close to 60 participants from 16 countries, with 32 oral presentations and 24 posters. All the information shared during the meeting is now available through these proceedings. This includes full papers as well as extended and short abstracts. In addition, a few papers were added from members who were not able to attend the meeting. The responsibility for the published papers lies with the authors.

The business meeting of the IUFRO Working Party 7.02.02 was held after the poster session near the end of the meeting. The coordinator presented a brief history of the WP. After four years as deputy and ten years as coordinator of this WP, he has resigned from his position as coordinator. This will give him more time to work as the coordinator of the IUFRO Pathology section 7.02, which includes 11 WPs. The participants elected Dr. Antti Uotila, from Finland, as the new coordinator of the WP. Dr. Mike Ostry, from the United States, will remain deputy, and the second deputy elected to replace Dr. Uotila was Dr. H. Tugba Dogmus-Lehtijärvi, from Turkey. The participants have also agreed to meet in the Isparta region of Turkey in May 2009; Dr. H. Tugba Dogmus-Lehtijärvi will be the local organizer.

Finally, we welcome research scientists dealing with foliage, shoot and stem diseases from all over the world to join us at the next meeting. This is an excellent opportunity to meet with many specialists working on diseases affecting these various tree parts; a critical mass of such scientists can be found only at these IUFRO meetings.

Gaston Laflamme
Coordinator
IUFRO WP 7.02.02

Ilona Szabó
Local Organizer
Sopron, Hungary

FULL PAPERS

Foliage diseases – Conifers

10.37045/aslh-2007-0009 *Acta Silv. Lign. Hung., Spec. Edition (2007) 17-21*

The Contemporary Situation of Dothistroma Needle Blight Outbreak in the Czech Republic

Miroslava BEDNAROVA – Irena BODEJCKOVA – Dagmar PALOVCIKOVA – Libor JANKOVSKÝ*

Department of Forest Protection and Game Management, Faculty of Forestry and Wood Technology, Mendel University of Agriculture and Forestry, Brno, Czech Republic

Abstract – Dothistroma needle blight *Mycosphaerella pini* E. Rostrup resp. its anamorphic stage *Dothistroma septospora* (Dorog.) Morelet was for the first time noted in the region of the Czech Republic in a consignment of imported plants of Austrian pine *Pinus nigra* Arnold in 1999. In 2000, it was also found on *Pinus nigra* in an open planting in a plantation of Christmas trees near the village of Jedovnice by Brno in the South Moravia. In the Czech Republic, Dothistroma needle blight was identified on 19 species of pines and 5 species of spruces. The critical period for infection is in the Czech Republic from the second half of May until the end of June, when the new shoots and needles develop. The incubation period lasts about 2–4 months depending on climatic conditions. The first symptoms on the needles infected in the current year appear in August being clearly expressed from September to November. In the CR, Dothistroma needle blight spread probably with infected planting stock obtained from import at the end of the 80s and at the beginning of the 90s.

Dothistroma needle blight / *Mycosphaerella pini* / *Dothistroma septospora* / host spectrum / diseases of Pines

Kivonat – A dothisztrómás tűhullás kitörésének jelenlegi helyzete a Cseh Köztársaságban. A dothisztrómás tűhullást, a *Mycosphaerella pini* E. Rostrup anamorf stádiumát 1999-ben jegyeztük először a Cseh Köztáraság területén import feketefenyő (*Pinus nigra* Arnold) növényeken. 2000-ben egy *Pinus nigra* karácsonyfa telepen is megtaláltuk Jedovnice falu közelében, Brno mellett. A Cseh Köztársaságban a dothisztrómás tűhullást 19 *Pinus* és 5 *Picea* fajon azonosítottuk. A fertőzés kritikus időszaka május második felétől június végéig tart, ami egybeesik a hajtás- és tűnövekedés időszakával. Az inkubációs idő hossza, az időjárási körülmények függvényében, 2-4 hónap. A folyó év során fertőzött tűkön az első tünetek augusztusban jelentkeznek, szeptember- novemberre teljesen kialakulnak. A Cseh Köztársaságban a betegség valószínűleg importból származó, fertőzött szaporítóanyaggal terjedt el a 80-as évek végén és a 90-es évek elején.

Dothisztrómás tűhullás / *Mycosphaerella pini* / *Dothistroma septospora* / gazdanövénykör / *Pinus*-ok betegségei

* Corresponding author: jankov@mendelu.cz; Zemedelska 3, 613 00 Brno, Czech Republic

1 INTRODUCTION

The Dothistroma needle blight caused by *Mycosphaerella pini* E. Rostrup was described from Europe, more exactly from Russia, as *Cytosporina septospora* Dorog in 1911 (DOROGUINE 1911). SACCARDO (1920) described the Dothistroma needle blight fungus found on *P. ponderosa* in Idaho as *Actinothyrium marginatum* Sacc. *Cytosporina septosporum* was later transferred to the genus *Septoriella* Oudem. as *S. septosporum* (Dorog.) Sacc. (TROTTER 1931). Later, this anamorphic stage was described as *Dothistroma pini* Hulbary (HULBARY 1941). The connection between the American and European pathogen was given when GREMMEN (1968) and MORELET (1968) realized that the fungus described in Europe as *C. septosporum* was the same as *D. pini* causing Dothistroma needle blight in the United States. MORELET (1968) found that it referred to the same fungus and created a new combination *Dothistroma septospora* (Dorog.) Morelet. Both names are commonly used. Some papers note differences between these two anamorphs. Eg. Barnes et. al (2004) find on the bases of phylogenetic studies, that *D. septospora* and *D. pini*, make up two distinct phylogenetic lineages. *Dothistroma septosporum* has a worldwide distribution and it is the causal agent of the disease that has severely damaged plantations of *P. radiata*, grown as an exotic in the Southern Hemisphere. In contrast, *D. pini* is a serious pathogen of pines that currently appears to be restricted in distribution to the North Central United States. The species found in the Czech Republic is classified as *Dothistroma septospora.*

A sexual stage was first described as *Scirrhia pini* Funk and Parker but subsequently it was included in the genus *Mycosphaerella* as *Mycosphaerella pini* E. Rostrup apud Munk. BARR (1996) reclassified the teleomorph based on the study of diversity of the genus *Mycosphaerella* to a new genus *Eruptio* as a species *Eruptio pini* (Rostr. apud Munk) M. E. Barr. Subsequent phyllogenetic analyses have proved that classifying into the genus *Mycosphaerella* is much suitable (GOODWIN et al. 2001). The anamorphic stage was divided to three varieties on the basis of differences in the length of conidia. THYR and SHAW (1964) distinguished within *D. pini* Hulbary a variety *pini* (syn. *D. septospora* var. *septospora*) with the length of conidia 15.4 – 28.0 (mean 22.4) μm and *D. pini* Hulbary var. *linearis* (syn. *D. pini* var. *lineare*) with the length of conidia 23.0 – 42.0 (31,9) μm. IVORY (1967) distinguished another variety *D. pini* Hulbary var. *keniensis* (syn. *D. septospora* var. *keniense*) with mean lengths of conidia 13.0 – 47.5 (28.9) μm. EVANS (1984) reasons that *D. pini* comes from mixt forests of Central America and occurs on isolated mountain "islands" at altitudes over 1500 m.

As compared with the anamorphic stage, the teleomorphic stage *Mycosphaerella pini* (syn. *Scirrhia pini*) occurs rather exceptionally. In the majority of countries with the occurrence of an anamorphic stage of *Dothistroma pini* or *D. septospora* a teleomorph was not found at all. A perfect stage is mentioned from Canada, parts of the USA, Germany, Yugoslavia, Poland and Portugal (BRADSHAW 2004). The perfect stage of *M. pini* is related to *D. pini* var. *linearis*. In *D. pini* var. *pini* and *D. pini* var. *keniensis*, a perfect stage has not been described (IVORY 1967).

Virtually, about 80 host species of Dothistroma needle blight are mentioned from all continents (Bednarova et al. 2006). Particularly various species of pine are hosts of the needle blight. Dothistroma needle blight is also mentioned from *Picea abies* (L.) Karst. (LANG 1987), *P. omorika* (Pančić) Purkyně (KARADŽIĆ 1994), *P. pungens* Engelm. (JANKOVSKÝ, BEDNÁŘOVÁ, PALOVČÍKOVÁ 2004), *P. sitchensis* (Bong.) Carr. (GADGIL 1984), *P. schrenkiana* Fisch. & C. A. Mey (Bednářová 2006), *Pseudotsuga menziesii* (Mirb.) Franco (DUBIN and WALPER 1967), *Larix decidua* Mill. (BASSETT 1969) etc.

The aim of paper is evaluate situation about distribution of Dothistroma needle blight in the Czech republic, epidemic situation, ecology, pathology and its host spectrum.

2 MATERIAL AND METHOD

Within monitoring carried out in 2000 - 2007, pine needle samples were examined taken mainly in the region of southern and central Moravia, Silesia and eastern and central Bohemia, individually also from other regions of the CR. Samples were taken with symptoms of damage to the assimilatory apparatus of pines from more than 60 localities.

The presence of the pathogen was always investigated according to characteristic symptoms such as red bands, dying tips of needles or the occurrence of subepidermal sporocarps, acervuli. A precise identification was proved on the basis of microscopic analyses of conidia. Records of the study are deposited in the herbarium of the Department of Forest Protection, Faculty of Forestry and Wood Technology, Mendel University of Agriculture and Forestry Brno (BRNL)

3 RESULTS AND DISCUSSION

Dothistroma needle blight caused by *Mycosphaerella pini* (or its anamorph *Dothistroma pini*) was firstly recorded in the Czech Republic on an imported *Pinus nigra* in 1999. In 2000, it was found in the open planting. Its occurrence was noticed in more than 50 localities in the region of Moravia and Silesia and eastern Bohemia (JANKOVSKÝ, BEDNÁŘOVÁ, PALOVČÍKOVÁ 2004). At present, it is a serious problem particularly in Christmas tree plantations as well as in forest nurseries.

3.1 Host spectrum in the Czech Republic

In the Czech republic, Dothistroma needle blight was identified on 19 species of pine: *Pinus aristata* Engelm., *Pinus banksiana* Lamb., *Pinus cembra* L. var. *sibirica* (Du Tour) G. Don, *Pinus contorta* Douglas ex Loudon, *Pinus heldreichii* H. Christ, *Pinus heldreichii* H. Christ var. *leucodermis* (Antoine) Markgraf ex Fitschen, syn. *Pinus leucodermis* Ant., *Pinus jeffreyi* Grev. et Balf, *Pinus mugo* Turra, *Pinus nigra* Arnold, *Pinus ponderosa* Douglas ex Lawson, *Pinus pungens* Lambert, *Pinus rigida* Miller, *Pinus rotundata* Link = *Pinus mugo* nothosubsp. *rotundata* (Link) Janchen & Neumayer, *Pinus strobus* L. var. *chiapensis* Martinez, *Pinus sylvestris* L., *Pinus tabuliformis* Hort. ex Carrière, *Pinus taeda* L., *Pinus thunbergii* Parlatore, syn. *Pinus thunbergiana* Franco, *Pinus wallichiana* A. B. Jackson. *Pinus nigra* Arnold and *Pinus mugo* Turra are the most frequent hosts. As for species of other genera *Picea pungens* Engelm., *Picea asperata* Masters, *Picea omorika* (Pančić) Purkyně and *Picea abies* L. Karst. also were noted as hosts. *Picea schrenkiana* Fisch. & C. A. Mey as a very sensible host of Dothistroma needle blight is also a certain rarity. Unusual new hosts are mostly from arboretums or ornamental plantings (Bednářová et al 2003).

3.2 Biology of D. septosporum in the Czech Republic

The open acervuli of *D. septosporum* were noticed in the majority of localities in the CR already from mid-March in the year 2002. In some localities, the formation of conidia was noticed from the end of April. The critical period for infection is in the Czech Republic from the second half of May until the end of June (beginning of July). The incubation period lasts about 2–4 months depending on climatic conditions.

The first symptoms on the needles infected in the current year appear in August in the form of unspecific yellow spots on needles. Finally, the needles get dry from tips and dead tissues are at first of straw-brown color. In the course of September, at first dark brown and later narrow black strips are formed on dead parts of needles. In this stage, fruit bodies are

formed on needles in which conidia of *Asteromella* synanamorph are produced. On their place, acervuli are formed from October and characteristic red strips are created. In acervuli, *Asteromella* spermogonia can be formed at first together with conidia of the stage of *Dothistroma*. In this period, intensive development of infection occurs. During a week, a progress of damage to pines was noticed in localities under study. Infection demonstrations are particularly evident in early spring.

Under strong infection pressure, needles die already during the year of infection, namely rather early, from August till September. In the same year, acervuli can be formed even with accompanying symptoms as the occurrence of red strips. Heavily infected trees are weakened to such an extent that sufficiently large new current year shoots are not often formed. If the shoots grow they are shortened and stunted ("lion tails") and during the next year, they die under the infection pressure.

Ascospores of *Mycosphaerella pini* were noticed only once on fallen needles of *Pinus mugo* from the Říkovice locality, Eastern Bohemia in October 2001.

Results obtained correspond to observations of PETERSON (1967). Virtually the same results are given by KARADZIĆ (1989) from the region of Serbia. He mentioned that conidia of *Mycosphaerella pini* were in Serbia dispersed from the beginning of April until the end of October, and ascospores from the second half of June until the end of September.

In Germany, conidia of *M. pini* were detected between March and November being particularly abundant from April to June (LANG, KARADZIĆ 1987).

4 CONCLUSIONS

Dothistroma needle blight caused by *Mycosphaerella pini* resp. mostly by its anamorphic stage *Dothistroma septospora* is one of the most important harmful organisms making problems in decorative nursery practice and forestry. In the Czech Republic, the spectrum of hosts includes 19 species of pines and 5 species of spruce. It is not possible to exclude that the disease is neglected in the region of the CR already for several decades. Only inclusion into quarantine organisms brought about an interest in the pathogen. Roughly at the same time as in the Czech Republic, the disease was also noticed in neighboring countries. The spread of Dothistroma needle blight can be considered (in addition to trade with planting stock) to be the result of favorable climatic conditions when natural geographic and climatic barriers preventing the spread of the disease towards north were removed.

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Five-Year Impacts of Swiss Needle Cast on Douglas-fir in Interior Forests of Oregon, USA

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Abstract – In 2001 and 2006, we examined 590 Douglas-firs in 59 stands age 10-23 years in the northern Cascade Mountain foothills in Oregon, USA. Mean 5-year-dbh growth was 6.1 cm and total-height growth was 3.6 m. Mean needle-retention index increased by 3.4 over 5 years, and mid-crown retention increased by 1.2 years. Mean percentages of stomata occluded by pseudothecia of *Phaeocryptopus gaeumannii* were 13.6% for 2000-(2-year-old) needles and 1.7% for 2001-(1-year-old) needles sampled in 2002, and 13.3% for 2004 (2-year-old) needles sampled in 2006. Mean crown-length to sapwood-area ratio was 5.2 cm/cm^2 in 2006. There were poor correlations (R^2 <0.3) among all variables except for a moderate correlation between stand elevation and either 2000-stomata occluded (R^2 = 0.43) or 2004-stomata occluded (R^2 = 0.50), where there were fewer pseudothecia at the higher elevations. Either 5 years is not enough time to evaluate the affects of Swiss needle cast on Douglas-fir growth in the Oregon Cascades or there was no significant effect of Swiss needle cast during the latest outbreak on Douglas-fir growth. Based on our results and their interpretation, forest managers may need not alter their current practices in the northern Oregon Cascades, and managing a mix of Douglas-fir and western hemlock at lower elevations and noble fir at higher elevations will help offset any future stand-growth declines due to Swiss needle cast.

***Phaeocrytopus gaeumannii / Pseudotsuga menziesii* / tree growth loss**

Kivonat – A svájci tűhullás ötéves hatása a duglászfenyőre Oregon belső erdeiben. 2001-ben és 2006-ban 590 darab duglászfenyőt vizsgáltunk 59, 10-23 éves állományban, az északi Cascade-hegység lábainál, az USA Oregon államában. Az ötéves mellmagassági átmérő növedékátlaga 6,1 cm, az összes magassági növedék pedig 3,6 m volt. Az átlagos tű-megtartási mutató 3,4-re emelkedett az öt év alatt, és a közép korona-megtartás 1,2 évvel nőtt. A *Phaeocryptopus gaeumannii* pszeudotéciumai által eltömött sztómák átlagos aránya 2002-ben 13,6 % volt a 2000 évi (két éves) tűknél és 1,7% a 2001 évi (1 éves) tűknél. A 2006-ban gyűjtött tűknél ez az arány 13,3% volt a 2004-es (két éves) tűk esetében. A koronahossz és a szíjácsterület átlagos aránya 5,2 cm/cm^2 volt 2006-ban. A változók közötti korreláció gyenge volt (R^2 <0,3), kivéve az állományok tengerszint feletti magassága és a 2000 évi eltömött sztómák (R^2 = 0,43), illetve 2004 évi eltömött sztómák (R^2 = 0,50) közötti mérsékelt korrelációt, amely szerint magasabb helyeken kevesebb volt a pszeudotéciumok száma. Vagy az 5 év kevés volt a svájci tűhullás duglászfenyő növekedésére való hatásának

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kimutatására, vagy a betegség legutóbbi kitörésének nem volt szignifikáns hatása a fafaj növekedésére az oregoni Cascade-hegységben. Eredményeink alapján az erdőgazdálkodóknak nem kell megváltoztatniuk az eddigi gyakorlatot, vagyis az alacsonyabb helyeken duglászfenyőt nyugati hemlokfenyővel elegyesen, a magasabb helyeken *Abies procera*-t termesztve kivédhető az állományok svájci tűhullás okozta jövőbeni növedékcsökkenése.

***Phaeocrytopus gaeumannii* / *Pseudotsuga menziesii* / növedékvesztés**

1 INTRODUCTION

Swiss needle cast (SNC), caused by the fungus, *Phaeocryptopus gaeumannii*, is one of the most damaging diseases affecting Douglas-fir (*Pseudotsuga menziesii*) in the Pacific Northwest, USA (Hansen et al. 2000). Biological impact is particularly acute on the Oregon and Washington Coast, one of the most productive regions for forest growth in the temperate world. In 2006, aerial-detection surveyors mapped 131,360 ha of Douglas-fir forest with obvious symptoms of SNC. Annual Douglas-fir volume-growth losses from SNC are estimated at about 23% over 75,680 ha with some losses as high as 52% in northwest Oregon (Maguire et al. 2002).

Although impact from SNC occurs in interior forests in the northern Cascade Mountains of Oregon, it is assumed to be less than damage in the Oregon Coast Range. In 2001, baseline monitoring plots were established in 59 stands covering 809,400 ha in the Cascade Mountains. It was essential that these plots be re-measured in order to determine the 5-year change and biological impact of SNC on Douglas-fir growth in the Oregon Cascades. These plots are the only source of data for SNC impact in the Oregon Cascade Mountains. Therefore, objectives of our study were to determine changes after 5-years (2001 to 2006) in 1) tree diameter and total-height growth and 2) Swiss needle cast severity as estimated by needle retention, stomata occlusion, and crown length/sapwood area ratio in 59 stands in the northern Oregon Cascade Mountains.

2 METHODS

From April to June**,** 2001, prior to Douglas-fir budbreak, transects were installed in 59 stands. Sampled stands were 10- to 23-years old and contained more than 50% Douglas-fir. Stands were systematically located on lands administered by the USDA Forest Service, USDI Bureau of Land Management, Weyerhaeuser Corp., Port Blakely Trees Farms, and Longview Fibre in the northern Oregon Cascade Mountains (Freeman 2001). Each stand has one transect with five sample points located at 15-m intervals. Transects were established in a location representative of the stand. Stand data collected in 2001 included: 1) elevation, 2) slope aspect (8 cardinal points), 3) slope %, and 4) some Global Positioning System (GPS) coordinates at the start of each transect.

At each sample point, the nearest co-dominant or dominant Douglas-fir on each side of the transect was selected for a total of 10 trees per stand. Sample trees were without damage from agents other than SNC. Data collected for each tree in 2001 included: 1) diameter at breast height (dbh at 1.4 m above ground, nearest cm), 2) total height (nearest 0.3 m), 3) height to lowest live branch, 4) ocular estimation of foliage retention in the mid-crown (0 to 6 yrs), and 5) foliage–retention index of a sampled branch. Heights were measured with a clinometer. Live-crown ratios for each year were calculated by subtracting height to lowest live branch from total-tree height for live-crown length, and then dividing crown length by total-tree height and multiplying by 100.

Foliage-retention index was calculated for each sample tree as follows: a live branch at mid-crown was selected on the S side of the sample tree and cut from the stem with the pole pruner, if necessary. From the cut branch, a secondary lateral branch was selected, and the amount of foliage remaining in each needle age class was rated and recorded as: 0 = 0 to10% of full compliment present, 1 = 11 to 20% present, 2 = 21 to 30% present, 9 = 90 to 100% present. Ratings were summed for a minimum score of 0 and a maximum of 36 for each branch. Needle retention has been shown to be the most reliable and efficient variable when estimating SNC severity in terms of tree volume-growth loss (Filip et al. 2000, Hansen et al. 2000, Maguire et al. 2002). Needle retention as estimated from the mid-crown is considered more reliable than upper- or lower-crown estimates, especially for larger trees.

In 2002, 37 of the 59 stands were sampled for pseudothecia density. For 5 sample trees per stand (1 tree per plot pair), a live branch as sampled above was returned to the laboratory for pseudothecia estimates. Pseudothecia density, measured as the percentage of needle stomata occluded, is a direct method of determining the presence and severity of *P. gaeumannii*. Measurements were made on the last 2 years of needles only (1- and 2-year-old needles). Foliage from 10 of 37 stands was sampled for *P. gaeumannii* DNA (Freeman 2002, Winton et al. 2006).

From April 17 to June 17**,** 2006, the 59 stands sampled in 2001 were relocated using reference maps, aerial photos, and, if recorded, GPS coordinates. GPS coordinates were collected for all stands at the start of each transect. The same data as collected in 2001 were collected for each tree in the 59 stands. If a sample tree was dead, the cause was recorded, and a live Douglas-fir tree was selected near the dead tree. Total height, height 5 years ago (to verify past data), and height of the lowest live branch were measured with a laser height measurer (Laser Technology, Inc.). For three sample trees per stand, foliage from severed branches was placed in a sample bag, labeled as to stand number; and processed in the laboratory for pseudothecial counts, which differed from the 2002 sampling. Instead of ocular counts of pseudothecia as done in 2002, sampled needles were placed under an imager connected to a laptop computer, and the percentage of stomata occluded was estimated.

Crown-length to sapwood-area ratio (CL:SA) was estimated for one tree in a plot pair (5 trees per stand). CL:SA has been shown to effectively discriminate among stands with varying degrees of SNC (Maguire – Kanaskie 2002). Variables measured to estimate 2006 CL:SA were: 1) live-crown length (as calculated above) and 2) sapwood radius at dbh. Sapwood radius (nearest mm) and tree age at dbh were measured from an increment core taken on the side of the tree facing the transect for one tree in a plot pair (5 trees per stand).

Because some stands were thinned and stand density can influence tree growth, total basal area/ha and basal area/ha of Douglas-fir were calculated around one sample tree at each of the five sample points. Total plot basal area was measured around each sample tree by counting all in-trees with a prism and multiplying by the basal area factor (BAF=10). Only trees $\geq$2.5 cm dbh and all tree species including hardwoods were counted. All data were entered into an Excel spreadsheet where R^2 values were calculated from selected graphed data.

3 RESULTS AND DISCUSSION

We sampled 590 Douglas-firs in 59 stands from April 17 to June 17, 2006. Stands ranged in elevation from 150 to 1300 m and % slope from 0 to 60. Total basal area (m^2) per ha averaged 18.2 with a range of 4.6 to 36.3. Some stands had been precommercially thinned either before or after initial plot establishment in 2001. Some plot trees were accidentally felled, and these were replaced with other trees in 2006. Douglas-fir basal area (m^2) per ha averaged 16.1 with

a range of 4.6 to 36.3. Other stand species included western hemlock (*Tsuga heterophylla*) at the lower elevations and noble fir (*Abies procera*) at the upper elevations.

Mean 5-year-dbh growth was 6.1 cm (range = 3.0 to 8.6) and total-height growth was 3.6 m (range = 2.3 to 4.7). Mean live-crown ratio (LCR) decreased by 9.1% (range = 3.7 to -28.0) over 5 years, but 7 of 43 (16%) stands increased in mean LCR. Although the trend was for tree growth to increase with decreasing stand density, correlations were poor for both 5-year-dbh growth (R^2 = 0.05) and total-height growth (R^2 = 0.02). Recently thinned stands (lower basal areas) may not have had enough time to show any density-reducing effect. Also, diameter growth has been shown to substantially increase with precommercial thinning, but height growth of young Douglas-fir was independent of stand density for the ranges tested (11.5-63.3 m^2/ha) (Tappeiner et al. 1982).

Mean needle-retention index increased by 3.4 (range = -3.4 to 11.8) over 5 years, and mid-crown–foliage retention increased by 1.2 years (range 0.2 to 2.3). In 2006, many trees had a partial fifth-year and some a partial sixth-year complement of needles, but these were not reflected in retention indexes that score only the last 4 years of needles. Mid-crown-retention ratings, however, did capture 5 and 6-year needles. Needle retention in healthy Douglas-fir does not increase with tree age, at least over a relatively short period (5 years), so the observed increase is probably due to decreasing defoliation by SNC.

Mean percentages of stomata occluded by pseudothecia were 13.6% for 2000-(2-year-old) needles and 1.7% for 2001-(1-year-old) needles sampled in 2002, and 13.3% for 2004-(2-year-old) needles sampled in 2006. There was a poor correlation between the 2001-foliage retention and percentage of 2000-needle (2-year-old) stomata occluded (R^2 = 0.15, *Figure 1*) and 2001-needle (1-year-old) stomata occluded (R^2 = 0.03). Correlation between 2006-foliage retention and 2004-(2-year-old) needle stomata occlusion was slightly better (R^2 = 0.22). In the Oregon Coast Mountains, Hansen et al. (2000) showed that increasing proportions of stomata occupied by pseudothecia were associated with increasing defoliation. They recorded, however, mean pseudothecia densities up to 50% in 1-year-old foliage and foliage retention as low as 1 year, whereas our highest mean pseudothecia density was 11% in 1-year-old needles and our lowest mean foliage retention was 2.3 years. All pseudothecia collected in the Cascade Mountains in 2002 were from lineage 1 (Winton et al. 2006).

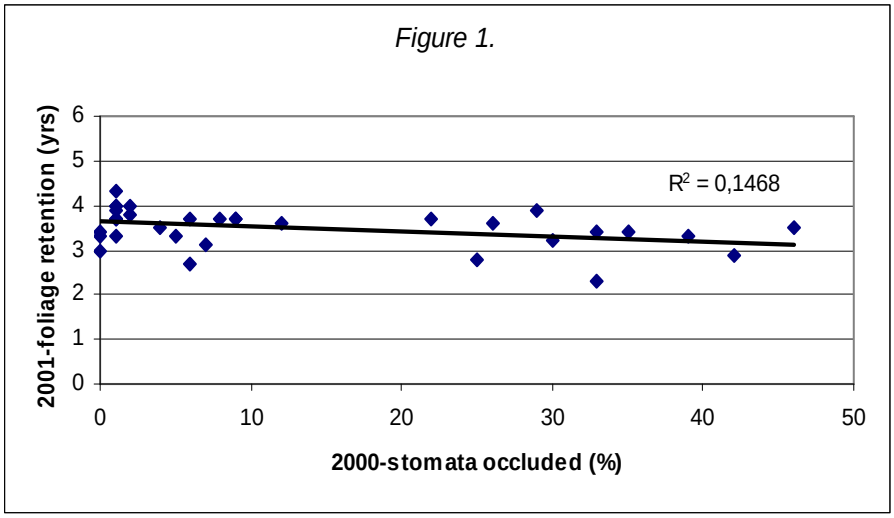


Figure 1. Graph showing correlation between the number of years of 2001-foliage retention at mid-crown and the percentage of 2000-(2-year-old) needles occluded by pseudothecia of Phaeocryptopus gaeumannii.

There was a moderate correlation between stand elevation and 2000-stomata occlusion (R^2 = 0.42) or 2004-stomata occlusion (R^2 = 0.50), where there were fewer pseudothecia at the higher elevations (*Figure 2*). Although correlations were poor (R^2 = 0.14 for 2001 and 0.21 for 2006), the trend was for foliage retention to also increase with elevation. Correlations between slope percent and either 2000-stomata occluded (R^2 = 0.25) or 2004-stomata occluded (R^2 = 0.14) were poor with occlusion decreasing with slope percent. Correlations between slope percent and either 2001-foliage retention (R^2 = 0.14) or 2006-foliage retention (R^2 = 0.05) were also poor with foliage retention increasing slightly with slope percent.

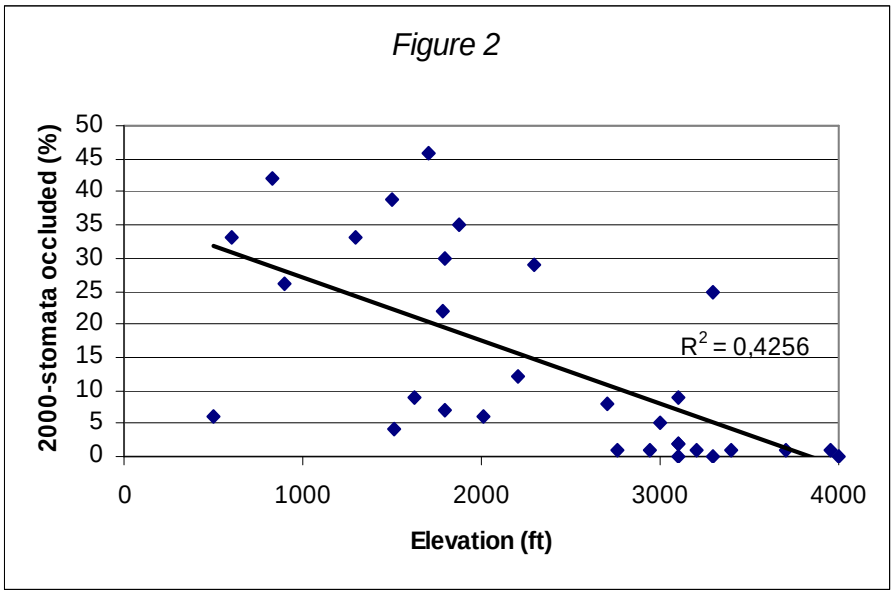


Figure 2. Graph showing correlation between the percentage of 2000-(2-year-old) needles occluded by pseudothecia of Phaeocryptopus gaeumannii and mean stand elevation. Pseudothecia decreased with increasing elevation.

Crown-length to sapwood-area ratio at dbh (CL:SA) averaged 5.2 cm/cm^2 (range 2.3 to 9.0) in 2006. Higher CL:SA values usually indicate poorer-growing stands; however, all of the Cascade stands sampled were in the lower range of CL:SA values for coastal Douglas-fir stands age 3-28 years that range from 3 to 24 CL:SA at crown base (Maguire – Kanaskie 2002) and for commercially thinned coastal Douglas-fir age 28-69 years that ranged from 5-18 CL:SA (Mainwaring et al. 2005). Although the trend was higher CL:SA values with poorer growing Cascade stands, correlations were poor with both 5-year-dbh growth (R^2 = 0.04) and total-height growth (R^2 = 0.05). There were also poor correlations between 2006 CL:SA and 2001-foliage retention (R^2 = 0.003), 2006-foliage retention (R^2 = 0.02), 2000-stomata occluded (R^2 = 0.20), or 2004-stomata occluded (R^2 = 0.18) .

There were poor correlations between 2001-foliage retention and 5-year-dbh growth (R^2 = 0.02, *Figure 3*) and total-height growth (R^2 = 0.01, *Figure 4*), between 2000-stomata occluded and 5-year-dbh growth (R^2 = 0.02) and total-height growth (R^2 = 0.03), and between 2004-stomata occluded and 5-year-dbh growth (R^2 = 0.02) and total-height growth (R^2 = 0.04). Either 5 years is not enough time to evaluate the affects of Swiss needle cast on Douglas-fir dbh growth in the Oregon Cascades, or there was no significant effect of Swiss needle cast on Douglas-fir growth during the latest outbreak.

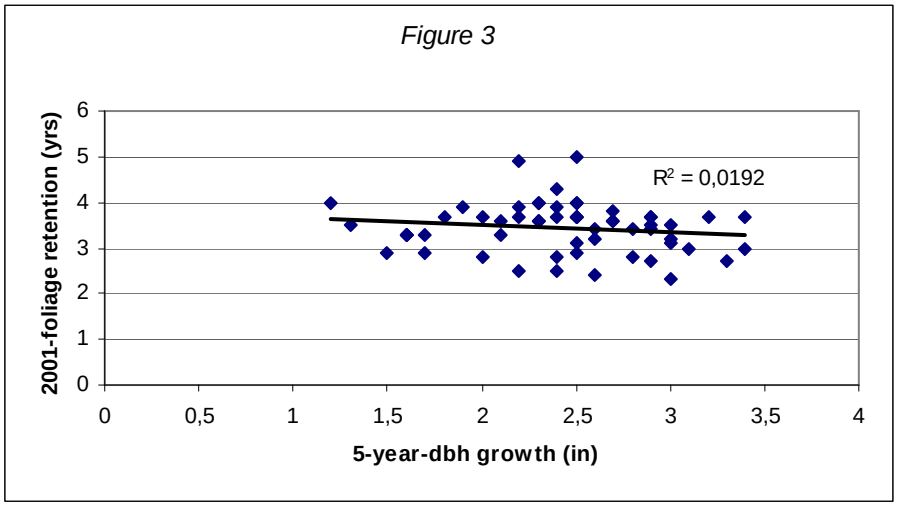


Figure 3. Graph showing correlation between the number of years of 2001-foliage retention at mid-crown and 5-year-dbh growth of Douglas-fir from 2001 to 2006

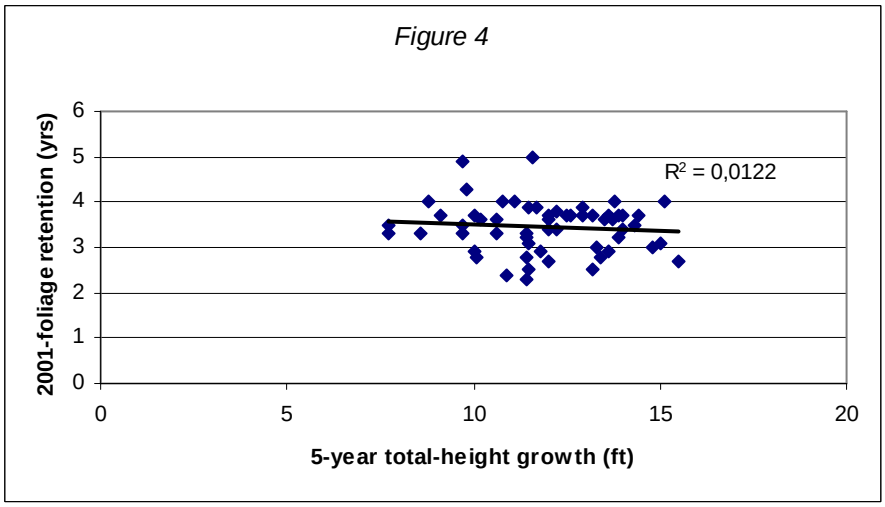


Figure 4. Graph showing correlation between the number of years of 2001-foliage retention at mid-crown and 5-year total-height growth of Douglas-fir from 2001 to 2006

4 CONCLUSIONS

There are at least two possible reasons why there may be no appreciable affect of Swiss needle cast on Douglas-fir 5-year-diameter and height growth during the latest SNC outbreak in the Cascade Mountains:

1) Oregon Cascade site characteristics, including plant associations, soil chemistry and parent material, air temperatures, and monthly precipitation and leaf wetness may not be as conducive to elevated populations of the causal fungus, *Phaeocryptopus gaeumannii*, and subsequent severe defoliation, as in the Coast Range.
2) The genetics (lineage 1) of isolates of the causal fungus in the Oregon Cascade Mountains more closely resemble isolates from New York, Europe, and New Zealand than isolates from the Oregon Coast Mountains (Winton et al. 2006). Also, lineage 2, which is abundant in the Oregon Coast Mountains, has not been reported in interior Oregon (Cascade Mountains) or elsewhere in the world.

Based on our results and their interpretation, forest managers may need not alter their current practices in the northern Oregon Cascades, and managing a mix of Douglas-fir and western hemlock at lower elevations and noble fir at higher elevations will help offset any future stand-growth declines due to Swiss needle cast or other pest outbreaks. On the other hand, we report only 5-year results, and more time may be needed to adequately detect any significant effects from Swiss needle cast in the Cascade Mountains. Plans are to resample Cascade stands in 5 years (2011).

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Retrospective Analysis of *Lophodermium seditiosum* Epidemics in Estonia

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Abstract – The needle trace method (NTM), created and developed by the Finnish forest pathologists prof. T. Kurkela, dr. R. Jalkanen and T. Aalto during the last decade of the XX century, has been already used by several researchers of different countries for retrospective analysis of needle diseases (*Hypodermella sulcigena*, by R. Jalkanen et al. in Finland) or herbivorous insect pests of Scots pine (*Diprion* pini, by T. Kurkela et al. in Finland; *Bupalus piniaria*, by H. Armour et al. in Scotland), but as well of pests of Sitka spruce (*Gilpinia hercyniae*, by D.T. Williams et al. in England). Scots pine in forest nurseries and young plantations of Estonia is often but irregularly suffering from the epidemics of the needle cast fungus *Lophodermium seditiosum*. Current environmental regulations exclude from the regulatory (control) measures all the others except of well-argued prophylactic systems, built up on reliable prognoses. The last is inconceivable without the availability of a reliable, as well, and long-lasting retrospective time-series of *L. seditiosum* epidemics, which, as it is known from the last half of the XX century, are occupying large forest areas, usually not least than a half of (the small) Estonia. An appropriate time-series would be useful, as well, for the more basic understanding of the accelerated mortality processes during the stand formation in early pole-age Scots pine plantations. Methodological principles of the use of NTM in an appropriate investigation together with the preliminary results of our research work, looking back for more than a century, are introduced and discussed in this investigation.

needle trace method (NTM) / needle diseases / annual needle loss / *Lophodermium seditiosum* / *Pinus sylvestris* / *P. contorta*

Kivonat – A *Lophodermium seditiosum* járványok visszatekintő elemzése Észtországban. A tűnyomok módszerét (NTM) finnországi kutatók (T. Kurkela, R. Jalkanen és T. Aalto) fejlesztették ki a XX. század utolsó évtizedében. Különböző országokban több kutató alkalmazta a tűbetegségek (*Hypodermella sulcigena*, R. Jalkanen et al. Finnországban), az erdeifenyő rovarkárosítások (*Diprion* pini, T. Kurkela et al. Finnországban; *Bupalus piniaria*, H. Armour et al. Skóciában), a szitkaluc károsítások (*Gilpinia hercyniae*, by D.T. Williams et al. Angliában) utólagos elemzésére. A tűkarc gomba (*Lophodermium seditiosum*) gyakran, de nem rendszeresen támadja az erdeifenyőt az észtországi csemetekertekben és fiatal erdősítésekben. A jelenlegi környezetvédelmi szabályok kizárják a védekezési módszereket, a megbízható előrejelzésen alapuló, jól megindokolt megelőző rendszerek kivételével. Ez utóbbiakhoz elengedhetetlen, hogy rendelkezzünk a *L. seditiosum* járványok megbízható, és hosszú távra visszatekintő idősoraival. E járványok a múlt század második felében nagy, rendszerint fél Észtországnyi területeket sújtottak. Egy megfelelő idősor hasznos lehet a felgyorsult pusztulási folyamatok alaposabb megértéséhez is az erdeifenyő erdősítések állománnyá

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alakulása során. Bemutatjuk és megvitatjuk az NTM alkalmazásának metodológiai elveit, kutatómunkánk több mint egy évszázadra visszatekintő előzetes eredményeivel együtt.

tűnyomok módszere (NTC) / tűbetegségek / éves tűvesztés / *Lophodermium seditiosum* / *Pinus sylvestris* / *P. contorta*

1 INTRODUCTION

Scots pine (*Pinus sylvestris* L.) is one of the main commercial tree species in Estonia, growing here in congenial to this species environment – climate and soils. The most common foliage disease in young Scots pine (*Pinus sylvestris* L.) plantations and forest nurseries of Estonia is caused by *Lophodermium seditiosum* Minter, Staley and Millar (*Rhytismatales, Ascomycota*). Occurrence of the needle cast disease and the disease agent (historically: *Lophodermium pinastri*) on the territory of Estonia was first documented at the turn of the XIX-XX century, both nearly at the same time, by the Baltic German foresters (Weiß 1902) and mycologists (Vestergren 1903), respectively. In Germany the problem of *Lophodermium* needle cast ("Kiefernschütte") had risen for the first time ca 50 years before, accompanying the beginning of the large-scale afforestations of the cutted areas by the use of nursery-grown pine seedlings (Stein, 1852. Thar. Forstl. Jahrb., Bd. 8; Holzner, 1877. Die Beobachtungen über die Schütte der Kiefer. Freiburg).

By today we know, that: 1) *Lophodermium* needle cast epidemics occur in Estonia almost regularly, but not precisely after definite intervals, and 2) needle cast epidemics are much more frequent in forest nurseries than in plantations, sometimes so frequent that the successive epidemics can not be distinguished from each other.

Still ignoring the possibility of genetic improvement of the pathogens virulence before the next-in-order epidemic, the explanation for the rise of a new epidemic has been still behold solely in the peculiar meteorological preconditions - rainy summers and following mild winters - of the years before the epidemics.

2 MATERIAL AND METHODS

2.1 Time-series and additional data sources

In this investigation we followed the traditional approach in the explaining of causes of the epidemics, i.e. considering the meteorological conditions of the preceding years. We tried to specify retrospectively the potential epidemic years of *Lophodermium* needle cast by juxtaposing two time-series: 1) the meteorological data (by selecting out the years with rainy summer + mild winter), and 2) the needle characteristic (extensive needle loss of the year, behold as a potential epidemic year).

This experimental approach could become possible only after the discovering and developing of the needle trace method (NTM, short description will follow) by the Finnish forest pathologists prof. T. Kurkela, dr. R. Jalkanen and T. Aalto (Kurkela and Jalkanen 1990, Aalto and Jalkanen 1996, 1998).

All available published data on the occurrence of *Lophodermium* needle cast disease during the last century in Estonia were used as additional sources for comparison with our experimental results with the purpose to make up the retrospective list of the disease epidemics.

During more than 40 years the elder author (M. Hanso) had carried out several investigations (Hanso, 1963, 1968, 1970, 2001, 2003) about the biology, ecology and regulation (control) of *Lophodermium* needle cast fungus and the disease, respectively, on

pine. Additionally, his laboratory served in the years from 1972 to 1985 as the diagnostic centre for the whole forest nursery management system in Estonia. All the appropriate materials were used in this investigation, as the additional data.

2.2 Experimental study areas

The retrospective NTM data were obtained from six pine stands, of which four *Pinus sylvestris* stands were growing in Konguta, Elva Forest District, south-eastern Estonia, but two stands (*P. sylvestris* and *P. contorta*, respectively) in Järvselja Training and Experimental Forestry District (*Figure 1*). Eight model pine trees from stand 1 (situated at 58°13' N, 26°10'E) were 109–115 years, eight trees from the stand 2 (58°12' N, 26°08' E) - 95-105 years old. The area of stand 2 had been more than a century ago in agricultural use. Eight model trees from stand 3 (58°16' N, 27°19' E) were ca 70 years old. The age of three model trees from stand 4 (neighbouring to stand 1) was about 45-50 years. Stand 5 (neighbouring to stands 1 and 4 from the other side) is a Scots pine provenance experiment and the age of altogether 68 model trees was 14 years. The age of eight model trees (*P. contorta*) from stand 6 (neighbouring to stand 3) was about 60-70 years. All stands were established by planting. All sample trees were growing in the main storey, each had a straight healthy stem.



Figure 1. Location of the investigated by the needle trace method (NTM) stands of Pinus sylvestris (in Konguta and Järvselja) and P. contorta (in Järvselja), and location of the meteorological station (Tõravere).

NTM data from the *P. contorta* stand were included, as this pine species was considered to be resistant to *Lophodermium* needle cast. Comparison of the needle cast dynamics in *P. contorta* and *P. sylvestris* stands could hopefully serve therefore as an additional argument in making decisions, e.g., was a hard needle loss in *P. sylvestris* stand caused by *Lophodermium* needle cast or by some other, more universal agents.

In this work an epidemic has been defined as any increase of disease in a population (Agrios 2005). The term “needle” actually means the term “fascicle” (or “short shoot”), less used in forest pathology than in plant anatomy.

2.3 Meteorological data

Meteorological data were obtained from the Tartu-Tõravere Meteorological Station, which is situated nearly between the investigated stands (*Figure 1*). For comparisons with the appropriate meteorological characteristics of the interesting us years ("pointer years") the long-period (1884-2004) mean monthly temperatures and precipitation sums were calculated first.

The pathogen needs both, the high precipitation for successful infection of pine needles in summer and the mild winter for successful colonisation of infected needles before the new vegetation of pine.

This way the potential *Lophodermium* needle cast epidemic years were considered to be probable, if the combination of wet (and warm?) summers and following mild winters will be met before the years of high needle loss. Epidemics of other infectious needle diseases of pine are rear in Estonia, but insect pests are triggered, on the contrary, by warm and dry summers.
Regarding the meteorological conditions, eight combinations of summers (between May and September) and following winters (from the previous year December to the pointer year March), were taken into the calculations to find out, which combinations were followed by the high needle loss in the next year. A year was classified as a “high precipitation” year and the following winter as a “mild” winter, if they were characterized by higher values of the respective meteorological characteristics, than the long-period (1882-2004) means +/- standard errors of the appropriate means.

Inside all the 8 combinations, higher (compared to long period mean, $p<0,001$) annual needle loss values followed the years with high precipitation in the period from:

a) July to September (VII-IX) + mild winter, and
b) July to August (VII-VIII) + mild winter.

Summer warmth, at the same time and concerning the same combinations of months inside “summer” (i.e.: a and b) had no effect on the amount of needle loss in the next (pointer) year, with no statistical differences between the warmer years and long period mean.

2.4 NTM analysis

One hundred and three sample trees from six pine stands were chosen and analysed according to NTM protocols (Aalto and Jalkanen 1998), including 95 sample trees of *Pinus sylvestris* and 8 of *P. contorta*. According to NTM protocols, a stand is already sufficiently represented by 5 to 10 model trees per age class (Jalkanen at al. 2000). Only 3 sample trees were analysed from the stand 4, which means that in our list the years 1958-1980 are represented insufficiently. The periods 1926-1937 and 1981-1994 are still not covered by NTM analysis at all.

After selecting out in a stand and felling of a sample tree the total height and annual height increments (the distances between the neighbouring branch whorls) were measured. From every annual shoot a 10-25 cm long section (bolt) was cut off. The western, northern and southern sides of each bolt were removed by axe already in forest, and only eastern side was transported to and analysed in laboratory according to NTM. Although all the bolts were marked (numbered), the full eastern side served for the control of the correct bolt age, i.e. the definite calendar year, as well. In laboratory, before the analyzes, only ten innermost rings of each bolt were left intact, because needles never keep attached to pine stem longer than 10 years (in Estonia they stay normally only 2.0 – 3.0 years).
One disk per every sample tree was sawed from the main stem for measuring the annual radial increments.

Needle trace method (NTM) was created for retrospective uncovering of different needle characteristics, from which only the characteristic "needle loss" was used in this investigation. NTM is based on the fact that the short shoots of living conifer needles remain attached to the pith of the main stem through the vascular tissue (Kurkela – Jalkanen 1990; Jalkanen – Kurkela 1992; Williams et al. 2003). The vascular connection extends through the each tree-ring, leaving there a trace for so long time as the needle is alive and functioning (*Figure 2*). When the needle dies the needle trace stops growing (into the next year ring). A needle trace is observed as a brown dot on the surface of the wood and can be seen with naked eye.

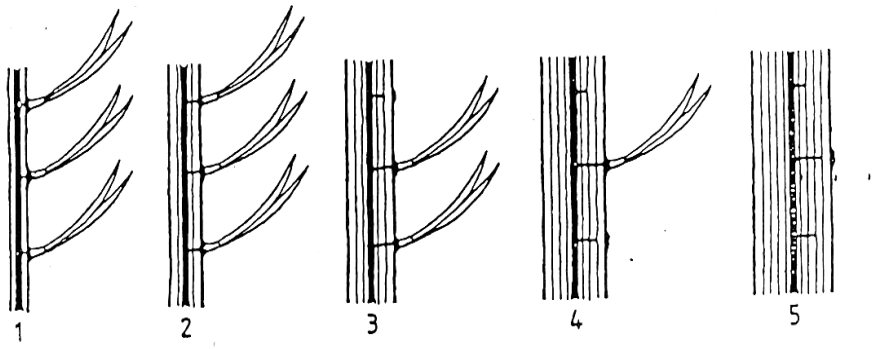


Figure 2. Pine shoots with attached needles and with the remains (needle traces) of fallen needles in wood in five years (Aalto and Jalkanen 1996).

Every annual section of tree have to be analysed, tree ring by tree ring, towards the pith to get the retrospective dataset of needle characteristics. An angle (usually from 45 to 90 degrees) has to be drawn on the both ends of the bolt, extending it from the pith outwards. Lines to connect the two angles have to be then drawn on the longitudinal surface of the eastern side. All needle traces within viewing plane have to be counted and the data have to be entered to the computing program NTMENG.

NTM has been already successfully used in retrospective studies of the growth losses of host trees, subsequent to the defoliation of pines by *Lophodermella* needle-cast disease and by insect *Diprion pini*, both in Finland (Jalkanen et al. 1994 and Kurkela et al. 2005, respectively), but, as well, to the defoliation of spruces by *Gilpinia hercyniae* in Wales (Williams et al. 2003) and of Scots pines by *Bupalus piniaria* in Scotland (Armour et. al. 2003). The first trial of retrospective analyses of *Lophodermium* needle cast in Estonia was undertaken some years ago (Drenkhan – Hanso 2003).

2.5 Data analysis

Different years from 1887 to 2003 were covered by different numbers of the model trees, analysed by NTM (*Table 1*, column 5). Only young age periods of tree life (from 5…7 to 22…25 years in stands 1. – 4., from ca 3 to 13 years in stand 5.) in *P. sylvestris* stands (*Figure 3*) and from 5…7 to 22…25 years in *P. contorta* stand were included into the computations. According to the NTM protocol the radial growth of sample trees was analysed on the breast height but in the youngest (5.) stand the radial growth was analysed on the stump height.

Although the older sample trees were analysed by NTM until the age of 35-45 years, the needle data, used in this investigation, were restricted by the younger period of tree life, not more than 25 years, because *Lophodermium* needle cast is not dangerous to older Scots pines. In this work recording of the needle trace data were used for calculating annual needle retention and, after that, the annual needle loss.

Annual needle retention (ANR), defined as the sum of the percentages of needles remaining on the leader sections of a tree in a particular year (Aalto and Jalkanen 1998; Pensa and Jalkanen 1999; Williams et al. 2003), was calculated using the equation:

$$ANR_t = \sum[x_t, (x\text{-}1)_t, \ldots, (x\text{-}n)_t]/100,$$

where ANR_t is the annual needle retention in year *t* (needle sets), x_t is the percentage of needles on leader section x present in year *t*, $(x\text{-}1)_t$ is the percentage of needles in year on the leader section formed on year earlier, etc.

Annual needle loss was calculated as:

$$ANL_t = (ANR_t – ANR_{t+1})+1,$$

where ANL_t is the total loss of needles (needle sets) in year *t*, and the term 1 represents the new flush of needles in year (t+1) (Aalto – Jalkanen 1998; Pensa – Jalkanen 1999).

The annual needle loss means the decrease in the amount of needle cohorts (defoliation) from the total main stem in a definite year, which is represented as the number of needle sets lost per year. The size of a needle set was calculated by evaluating a complete cohort of needles (i.e. a set of needles produced in a single year) with the value of 1 (100%), a needle cohort, where 75% needles had remained with the value of 0,75, and a needle cohort, where 50% of the needles had remained with the value of 0,5, etc. (Jalkanen 1998; Armour et. al. 2003).

NTM data were calculated by the special program NTMENG version 8 (Aalto – Jalkanen 2004). Statistical analyses were carried out by MS Excel and statistical program SAS.

3 RESULTS AND DISCUSSION

The development of *Lophodermium seditiosum* is very irregular and depends on environmental, particularly climatic factors (Martinsson 1979). The main source of *L. seditiosum* inoculum for infections in plantations is young infected fallen needles on which ascocarps of the fungus form in late summer and autumn (Diwani and Millar 1990). Wet and warm autumns provoke the epidemics in Estonia (Viirok 1931) as do the mild winters (Lepik 1930). Increased problems with *Lophodermium* needle cast in Sweden during the 1990s might be due in part to the occurrence of several consecutive mild winters (Stenström – Arvidsson 2001). Epidemiology of *Lophodermium* needle cast was first analysed by L. Lanier – G. Sylvestre (1971). A. van Maanen – F. Gourbière (2000) conducted an investigation into whether *Lophodermium pinastri* dynamics is determined by the balance between colonization and fructification. Both of these processes were found to be controlled by climatic factors, particularly rainfall. The conclusion was made, that spore production of the pathogen correlates with fructification, and colonization correlates with spore dispersal. It is the best short explanation, why the climatic factors determine the success of an epidemic.

In the earlier (first half of the XX century) literature *Lophodermium* needle cast has been mentioned in Estonia several times, sometimes presumably after the noticeable epidemics of the disease (Weiß 1902, Anonymous 1906 and 1907, S.-K. 1907, Krüger 1910, Aun 1922, Reim 1924 and 1925, Sepp 1928, Viirok 1931, Lepik 1930, Kohh 1933, Stegman 1936). During the Soviet occupation time (i.e. until the 1990s) *Lophodermium* needle cast has been investigated in Estonia in several works (Hanso 1963, 1968, 1970 and 1995, Hanso – Hanso 2001, 2003), but official registration of the data concerning the epidemics and economic losses in forest nurseries of the former (i.e. Soviet) Estonia has been always concealed and/or neglected. Therefore there is still a blank in our knowledge, although theoretical approaches

by that time were solved sufficiently, if not even well, and the disease and its control peculiarities introduced to the practical foresters.

Table 1 represents the potential epidemic years of the disease, therefore the preceding years, supporting the rise of the epidemic through its meteorological peculiarities (two combinations of the summer months and following mild winters, cf. columns 1 and 3, respectively) are not indicated separately. If the NTM data in a potential epidemic year (i.e. after a year with high precipitation in summer and following mild winter) showed, as well, a "high needle loss" (i.e. higher than the mean needle loss of the years of all the period, covered by our NTM data), then the year was classified as an epidemic year for pine plantations (bold numbers in column 7 of the *Table 1*). Following solely the "high precipitation and mild winter" years (i.e. without high needle loss) were classified as potential epidemic years only for forest nurseries (indicated in the *Table 1* with "?" after the year number). Letters after the year numbers in column 7 indicate other important threshold years, concerning *Lophodermium* needle cast in Estonia (e.g. first records, published in German and Estonian, respectively, cited in literature epidemic years, etc., the piths of the letters are specified under the *Table 1*).

Table 1. The result table

High precipitation in VII-IX + mild winter	High precipitation in VII-IX + mild winter + high annual needle loss	High precipitation in VII-VIII + mild winter	High precipitation in VII-VIII + mild winter + high annual needle loss	The number of sample trees, which NTM data cover the appropriate year	High annual needle loss in *Pinus contorta*	Important threshold and epidemic years in history attributed to *Lophodermium seditiosum*
1	**2**	**3**	**4**	**5**	**6**	**7**
1884	1884	**1884**	1884		1884	1884 **?**
1885	1885	1885	1885		1885	1885
1886	1886	1886	1886		1886	1886
1887	1887	**1887**	1887	1	1887	1887 **?**
1888	1888	1888	1888	4	1888	1888
1889	1889	1889	1889	4	1889	1889
1890	1890	1890	1890	6	1890	1890
1891	1891	1891	1891	6	1891	1891
1892	1892	1892	1892	7	1892	1892
1893	1893	1893	1893	8	1893	1893
1894	**1894**	1894	1894	8	1894	**1894**
1895	1895	1895	1895	8	1895	1895
1896	**1896**	**1896**	**1896**	8	1896	**1896**
1897	1897	1897	1897	8	1897	1897
1898	1898	**1898**	1898	8	1898	1898 **?**
1899	1899	**1899**	1899	8	1899	1899 **?**
1900	1900	1900	1900	8	1900	1900
1901	1901	1901	1901	8	1901	1901
1902	1902	1902	1902	8	1902	1902[a]
1903	1903	**1903**	1903	8	1903	1903[b] **?**
1904	**1904**	**1904**	**1904**	8	1904	**1904**
1905	1905	**1905**	1905	4	1905	1905 **?**
1906	1906	**1906**	1906	4	1906	1906 **?**
1907	1907	1907	1907	7	1907	1907[c]

Table 1. cont. The result table

High precipitation in VII-IX + mild winter	High precipitation in VII-IX + mild winter + high annual needle loss	High precipitation in VII-VIII + mild winter	High precipitation in VII-VIII + mild winter + high annual needle loss	The number of sample trees, which NTM data cover the appropriate year	High annual needle loss in *Pinus contorta*	Important threshold and epidemic years in history attributed to *Lophodermium seditiosum*
1	**2**	**3**	**4**	**5**	**6**	**7**
1908	1908	1908	1908	8	1908	1908
1909	1909	1909	1909	8	1909	1909
1910	1910	**1910**	1910	8	1910	1910 **?**
1911	1911	**1911**	**1911**	8	1911	**1911**
1912	1912	1912	1912	8	1912	1912
1913	1913	1913	1913	8	1913	1913
1914	1914	1914	1914	8	1914	1914
1915	1915	1915	1915	8	1915	1915
1916	1916	1916	1916	8	1916	1916
1917	1917	1917	1917	8	1917	1917
1918	1918	**1918**	1918	8	1918	1918 **?**
1919	1919	1919	1919	7	1919	1919
1920	1920	1920	1920	7	1920	1920
1921	1921	1921	1921	7	1921	1921
1922	1922	1922	1922	5	1922	1922[d]
1923	1923	1923	1923	4	1923	1923[e]
1924	1924	1924	1924	2	1924	1924[f]
1925	1925	**1925**	1925	1	1925	1925 **?**
1926	1926	1926	1926		1926	1926
1927	1927	1927	1927		1927	1927
1928	1928	1928	1928		1928	1928
1929	1929	1929	1929		1929	1929[g]
1930	1930	**1930**	1930		1930	1930 **?**
1931	1931	1931	1931		1931	1931
1932	1932	1932	1932		1932	1932
1933	1933	1933	1933		1933	1933
1934	1934	**1934**	1934		1934	1934 **?**
1935	1935	**1935**	1935		1935	1935 **?**
1936	1936	**1936**	1936		**1936**	1936
1937	1937	**1937**	1937		1937	1937 **?**
1938	1938	**1938**	1938	5	**1938**	1938
1939	**1939**	**1939**	**1939**	7	1939	**1939**
1940	1940	1940	1940	8	1940	1940
1941	1941	1941	1941	8	1941	1941
1942	1942	1942	1942	8	1942	1942
1943	**1943**	**1943**	**1943**	8	1943	**1943**
1944	**1944**	**1944**	**1944**	8	**1944**	1944
1945	1945	1945	1945	8	1945	1945
1946	1946	1946	1946	8	1946	1946
1947	1947	1947	1947	8	1947	1947
1948	1948	1948	1948	8	1948	1948

Table 1. cont. The result table

High precipitation in VII-IX + mild winter	High precipitation in VII-IX + mild winter + high annual needle loss	High precipitation in VII-VIII + mild winter	High precipitation in VII-VIII + mild winter + high annual needle loss	The number of sample trees, which NTM data cover the appropriate year	High annual needle loss in *Pinus contorta*	Important threshold and epidemic years in history attributed to *Lophodermium seditiosum*
1	**2**	**3**	**4**	**5**	**6**	**7**
1949	**1949**	**1949**	**1949**	8	1949	**1949**
1950	1950	1950	1950	8	1950	1950
1951	1951	1951	1951	8	1951	1951
1952	1952	1952	1952	8	1952	1952
1953	1953	1953	1953	8	1953	1953
1954	1954	1954	1954	8	1954	1954
1955	**1955**	**1955**	**1955**	8	1955	**1955**
1956	1956	1956	1956	6	1956	1956
1957	1957	**1957**	**1957**	1	1957	**1957**
1958	1958	1958	1958	2	1958	1958
1959	1959	1959	1959	2	1959	1959
1960	1960	1960	1960	2	1960	1960
1961	1961	**1961**	**1961**	2	**1961**	**1961**[h]
1962	1962	**1962**	1962	2	1962	1962 ?
1963	1963	1963	1963	2	1963	1963
1964	1964	1964	1964	3	1964	1964
1965	1965	1965	1965	3	1965	1965
1966	1966	1966	1966	3	1966	1966
1967	1967	1967	1967	3	1967	1967
1968	1968	1968	1968	3	1968	1968
1969	1969	1969	1969	3	1969	1969
1970	1970	1970	1970	3	1970	1970
1971	1971	1971	1971	3	1971	1971
1972	1972	1972	1972	3	1972	1972
1973	1973	1973	1973	3	1973	1973
1974	**1974**	**1974**	**1974**	3	1974	**1974**
1975	1975	1975	1975	3	1975	1975[i]
1976	1976	1976	1976	3	1976	1976
1977	1977	1977	1977	1	1977	1977
1978	1978	1978	1978	1	1978	1978
1979	1979	1979	1979	1	1979	1979[j]
1980	1980	1980	1980	1	1980	1980
1981	1981	1981	1981		1981	1981
1982	1982	1982	1982		1982	1982[k]
1983	1983	1983	1983		1983	1983
1984	1984	1984	1984		1984	1984
1985	1985	1985	1985		1985	1985
1986	1986	1986	1986		1986	1986
1987	1987	1987	1987		1987	1987
1988	1988	**1988**	1988		1988	1988 **?**
1989	1989	**1989**	1989		1989	1989 **?**

Table 1. cont. The result table

High precipitation in VII-IX + mild winter	High precipitation in VII-IX + mild winter + high annual needle loss	High precipitation in VII-VIII + mild winter	High precipitation in VII-VIII + mild winter + high annual needle loss	The number of sample trees, which NTM data cover the appropriate year	High annual needle loss in *Pinus contorta*	Important threshold and epidemic years in history attributed to *Lophodermium seditiosum*
1	**2**	**3**	**4**	**5**	**6**	**7**
1990	1990	1990	1990		1990	1990
1991	1991	**1991**	1991		1991	1991**?**
1992	1992	1992	1992		1992	1992
1993	1993	1993	1993		1993	1993
1994	1994	**1994**	1994		1994	1994 **?**
1995	1995	1995	1995	1	1995	1995 **?**
1996	1996	1996	1996	3	1996	1996
1997	1997	1997	1997	9	1997	1997
1998	1998	1998	1998	37	1998	1998
1999	**1999**	**1999**	**1999**	62	1999	**1999**[l]
2000	2000	2000	2000	68	2000	2000
2001	**2001**	**2001**	**2001**	67	2001	**2001**[m]
2002	2002	2002	2002	67	2002	2002
2003	2003	2003	2003	66	2003	2003
2004	2004	**2004**	2004		2004	2004[n]

Legend: The gray field covers the years, for which the NTM data are still missing.
The bold numbers in column 7 indicate the epidemic years in forest plantations.
The bold numbers and normal black numbers in column 7 indicate the possible epidemic years in forest nurseries.
The bold numbers in column 6 indicate the years with high needle loss in the *Pinus contorta* stand.

a – the first published information about Lophodermium needle cast in Estonia (in German),
b – the first published record of the agent fungus in Estonia,
c – the first published in Estonia instructions for the control of the disease,
d – the first published information about Lophodermium needle cast in Estonia (in Estonian),
e – **k** - documented epidemics of Lophodermium needle cast in Estonia.

Our experimental material (NTM data concerning the dynamics of needle loss in pines) is presented on *Figure 3*. According to our computations, including and considering, as well, the literature and other data, the definite Lophodermium needle cast epidemic years in Estonia were: 1) in young pine plantations: 1894, 1896 (the both before the first records of the disease and its agent in literature!), 1904, 1911, 1939, 1943, 1949, 1955, 1957, 1961, 1974, 1999 and 2001; and 2) in forest nurseries: 1884, 1887, 1894, 1896, 1898, 1899, 1903, 1904, 1905, 1906, 1910, 1911, 1918, 1925, 1930, 1934, 1935, 1937, 1939, 1943, 1949, 1955, 1957, 1961, 1962, 1974, 1988, 1989, 1991, 1994, 1995, 1999, 2001 and 2004.

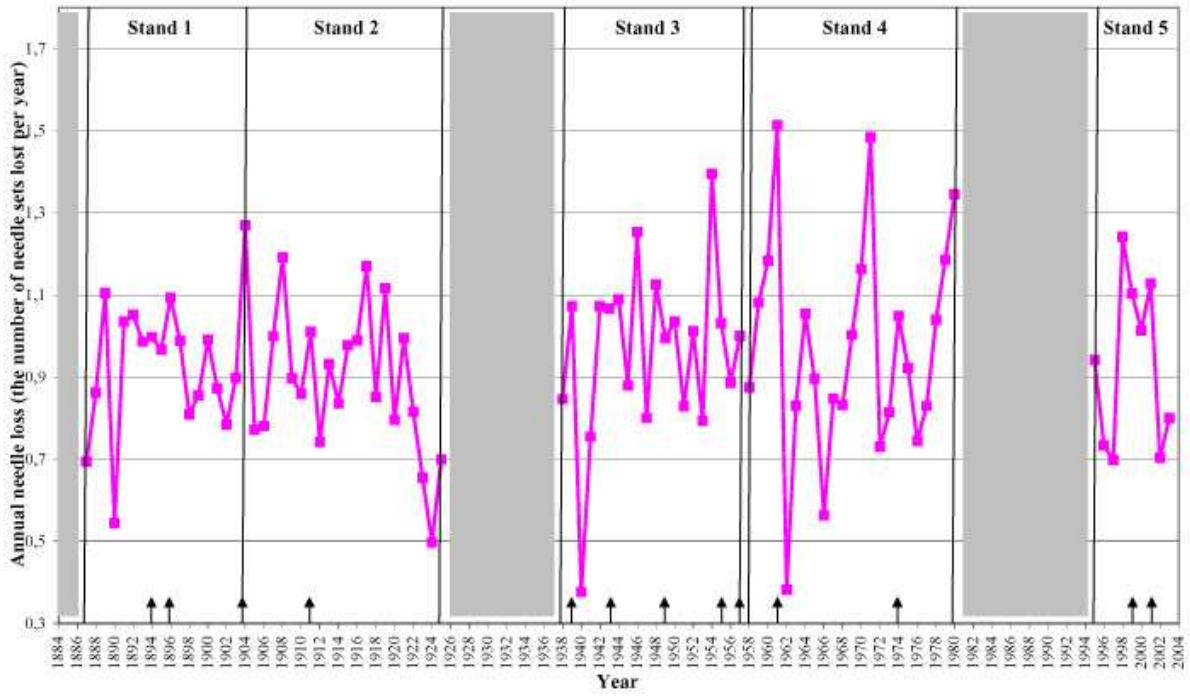


Figure 3. Annual needle loss dynamics during the young age (<25 years) of the analysed by NTM five Scots pine stands in South-East Estonia. Arrows below indicate the possible Lophodermium needle cast epidemic years (on the base of meteorological + NTM data). Grey fields cover the years, for which the NTM data are still missing.

Inside the long period (1884-2004) the documented in the literature (or by the services in forest pathology) epidemic years of Lophodermium needle cast in Estonia were 1923, 1924, 1929, 1961, 1975, 1979, 1982, 1999, 2001 and 2004 (*Table 1*). The years of high annual needle loss coincided with the documented epidemic years in 1961, 1979, 1999 and 2001 (*Figure 3*), which mean values (1,233 +/- 0,1) were higher than the long period (1887-2003) mean annual needle loss (0,966 +/- 0,013). Regarding the meteorological characteristics, the years 1961, 1999, 2001 and 2004 were selected out namely as the years, characterized by the combinations of high precipitation summer (VII-IX or VII-VIII) of the preceding year and following mild winter. At the same time the years 1923, 1924, 1929, 1975, 1979 and 1982, although documented in the literature as epidemic years, were not characterized by the supporting the pathogen preceding conditions (wet summers + mild winters). The years 1923, 1924 and 1975 (but not 1979) had low annual needle loss values compared to the long period mean. The reasons of these low values would be: a) trees in the appropriate age were not any more seriously affected by the Lophodermium needle cast, b) the amount of sample trees, covering the appropriate years were not sufficiently represented in the NTM material (at least 5 trees per year have to be analyzed, but e.g. the year 1979 was represented by only a single sample tree, although with high needle loss) and c) the weather data combinations were not perfectly selected for opening the potential epidemic years, in other words: we still do not know the actual needs of the pathogen. For the years 1929, 1982 and 2004 the NTM data are missing (*Table 1*).

Comparison of the appropriate meteorological data of documented Lophodermium needle cast epidemic years with the long period (1884-2004) means showed us, that: 1) the both combinations of summers of epidemic years had the mean precipitation values close to the long period mean (except of 1975), and 2) the mean winter temperatures had even lower values than long period mean in the epidemic years 1923, 1924, 1929, 1979 and 1982 (*Table 2*).

The low winter temperature, therefore, seems not to be a serious problem for the pathogen in raising a new epidemic, and if the level of summer precipitation is close to the long period mean, the epidemic can get start.

Table 2. Mean meteorological characteristics (summer precipitation and winter temperature) of the years preceding to the documented Lophodermium needle cast epidemic years

Year	Precipitation sum during VII-IX (mm)	Precipitation sum during VII-VIII (mm)	Monthly XII-III temperature (°C)
1923	216,0	189,0	-5,4
1924	270,0	185,0	-6,6
1929	329,0	217,0	-7,9
1961	186,0	157,0	-0,1
1975	190,0	145,0	-0,7
1979	381,0	272,0	-8,4
1982	247,0	181,0	-5,2
1999	292,0	268,0	-3,6
2001	237,0	225,0	-2,2
2004	201,5	187,2	-3,1
Long period (1884-2004) mean	220,1+/-6,6	157,9+/-5,5	-4,9+/-0,2

Our NTM material concerning *Pinus contorta* (stand 6) betrayed high annual needle loss values of this pine species in the years 1936, 1938, 1944 and 1961 (*Table 1*, column 6). These losses were caused, seemingly, by the other agents (perhaps by the starting epidemic of *Gremmeniella abietina*, which was first documented in Estonia in 1964, cf. Hanso, 1969, 1972). *P. contorta* is considered to be resistant to Lophodermium needle cast. However, our hopes to find clear differences in the dynamics of needle losses in these pine species (*P. sylvestris* and *P. contorta*) failed, as during some of the presumably epidemic years of Lophodermium needle cast (e.g. 1961) both the pine species had high needle loss. During the years of diagnostic service in the forest nursery management system of Estonia (1972-1985) we had diagnosed (by personal communication of the elder author) serious Lophodermium needle cast attack on *Pinus contorta* seedlings twice, in 1978 and 1979.

We can set aside the possibility, that any insect defoliator could cause the extensive needle loss of pines during the study years, as it is generally known, that insect pests accompany namely hot and dry, and not wet summers as do fungal diseases. However, we decided to look for the appropriate (i.e. hot and dry) summers and see, which kind of needle losses were following these, presumably congenial to insects, years. It was found, that all the years with hot summers were followed by the years with annual needle loss, less than the long period mean. Therefore we can believe, that none of our five Scots pine stands had lost considerable amount of needles in their youth through the insect attack.

Large scale epidemics of Coleosporium needle rust – another possible interfering our conclusions fungal disease of pine needles - are seldom in Estonia. Although the fungus (fungi) can be found year by year, they are few in numbers (personal communication by the elder author). Epidemics of *Hypodermella sulcigena* or *H. conjuncta* are rear, as well, in Estonia.

4 CONCLUSIONS

As the bases of the modern disease regulation (control) system in growing pine seedlings in forest nurseries can be, only and strictly, prophylactic, the prognoses must lie in the foundation of an appropriate system. A retrospective list of epidemics from the history will hopefully improve the raising of a more acceptable prognostic system than it exists today.

According to our computations by the use of NTM (needle trace method), including and considering, as well, the literature and other data, the definite Lophodermium needle cast epidemic years in Estonia were:
1) in young pine plantations: 1894, 1896 (the both before the first records of the disease and its agent in literature!), 1904, 1911, 1939, 1943, 1949, 1955, 1957, 1961, 1974, 1999 and 2001; and 2) in forest nurseries: 1884, 1887, 1894, 1896, 1898, 1899, 1903, 1904, 1905, 1906, 1910, 1911, 1918, 1925, 1930, 1934, 1935, 1937, 1939, 1943, 1949, 1955, 1957, 1961, 1962, 1974, 1988, 1989, 1991, 1994, 1995, 1999, 2001 and 2004.

The appropriate analyses, represented in this investigation, is not ended as there are some large periods which are not covered at all or are covered by insufficient amount of the NTM material.

Hopefully, the NTM will serve us in the future as well in the investigations, which have to elucidate the role of Lophodermium needle cast epidemics in the selection of surviving and failing trees during the rich-in-victims formation of a pine stand. As well the question: how large territory is occupied by a Lophodermium needle cast epidemic, is still not answered.

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Susceptibility of Different Clone Groups of Austrian Pine to *Mycosphaerella pini* E. Rostrup and *Sphaeropsis sapinea* Dyko & Sutton

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Abstract – Necrosis of needles and shoots caused by various damaging agents, have emerged about fifteen or twenty years ago in the Hungarian Austrian pine stands. Damages caused mainly *Sphaeropsis sapinea* Dyko & Sutton, *Mycosphaerella pini* E. Rostrup (its anamorph *Dothistroma septospora* /Dorog./ Morlet), and *Sclerophoma pithyophila* (Corda) Höhn, and *Cenangium ferruginosum* Fr. ex Fr. Appearance and rapid spread of the fungi may surely be attributable to the anomalies in weather conditions. Since, the prevailing conditions, according to the long term predictions, have a fair chance of becoming permanent, it seems to be rational to select resistant or less susceptible species in producing propagation material of Austrian pine in the future. This brought up the investigation on the susceptibility of those clones, which are considered as basis for the elite propagation material of Austrian pine, to individual damaging agents. The investigations were carried on clone bank of Kisunyom. The results showed the susceptibility to the damaging agents considerable differences prevailed among the clone-groups, so it has been confirmed that in the process of genetic improvement when individuals are selected for cultivation, their susceptibility to the damaging agents should also be considered.

***Sphaeropsis sapinea* / *Dothistroma septospora* / *Pinus nigra* / Austrian pine / infection / investigation of clone / resistance breeding**

Kivonat – Különböző feketefenyő klóncsoportok fogékonysága a *Mycosphaerella pini* E. Rostrup és a *Sphaeropsis sapinea* Dyko & Sutton iránt. Magyarország feketefenyő állományai az 1980-as évek közepéig erdővédelmi szempontból a legstabilabb kultúrának számítottak. Többnyire *Heterobasidion annosum* (Fr.) Bref. fertőzés, illetve lokális jelleggel szú károk fordultak elő az állományokban. Mintegy tizenöt, húsz évvel ezelőtt jelentkeztek az első komolyabb hajtás és tűelhalások melyet különböző kórokozók idéztek elő. Kezdetben a *Sphaeropsis sapinea* Dyko & Sutton majd néhány évvel később a *Mycosphaerella pini* E. Rostrup (anamorf alakja *Dothistroma septospora* /Dorog./ Morlet), és *Sclerophoma pithyophila* (Corda) Höhn fajok okoztak helyenként jelentős károkat. 1997-1998 során a *Cenangium ferruginosum* Fr. ex Fr. is megjelent és elterjedt országszerte, a különböző korú feketefenyő állományokban. A gombák megjelenése és gyors elterjedése nagy valószínűséggel az időjárási anomáliáknak köszönhető. Mivel a hosszú távú előrejelzések szerint ez az állapot állandósulhat, a jövőben a feketefenyő szaporítóanyag előállításakor célszerű lenne rezisztens vagy kevéssé fogékony fajokat szelektálni. Ez indított minket arra, hogy megvizsgáljuk az elit szaporítóanyag alapjául szolgáló feketefenyő klónok érzékenységét az egyes kórokozókkal szemben. Ezeket a kutatásokat a Kisunyomban létesített feketefenyő klón-gyűjteményben végeztük. A kutatások eredményei alapján bizonyossá vált, hogy a nemesítési eljárás

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során, a szaporítóanyag termelés céljára kiválasztandó egyedek esetében célszerű figyelembe venni a kórokozókkal szemben tanúsított érzékenységet is, mivel ebben igen nagy különbségek mutatkoznak az egyes feketefenyő klóncsoportoknál.

***Sphaeropsis sapinea* / *Dothistroma septospora* / *Pinus nigra* / feketefenyő / gombafertőzés / klónvizsgálatok / rezisztencia nemesítés**

1 INTRODUCTION

From the aspects of plant pathology, the stands of Austrian pine in Hungary had been considered as the most stable man-made plant formations till the mid 1980s. Damages caused mainly by *Heterobasidion annosum* (Fr.) Bref. and wood-beetle locally can be well identified in the stands. (Pagony 1983) The first notable necrosis of needles and shoots caused by various damaging agents, have emerged about twenty or twenty five years ago. At first, the species *Sphaeropsis sapinea* Dyko & Sutton, then a few years later the *Mycosphaerella pini* E. Rostrup (its anamorph *Dothistroma septospora* /Dorog./ Morlet), and *Sclerophoma pithyophila* (Corda) Höhn caused considerable damages at certain locations. (Szabó 1991, 1997; Koltay 1990, 1997) Some years later *Cenangium ferruginosum* Fr. ex Fr. has also appeared and dispersed in various age of Austrian pines stands. (Koltay 1999)

Appearance and rapid spread of the fungi may surely be attributable to the anomalies in weather conditions. The damaging agents emerged in the last decades are so-called degradation parasites. As a result of the stress caused by the hot and dry weather, the degraded trees have become more susceptible to these agents. (Gilmour 1981; Gibson 1979; Nicholls – Ostry 1990) Since, the prevailing conditions according to the long term predictions have a fair chance of becoming permanent, it seems to be rational to select resistant or less susceptible species in producing propagation material of Austrian pine in the future. Up to now, the needed knowledge on the Austrian pine has not been at hand yet. This brought up the investigation on the susceptibility of those clones, which are considered as basis for the élite propagation material of Austrian pine, to individual damaging agents. For the research work, the clone bank of Austrian pine established at Kisunyom, near to town Szombathely in Western Hungary, proved to be the most appropriate choice. (Varga 2000)

2 MATERIAL AND METHODS

In Hungary, the programme aimed at genetic improvement of conifers has been launched in the early 1950s with selecting and marking the stock trees. Later on, clone banks were established by collecting individuals from domestic and other foreign populations. The clone bank of Kisunyom was established in 1965-1968. Currently, there are 4 domestic and 6 European clone-groups with 680 individual clones in the collection here. (*Table 1.*) The groups were planted with different representation but with a uniform spacing of 6 by 4 metre. (Bánó 1970; Bánó – Mátyás 1987)

Table 1. Type and number of Austrian pine clones

	Hungarian clones				European clones						
Clones	101	108	104	103	YU	F	A	TR	CY	E	Total
piece	98	176	52	17	46	168	39	10	43	31	680

In 1996, a medium, then in 1997, a very intensive reddish discoloration occurred on the individuals of the clone bank. On the basis of the symptoms as well as on the collected samples is became evident that needle and shoot necrosis of the trees were caused by two damaging fungus species *Dothistroma septospora* (Dorog.) Morlet and *Sphaeropsis sapinea* Dyko & Sutton. Occurrence of the *D. septospora* on the infected trees were considerably higher than that of *S. sapinea*. The different rates of infection among the clone-groups were eye-catching even in the first stage of investigations. It lead to the conclusion that in order to reveal the susceptibility of individual clones to the infection of fungi, a very thoughtful investigation was needed.

The assessment on the conditions of infection was launched in the autumn of 1997. The rates of rubescence and needle necrosis in the crown have been established for each individuals with a 10 % accuracy. On the basis of the different symptoms caused by the two damaging species, the actual infections have been identified and the findings were analysed according to species. (*Table 2 - 3.)*

Table 2. Distribution (%) of Dothistroma pini infection

Infection	Type of clones									
(%)	101	108	104	103	YU	F	A	TR	CY	E
0	0,0	35,2	13,5	17,5	8,7	2,4	25,6	40,0	0,0	9,7
5	1,0	33,5	55,8	5,9	32,6	7,7	12,8	0,0	0,0	3,2
10	6,1	6,8	11,5	11,8	26,1	12,5	30,8	0,0	0,0	41,9
20	16,3	5,1	9,6	11,8	13,0	14,3	17,9	0,0	0,0	25,8
30	20,5	7,4	7,7	11,8	15,2	11,3	10,3	10,0	9,3	3,2
40	18,4	6,8	1,9	0,0	4,3	14,9	0,0	0,0	4,7	12,9
50	9,2	1,1	0,0	11,8	0,0	14,9	0,0	20,0	14,0	3,2
60	2,0	1,1	0,0	5,9	0,0	2,4	2,6	10,0	11,6	0,0
70	13,3	1,7	0,0	11,8	0,0	11,9	0,0	10,0	11,6	0,0
80	12,2	1,1	0,0	11,8	0,0	7,7	0,0	10,0	16,3	0,0
90	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	32,6	0,0
100	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 3. Distribution (%) of Sphaeropsis sapinea infection

Infection	Type of clones									
(%)	101	108	104	103	YU	F	A	TR	CY	E
0	78,6	27,8	19,2	76,5	17,4	90,5	64,1	20,0	100,0	100,0
5	21,4	10,2	28,8	23,5	60,9	9,5	15,4	40,0	0,0	0,0
10	0,0	26,7	17,3	0,0	8,7	0,0	5,1	0,0	0,0	0,0
20	0,0	6,8	13,5	0,0	2,2	0,0	2,6	0,0	0,0	0,0
30	0,0	14,2	7,7	0,0	6,5	0,0	5,1	10,0	0,0	0,0
40	0,0	5,1	9,6	0,0	4,3	0,0	0,0	20,0	0,0	0,0
50	0,0	1,1	1,9	0,0	0,0	0,0	2,6	0,0	0,0	0,0
60	0,0	0,0	1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0
70	0,0	1,1	0,0	0,0	0,0	0,0	0,0	10,0	0,0	0,0
80	0,0	2,8	0,0	0,0	0,0	0,0	2,6	0,0	0,0	0,0
90	0,0	2,8	0,0	0,0	0,0	0,0	2,6	0,0	0,0	0,0
100	0,0	1,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

The basic question to be answered in the investigations was whether there were any accountable differences among the susceptibility of the clone-groups? Further, we wanted to clear up the magnitude of differences, if any, i.e. what was the range of susceptibility of the individual clone-groups to the two species of fungus. Finally, we studied within the individual clone-groups whether there was any indicative relationship between the susceptibility to the two species?

3 RESULTS AND DISCUSSION

To answer the problems, at first, on ***K*** (actually for 10 different clone groups of Austrian pine) patterns of frequency distribution represented by ***v*** classes (12 grades of infection) the test of homogeneity has been performed using a contingency table of fields ***K*** x ***v.*** As results, the following conclusions can be drawn:

In the case of *D. septospora* the calculated value $\chi^2 = 653.7$ exceeds the critical table value P 0.1% = 149.4. That means, as for the damaging agent, reactions of clone groups of Austrian pine to the actual fungus were not homogeneous, i.e. their susceptibility varied considerably.

In the case of *S. sapinea* the calculated value $\chi^2 = 494.5$ is also much more higher than the critical table value P 0.1% = 149.4. Thus, the clone-groups show up various susceptibility to this fungus too.

As next step the ***infection index*** of individual clones have been calculated. Infection index = rate of infection per cent multiplied by band of per cent (as weight). With using that formula, susceptibility of clone-groups to individual fungus species could be accounted. Ranking the clone-groups it was quite clear that *D. septospora* occurred in all of the clone-groups, but at the same time the rate of infection, which is equal to the susceptibility to the damaging agent considerable differences, prevailed among the clone-groups. Analysing the occurrence of species *S. sapinea* brought that rate of infection was less for all of the clone-groups and there were two clone-groups in which damaging agent could not be detected at all. (*Table 4.*)

Table 4. Susceptibility of clones against *Dothistroma p.* and *Sphaeropsis s.*

Type of clones	Order of Susceptibility	Infection index of *Dothistroma p.*	Order of Susceptibility	Infection index *Sphaeropsis s.*
CY	***1***	6907,0	***9,5***	0,0
101	***2***	4320,0	***7***	107
F	***3***	3723,2	***8***	47,6
103	***4***	3449,9	***6***	117,6
TR	***5***	3400,0	***1***	2000,0
E	***6***	1725,8	***9,5***	0,0
YU	***7***	1315,2	***5***	804,3
A	***8***	1192,3	***4***	897,4
108	***9***	1167,6	***2***	1818,2
104	***10***	894,2	***3***	1413,5

Finally, in studying the relation between the two species of fungus, rank correlation was calculated according to *Sperman.* The value of the rank correlation coefficient "$\boldsymbol{R}^{\text{rank}}$ " proved to be - 0.655. Its absolute value exceeded the "***R***" value of 0.631 from the table. In

accordance with this finding, a correlation seems to be existing, between the susceptibility of Austrian pine clones to the two species of fungus. Here, the minus sign illustrates that the above relation means: when a specific clone of Austrian pine is susceptible to the infection of the *D. septospora*, it tends to be more resistant to the infection of *S. sapinea* and vice versa. This trend, however, could not be confirmed properly, because, apart from other causes, the phenomena can also be understood that, as a result of an earlier attack by one of the fungus species, the other would not find enough healthy needles and shoots, or the two species may be antagonist of each other. Further investigations are needed.

On the basis of the recent research findings, it has been confirmed that in the process of genetic improvement when individuals are selected for cultivation, their susceptibility to the damaging agents should also be considered, because the clone-groups of Austrian pine have large-scale differences in this respect.

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The Most Frequent *Lophodermium* spp. on Scots Pine and Austrian Pine and Their Role in the Appearance of Other Fungi on the Needles

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Abstract – Different critical periods for infection, the difference in morphological-anatomical and eco-physiological characteristics and other distinguish support the taxonomic concept on the existence of several *Lophodemium* spp. on pines.
In addition to the study of *Lophodemium* spp. on secondary needles, the study was extended also to primary needles. The results of our long-term research, compared to the results obtained by other researchers in Europe, show that in Serbia there are at least two widely distributed *Lophodemium* species infecting Scots pine and Austrian pine needles. This study is supporting with identification of *L. seditiosum* and *L. pinastri* using species-specific PCR primers from the ribosomal ITS region. In pine plantations were established connection between *Lophodemium* species and other fungi on needles Scots and Austrian pine. On needles of Scots pine, except *L. pinastri* and *L. seditiosum* mostly were established following more important fungi: *Sphaeropsis sapinea*, *Cyclaneusma minus* and *Sclerophoma pythiophylla*. On needles of Austrian pine, except *L. pinastri*, were established following more important fungi: *Dothistroma pini, Sphaeropsis sapinea, Cyclaneusma niveum* and *Cytospora friesii.*

***Lophodermium* spp. / Scots pine / Austrian pine / needle fungi**

Kivonat – A leggyakoribb *Lophodermium* fajok az erdeifenyőn és a feketefenyőn, szerepük más tűgombák megjelenésében. Különböző kritikus fertőzési időszakok, morfológiai-anatómiai és öko-fiziológiai jellegek és egyéb megkülönböztető bélyegek támasztják alá a több *Lophodermium* faj létezésének taxonómiai elvét a *Pinus* fajokon. A *Lophodermium* fajok másodlagos tűkön végzett vizsgálatát az elsődleges tűkre is kiterjesztettük. Hosszú távú kutatásaink eredményei, összhangban más európai kutatók eredményeivel azt mutatják, hogy Szerbiában legalább két széleskörűen elterjedt *Lophodermium* faj fordul elő az erdeifenyő és a feketefenyő tűin. Ezt a *L. seditiosum* és *L. pinastri* riboszomális ITS szekvenciáin alapuló, fajspecifikus PCR primereket alkalmazó, molekuláris azonosítása is alátámasztja. *Pinus* erdősítésekben összefüggést állapítottunk meg a *Lophodermium* fajok és az egyéb gombák között az erdei- és feketefenyő tűin. Az erdeifenyő tűin, a *L. pinastri* és *L. seditiosum* fajokon kívül a következő jelentősebb gombák telepedtek meg: *Sphaeropsis sapinea, Cyclaneusma minus* és *Sclerophoma pityophila*. A feketefenyő tűin a *L. pinastri*-n kívül a *Dothistroma pini, Sphaeropsis sapinea, Cyclaneusma niveum* és *Cytospora friesii* fajok jelentek meg gyakrabban.

***Lophodermium spp.* / erdeifenyő / feketefenyő / tűgombák**

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1 INTRODUCTION

Lophodermium spp. are identified on all five Continents, on about 30 pine species and varieties, and the most susceptible are *Pinus sylvestris* and *P. resinosa.* More susceptible than other species are *P. brutia, P. densiflora, P. montana, P. mugo, P. nigra, P. tabuliformis, P. contorta, P. halepensis, P. pinea, P. radiata, P. montezumae, P. virginiana.* Pine needles are infested by numerous *Lophodermium* species: *L. pinastri, L. nitens, L. australe, L. pini-excelsae, L. pini-pumilae, L. conigenum, L. seditiosum,* etc. In Europe the most significant species in this genus is *L. seditiosum* Minter, Staley a. Millar (anamorph stage *Leptostroma rostrupii* Minter / = *L. austriacum* Oud./), and the most widespread is *L. pinastri* (Schrad. ex Hook.) Chev. (anamorph stage *Leptostroma pinastri* Desm). Along with these two species, *L. conigenum* (Brunaud) Hilitzer and *L. pini-excelsae* Ahmad are more frequent than other species on pine needles in Europe.

In Europe, *Lophodermium* spp. have been a serious problem in the nurseries for more than 100 years, while in North America they have been significant since 1966. Infected seedlings are often transported before the visible symptoms, and they die after planting. This method of dissemination results in the occurrence of local epidemics.

The species in the *Lophodermium* genus, as well as other species of widely widespread micro-organisms, are characterised by high variation of bioecological characteristics (morphological characters, host spectre, degree of virulence, point of attack, etc), which points to the presence of numerous species. The determination of the species which differ by the above bioecological characteristics, and especially by pathogenic properties, explains the contradiction between the European and the North American descriptions of this disease, i.e. its parasitic and saprophytic characteristics, as well as the existence of the pathogenic species which is very aggressive (Millar – Watson, 1971; Staas Ebregt – Gremmen, 1975; Staley 1975; Minter 1981; Lazarev 1980, 2004). However, the symptoms of this species have been masked for a long time or covered by the symptoms of widespread species which are frequent on the cast needles.

The variation of *Lophodermium* spp. is proved also by the identification of *L. seditiosum* and *L. pinastri* by the species-specific PCR primers from ribosomal ITS region (Stengström – Ihrmark, 2005).

During the multiannual study of *Lophodermium* species life cycle on pine needles, several species of fungi were found on the same needles. For this reason, this paper presents, along with bioecological characteristics of the most frequent and most significant *Lophodermium* species which infest Scots pine and Austrian pine needles, also a survey of succession and connexion of other fungal species identified on the needles.

2 MATERIAL AND METHODS

Scots pine and Austrian pine needles of different ages, infected by disease agents, are analysed in all development stages (primary and secondary needles) and a year after shedding. The collected samples originate from the nurseries, plantations and stands. During the three-year research, each year and each month, 100 needles of different ages were analysed from each of the 12 plots (4 nurseries, 4 plantations and 4 stands). The development of the disease symptoms was monitored on the still living and on the dead needles, as well as the time of occurrence and ripening of the fruiting bodies in relation to the type, location and needle age. From each needle group (green, chlorotic, yellow, brown, red, with and without fruiting bodies) 10 samples were selected which were superficially sterilised in sodium hypochlorite

for 5 minutes, washed in distilled water, cut into 3 mm long segments and placed on artificial media potato dextrose agar (PDA) and malt agar (MA).

The morphological-anatomic characteristics of fruiting bodies and reproductive organs on the needles were analysed by standard mycological and phytopathological techniques.

The succession of fungi on individual needles was observed based on the analysis of the isolates from the infected needles with and without disease symptoms, every 15 days, and based on the fruiting bodies which appeared in different times of the year.

3 RESULTS

The principal diagnostic characteristics of the most significant and most widespread *Lophodermium* species in our conditions (*L. pinastri* and *L. seditiosum*) on Scots pine primary and secondary needles, as the most susceptible species, are presented in *Table 1*.

Table 1. The principal diagnostic characteristics of L. pinastri and L. seditiosum on primary and secondary needles

Characteristics	Primary needle		Secondary needle	
	L. pinastri	*L. seditiosum*	*L. pinastri*	*L. seditiosum*
Location of the pathogen fruiting bodies	coleoptiles	3 months old	in the litter and on needles of dead branches	needles on the plants in the nursery
Position of apothecia in the needles	partly subepidermal	wholly subepidermal	partly subepidermal	wholly subepidermal
Average clypeus thickness (μm)	70	60	95	115
The most frequent colour of lips of apothecia	greygreen	green	red	green
Thickness of apothecium basal layer (μm)	60	50	80	96
Apothecium length (μm)	600-800	500-700	700-1200	800-1500
Average hymenium thickness (μm)	35	30	48	58
Ascus length (μm)	105-135	120-160	110-155	140-170
Ascospore length (um)	60-120	80-120	70-110	90-120
Pycnidia length (μm)	180-250	200-280	300-400	300-500
Conidia length (μm)	3.0-4.5	5.0-6.5	4.5-6.3	6.0-8.0
Cross bands on needles as differential symptoms	not formed or several black bands formed	not formed	many black bands formed	lines not formed or several brown bands formed
Mycelium colour on 2 % malt agar	white with black margin	brown	white with black margin	brown
Period of ascospore dissemination	April-September	August-April	April-September	August-April
Critical period for infection	May-June	mid August-October	May-June	mid August-October

The differences in morphology, anatomic structure and other characteristics between the primary and the secondary needles point to the differences expressed by morphological and anatomical characters of *L. pinastri* and *L. seditiosum* fruiting bodies and reproductive organs on these types of needles. However, the periods of spore dissemination from the fruiting bodies, critical periods of infection and the characteristics of the cultures of both needle types are the same for each species.

The results of the multiannual study of succession and connexion of the more significant fungi on Scots pine and Austrian pine needles are presented in *Table 2.*

Table 2. Succession and connexion of the more significant fungi on Scots pine and Austrian pine needles

Pine species	Needle type and age	Succession and connexion of the pathogens
Pinus sylvestris	primary; younger than one year	*Lophodermium seditiosum (++);* isolated from the needles about 3 months old;
	primary; one year old	*Lophodermium pinastri (++);*
	secondary; younger than one year	*Lophodermium seditiosum (++);* *L. pinastri (+);* */*both fungi isolated from green needles/;
	secondary; one year old	*Lophodermium seditiosum (++)/*pycnidia/*; (+);* *Lophodermella sulcigena (+);* *L. pinastri (+)/*isolate/;
	secondary; two years old	*Lophodermium pinastri (++);* *Cyclaneusma minor (++);*
	secondary; more than two years old, in the litter	*Lophodermium pinastri (++);* *Cyclaneusma minor (++);* *Cytospora friesii (++);*
Pinus nigra	primary; younger than one year	*Lophodermium seditiosum (+);* isolated from the needles about 3 months old;
	primary; one year old	*Lophodermium pinastri (+);*
	secondary; younger than one year	*Lophodermium seditiosum (+);* *L. pinastri (+);* /both fungi isolated from green needles/
	secondary; one year old	*Dothistroma pini (++);* *Sphaeropsis sapinea (++);* *Lophodermium seditiosum (+)/*pycnidia/; *L. pinastri /*isolate/;
	secondary; two years old	*Dothistroma pini (++);* *ophodermium pinastri (++);* *Cyclaneusma niveus (++);*
	secondary; more than two years old, in the litter	*Dothistroma pini (++);* *Cyclaneusma niveus (++);* *Sphaeropsis sapinea (++),* *Lophodermium pinastri (+);* *Cytospora friesii (++);*

The data in *Table 2* indicate that the older primary needles of Scots pine and Austrian pine are infested by *Lophodermium pinastri*, and the younger ones by *L. seditiosum.* Based on the symptoms and the obtained isolates, it can be concluded that Scots pine seedlings (primary needles) are more susceptible to the attack of *Lophodermium* species than Austrian pine. The same conclusion also refers to secondary needles.

Primary pathogens on Scots pine secondary needles are: *Lophodermium seditiosum, Lophodermella sulcigena* and *Lophodermium pinastri* (on older needles), and on Austrian pine *Dothistroma pini* and *Sphaeropsis sapinea.*

The secondary pathogens, which occur massively on the needles diseased by primary pathogens, are the species in the genera *Cyclaneusma* and *Cytospora friesii.*

4 DISCUSSION AND CONCLUSIONS

Different periods of mass infection during a year, then different types of ascospore germination, penetration of germ tubes directly through the cuticle or through the stomata, significant anatomic-morphological differences of fruiting bodies (in particular apothecia) on primary and secondary needles, as well as numerous other ecological, biological, physiological and pathological characteristics point to the existence of a number of *Lophodermium* species which differ significantly (Millar – Watson 1971; Staley 1975; Minter 1981; Lazarev 1980, 2004).

The species in the *Lophodermium* genus can cause damage in different climate conditions. The intensity of attack depends, *inter alia*, on geographic position and macroclimate, first of all on the climate humidity. It can be taken that pines are more at risk in the more humid areas and in the higher mountain regions. The intensity of attack of the disease agents is maximal in the areas with precipitation 700-800 mm, while in the areas with precipitation between 600 and 700 mm, it is significantly lower (Lazarev 1981). The precipitation below 300 mm during the vegetation period is not sufficient for the infection (Pagony, 1975). Consequently, these pathogens are a far more difficult problem in the countries of North and central Europe (Germany, Holland, Sweden, Bosnia and Herzegovina) than in the countries of South Europe. The disease can reach the epidemic proportions in the nurseries during one year, because of the high density of seedlings and the favourable moisture, due to irrigation. In the plantations, epidemics last for 2-3 years, and they become severe in the effects of favourable microclimate and during the rainy period.

Pathogen biology is characterised by four phases of development: infection phase (in which pathogenic action starts), latent phase (latency), phase of transition to saprophyte feeding, and the phase of reproduction. Infection phase is restricted to the period of ascospore release and their dissemination. According to Raspopov (1966), the ascospore dissemination dynamics depends on the number of apothecia with mature ascospores, air and needle humidity, and air temperature. At mean daily air temperatures of 15-20^0 C, ascospore dissemination increases and it decreases at the temperatures of 10-12^0 C. Ascospore dissemination increases significantly after daily precipitation of 8-10 mm. R e n d l (1967) reports the positive correlation between humidity and intensity of attack. In his study of dissemination rhythm, Rack (1975) reports that apothecia contain up to 6,000 spores which are, at the optimal conditions of humidity and temperature, released in about 120 hours. The germination of ascospores on the surface of the needles starts in about 16-36 hours after the release from the apothecia, but no ascospores germinated after 48 hours. Although the above authors did not observe the infection breakthrough, they presumed the possibility of direct penetration through the cuticle. However, Lanier (1968, 1969) believes that penetration develops mainly through the natural openings (stomata). Staley (1975) shows that the germ

tube is formed first after the ascospore release, and then it elongates into a colourless appressorium with a relatively thin wall. Germ tube after germination grows through the cuticle and epidermis. In these ascospores there is no penetration through the stomatal aperture, even when the appressoria are formed in the direct vicinity of the stomata. Therefore, the species in the genus *Lophodermium* can have a different mechanism of infection. After fungus penetration in the needle, an amorphous mass appears in individual cells of the epidermis and between the cuticle and the epidermis. After 6-8 days, the needles first become yellow variegated, and then yellow-brown spots are formed, when usually the infection process terminates. It is taken that the disease requires minimum 10 infections per one needle (Rack, 1963).

In the latency phase, there are no visible changes on the infected needles. The decisive factors for the latency period are temperature conditions, but it usually lasts for 20-30 days. This phase, however, can also be absent.

The phase of transition to the saprophyte feeding habit includes at the beginning the pathogenic activity. The germ tubes, diameter 0.5-2 μm, develop from the amorphous mass and penetrate the epidermal cells creating a vigorous mycelium. The mycelium develops best periclinally along the axis of the needles, but it also develops radially filling the resin channels. In this phase, the chlorotic spots gradually become brownish-red and the pycnidia are formed in them. Primary necroses then unite, and at the end of this phase, the whole needle is necrotised.

The phase of generative reproduction is characterised by the formation of fruiting bodies - apothecia in which asci with ascospores are formed. Depending on the pathogen species and climate conditions, apothecia form on the needles during the year. When they are mature they open along the longitudinal aperture and asci with ascospores are released, and they then cause infection. In this phase, depending on the species, cross bands are formed on some needles, which are the differential symptoms of the disease, along with pycnidia and apothecia.

Ševčenko (1960) reports that the life cycle of the fungi in this genus depends for the most part on site conditions. In lowland regions, the development cycle lasts for one year, and in mountain regions, for two or more years (Lebkova 1967).

Lophodermium species, as the primary pathogens, have a significant role in the succession and inter-relations with other pathogens on the needles. This is especially the case in Scots pine, which is much more susceptible to the attack of *Lophodermium* species than Austrian pine. By all means, the succession and connexion of other fungi is affected by the factors mentioned in this paper.

Based on our study, the following can be concluded:

- that the older primary needles of Scots pine and Austrian pine are infested by *Lophodermium pinastri*, and the younger ones by *L. seditiosum*;
- Based on the symptoms and the obtained isolates, it can be concluded that Scots pine seedlings (primary needles) are more susceptible to the attack of *Lophodermium* species than Austrian pine. The same conclusion also refers to secondary needles;
- Primary pathogens on Scots pine secondary needles are: *Lophodermium seditiosum, Lophodermella sulcigena* and *Lophodermium pinastri* (on older needles), and on Austrian pine *Dothistroma pini, Sphaeropsis sapinea* and *Lophodermium pinastri*;
- The secondary pathogens, which occur massively on the needles diseased by primary pathogens, are the species in the genera *Cyclaneusma (C. minor, C. niveus)* and *Cytospora friesii*.

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Genetic Variation among European *Lophodermium piceae* Populations - Preliminary Results

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Abstract – *Lophodermium piceae* is a common needle endophyte of Norway spruce (*Picea abies)*. The aim of the present study was to examine the degree of differentiation within and among European populations separated by various distances and geographical obstacles. For this purpose, populations (including > 10 isolates/subpopulation) were collected along a north-south transect stretching from the northern timberline in Finnish Lapland to the southern border of the distribution area of Norway spruce in northern Italy. Differentiation between *L. piceae* populations was determined from DNA sequences of three genetic markers. One of the markers was the internal transcribed spacer (ITS) of the ribosomal DNA and the other two (LP1 and LP2) were based on sequence characterized amplified regions (SCAR) designed for *L. piceae*. Preliminary results including sequences of Finnish, Swiss and Italian isolates show low differentiation among populations. According to analysis of molecular variance the among population variation was 1%, 5% and 0% in ITS, LP1 and LP2 markers, respectively.

Norway spruce / Picea abies / endophyte / genetic differentiation / gene flow

Kivonat – Genetikai variáció a *Lophodermium piceae* európai populációi között - előzetes eredmények. A *Lophodermium piceae* a lucfenyő (*Picea abies*) tűiben élő gyakori endofita gomba. E tanulmány célkitűzése megvizsgálni a differenciálódás fokát a különböző távolságok és földrajzi akadályok által elválasztott európai populációk között, és azokon belül. Ennek érdekében populációkat (> 10 izolátum/alpopuláció) gyűjtöttünk egy észak-déli metszésvonal mentén, a finnországi lapp erdőhatártól Észak-Olaszországig, a lucfenyő elterjedési területének déli határáig. A *L. piceae* populációk közötti differenciálódást genetikai markerek DNS szekvenciája alapján határoztuk meg. Egyik marker a riboszomális DNS ITS szakasza, a másik kettő pedig (LP1 és LP2) a *L. piceae*-ra jellemző SCAR szekvenciák voltak. A finnországi, svájci és olaszországi izolátumok szekvenciáit tartalmazó előzetes eredmények alacsony differenciálódást mutatnak a populációk között. A molekuláris variancia-analízis szerint a populációk közötti variáció 1%, 5% és 0% volt az ITS, LP1 és LP2 markerek tekintetében.

lucfenyő / Picea abies / endofita / genetikai differenciálódás / génáramlás

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1 INTRODUCTION

This study is part of a project studying the differentiation and gene flow between distant populations of several fungal species occurring on Norway spruce and Scots pine (*Pinus sylvestris*). Knowledge on natural long-distance dispersal (>1000 km) of forest pathogenic fungi, i.e. by wind or animal vectors (insects, birds), is presently scant. Long-distance dispersal of pathogens attributable to human activities, e.g. by the import of seedlings or wood, has been disastrous in many instances, e.g. chestnut blight or Dutch elm disease. Sporal spread by wind over long distances is undisputable in the case of some pathogenic fungi of agricultural plants (Gregory, 1973; Schmale *et al*. 2006), but the dissemination potential of most forest pathogenic fungi and the significance of geographical barriers (e.g. areas without host trees, mountains or the sea) for dispersal is poorly known. Consequently, we do not know how rapidly these pathogens may invade new locations which have turned climatically suitable to them. Neither do we know what kind of barriers prevent long-distance translocation of fungi nor how different fungi vary in their ability to spread over long distances. Comparisons of genetic variation among and within populations from distant sites may help to reduce this lack of knowledge.

Lophodermium piceae is an extremely common endophyte in Norway spruce needles all over Europe, and the population size is relatively stable. It can generally be found in the majority of older (> 3 yr) needles of single trees and it may be one of the most numerous fungi in spruce forests. Locally, L. piceae is a highly diverse fungus and it is difficult to find identical (characterized by DNA markers) isolates even within a single needle. The fungus is transmitted by aerial spread of ascospores, which are formed on dead needles still attached to twigs in the tree crown or on fallen senescent needles. It is not known how the ascospores are dispersed over long distances. Remote populations are expected to differ distinctly genetically due to isolation if long-distance dispersal of ascospores is negligible. In this research, genetic differentiation and gene flow among several populations of *L. piceae* from Finland, Italy and Switzerland are studied using DNA sequence comparisons at three loci.

2 MATERIAL AND METHODS

One part of the isolates used in this study is identical to that described in Müller et al. (in press). The majority of the isolates were obtained from three to four year old healthy looking needles detached from mature Norway spruce trees. Each sampling site consisted of a mature forest with a size in the range of 2-100 ha. Two of the sampling sites included in this study represent extreme environments for Norway spruce. The permafrost site in Switzerland (Jura Mountains, Creux du Van, Neuchâtel) is a *Tofieldio-Piceetum* association on a cold scree slope at ca. 1200 m altitude. The presence of ice in the ground or between the blocks is often visible during early summer, and a cold wind is easily perceptible coming out of the block field (Pancza 1989). The mean annual ground temperature ranges between 0.5°C and 2.0°C with a maximum of 5.5°C (Delaloye et al. 2003). The Norway spruce trees at this site exhibit dwarfed growth. Trees older than 50 years, as determined by tree ring analysis, are only ca. 20 cm high. The North Finnish site at Ylläs is close to the timberline of Norway spruce in Finland. Sampling sites in Italy, Central Finland and North Switzerland are situated 20-100 km from each other, and those in South Finland maximally 280 km from each other. Surface sterilization of the needles and fungal isolation was carried out as described by Müller et al. (2001). Each isolate was obtained from a different tree individual within a sampling site. The number of isolates and origin are listed in *Table 1*.

DNA isolations, PCR, sequencing and design of the SCAR-markers LP1 and LP2 are described by Müller et al. (in press). Analysis of molecular variance (AMOVA) was calculated with Arlequin ver. 3.0 (Excoffier et al. 2005).

Table 1. Origin and number of isolates used for sequence analysis of three DNA-markers: ITS, LP1 and LP2.

Origin of isolates	Number of sampling sites	Number of isolates		
		ITS	LP1	LP2
Finland, North (at timberline)	1	13	12	12
Finland, Central	2	11	23	23
Finland, South	4	41	46	45
Switzerland, North	2	22	-	-
Switzerland, West (permafrost site)	1	13	11	11
Italy, North	2	25	23	23
Total number of isolates		125	115	114

3 RESULTS AND DISCUSSION

All three markers showed to be highly variable. Between 34 and 55 alleles could be detected among the examined isolates (Table 2). The majority of the alleles occurred in only one isolate each. The most common ITS, LP1 and LP2 allele occurred in 35, 23 and 25% of the isolates, respectively. Variation of allele frequencies was much greater within than among populations. Greatest among-population variation was found in LP1-sequences, amounting to 5% of the total variation (Table 2). All or almost all of the variation between LP2 and ITS allele frequencies was found within populations.

Table 2. Distribution (%) of the total variance among and within populations (AMOVA) and number of alleles found from each of the investigated markers

Source of variation	d.f.	Marker		
		ITS	LP1	LP2
Among populations	4-5	1	5	0
Within populations	109-119	99	95	100
Number of alleles		34	55	44

These results suggest significant gene flow among remote *L. piceae* populations in Europe. Presence of a diverse *L. piceae* population in the extreme climatic conditions at timberline in North Finland and the permafrost site in Switzerland is noteworthy. The mode of life of *L. piceae* includes periods dwelling at low activity as "dormant" microthalli within apparently healthy needles (Suske - Acker 1987, Suske - Acker, 1989, Müller et al. 2001). *L. piceae* is protected against harsh weather conditions during this dormant phase. *L. piceae* thalli resume growth in senescing needles and sporulate in spring that follows death of the needles. Weather conditions must probably be favourable only for a comparatively short period of time for sporulation and infection. Thus, as long as favourable conditions are sometimes met and if *L. piceae* ascospores get dispersed over long distances, isolation of *L. piceae* populations is prevented. It remains to be investigated in future studies, whether subpopulations of *L. piceae* show any phenotypic adaptation to extreme climatic conditions compared to subpopulations in favourable climatic conditions.

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Foliage diseases – Hardwoods

Pathogenicity of *Marssonina betulae* on *Betula pendula* and *Betula pubescens*

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Abstract – Studies were undertaken to investigate whether *Marssonina betulae* is a causal agent of dieback of young birch in Scotland. *Marssonina betulae* was inoculated onto shoots of *B. pendula* and *B. pubescens* and symptoms monitored over several seasons. On *B. pendula*, but not *B. pubescens*, lesions developed on young shoots which often girdled to cause dieback, and secondary, sunken cankers developed on main stems. These cankers expanded during subsequent seasons, and often coalesced, girdling stems to cause death of some seedlings. A survey of 900 trees at nine planting sites in Scotland found that 50% of *B. pendula* and 17% of *B. pubescens* had *M. betulae* foliar infections, and that 82% of these infected trees also had sunken cankers on shoots and stems. This study has shown that *M. betulae* is an aggressive pathogen on *B. pendula*, causing sunken stem cankers and progressive crown dieback which are symptoms commonly observed on young, planted birch in Scotland.

birch dieback / field surveys / pathogenicity tests

Kivonat – A *Marssonina betulae* patogenitása *Betula pendula* és *Betula pubescens* fajokon. Kutatásaink során azt vizsgáltuk, hogy a *Marssonina betulae* okozza-e a fiatal nyírfák pusztulását Skóciában. *B. pendula* és *B. pubescens* hajtásokat fertőztünk *M. betulae*-val és a tüneteket néhány éven át figyelemmel kísértük. A *B. pendula* fiatal hajtásain nekrózisok fejlődtek, a hajtások befűződtek, elhaltak, a fő szárakon másodlagos, besüppedő nekrózisok keletkeztek. A nekrózisok a következő évek során növekedtek, gyakran összefolytak, a szárat körülölelve a csemeték pusztulását okozták. Kilenc skóciai ültetvényben 900 fa felmérése alapján a *B. pendula* 50%-án és a *B. pubescens* 17%-án találtunk levélfertőzést, a fertőzött fák 82%-án besüppedő nekrózisokat, rákokat is találtunk a hajtásokon és a száron. Eredményeink azt mutatják, hogy a *M. betulae* a *B. pendula* agresszív kórokozója, a száron besüppedő rákokat és fokozódó koronapusztulást okoz, amely tünetek fiatal, ültetett nyírfákon Skóciában gyakoriak.

nyírpusztulás / terepi vizsgálat / patogenitási teszt

1 INTRODUCTION

Birch (*Betula* spp.) is a major component of native woodlands throughout Scotland, and is valued increasingly as a resource for biodiversity, conservation, habitat and landscape purposes (Green 2005). There has also been recent interest in the potential of silver birch (*Betula pendula*) as a timber species in the UK (Malcolm – Worrell 2001). Both silver birch

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and downy birch (*B. pubescens*) are two of the more important broadleaved species in recent native woodland afforestation schemes in Scotland. The area of new native woodland is projected to increase further with continued applications under the Scottish Forestry Grant Scheme. As a result, there are now large numbers of young birch trees on a wide variety of site types across Scotland (Green 2005).

During the last few years there have been reports of widespread die-back of young, planted birch in Scotland, with over 20 native woodland planting schemes reported as affected to date. These planting schemes vary in size from 10 ha to over 450 ha. Affected trees appear to grow well initially, but approximately 5-10 years after planting begin to exhibit shoot die back from the lower crown upwards and from the outer crown inwards (Green 2005). Symptoms include sunken cankers and fissures on stems and branches, and discrete lesions and tip die-back on young shoots. These disease symptoms suggest that attack by fungal pathogens may be an important element in the demise of the trees. However, very little is known about the fungi associated with shoots of birch in the UK, or to what degree pathogenic shoot fungi might be responsible for the observed crown die-back.

A survey of five affected planting schemes was conducted in Scotland in 2002 as the first stage in a research programme to determine the potential roles of fungal pathogens in causing birch dieback (Green 2004). A broad range of fungi were isolated from birch shoots with and without symptoms, and the most frequently isolated fungi were inoculated onto birch seedlings in pathogenicity tests (Green 2004). Subsequent observations of disease over a single growing season indicated a high degree of pathogenicity in *Marssonina betulae* (Green 2004). *Marssonina betulae* is a common foliar pathogen on birch, causing characteristic leaf spots (Bennell and Millar 1984) although this fungus has not been considered previously to be a causal agent of shoot dieback. This paper provides an overview of work already published which confirms the pathogenicity of *M. betulae* on silver birch and its impact on birch at planted sites in Scotland (Green – MacAskill 2007, DeSilva et al. submitted).

2 METHODS

2.1 Inoculation tests

Marssonina betulae was inoculated onto Scottish provenance seedlings of silver birch and downy birch in 2003 and 2004 and symptoms monitored over two to three years. All seedlings were inoculated on the leading shoot, 2-3 cm above the base of the current year's shoot extension. In early June 2003, one-year-old seedlings were inoculated in an experiment designed to test the pathogenicity of *M. betulae* with the following main factors; i) birch species (silver birch or downy birch), ii) inoculum type (conidia or mycelium), iii) wounding or non-wounding of the inoculation site, and iv) age of leading shoot (3, 6 or 9 weeks post-flushing) (Green – MacAskill 2007). In late July 2004, two-year-old seedlings of silver birch were inoculated with conidial suspensions of five isolates of *M. betulae* and in mid-November 2004, two-year old silver birch seedlings were inoculated with mycelial plugs of four isolates of *M. betulae* (Green – MacAskill 2007).

2.2 Field survey

In August and September 2004, 100 birch trees at each of nine WGS plantings in Scotland were surveyed to evaluate the frequency and severity of crown die-back, to record the incidence of *Marssonina betulae* foliar disease and severity of sunken shoot and stem cankers, and to determine whether a relationship exists between incidence of *M. betulae* foliar disease and incidence of sunken cankers on shoots and stems (DeSilva et al. submitted). Eight of the

sites were planted between 1989 and 1995, and one site comprised late-1980's naturally regenerated downy birch of local origin.

3 RESULTS

3.1 Inoculation tests

Inoculation of silver birch seedlings with *M. betulae* resulted in the development of lesions at the inoculation site and secondary, sunken stem cankers, which continued to expand up to two years after initial infection and often girdled, causing extensive shoot die-back and the death of some seedlings (Green – MacAskill 2007). These secondary stem cankers were often centred about the base of a side shoot. Non-wounded shoots inoculated with conidial suspensions caused disease and young shoots inoculated in early June were more susceptible than shoots inoculated in late July which had ceased extension growth. All isolates of *M. betulae* tested caused disease on silver birch (Green – MacAskill 2007). Disease did not develop on downy birch after inoculation.

3.2 Field survey

At six of the nine sites surveyed in 2004 at least half of all birch trees had 40 % or greater crown die-back. In total, 61 % of silver birch (n = 291) and 41 % of downy birch (n = 609) had 40 % or greater crown die-back. Overall, 28 % of the 900 trees surveyed had *M. betulae* foliar disease, with incidences of infection varying quite widely from site to site (DeSilva et al. submitted). *Marssonina betulae* foliar disease occurred more frequently on silver birch (50% affected) than on downy birch (17% affected). The incidence of sunken shoot and stem cankers was also greater on silver birch (63 % affected) than on downy birch (23 % affected). There was a significant interaction ($P < 0.0001$) between the incidence of *M. betulae* foliar disease and incidence of sunken shoot and stem cankers, with 82 % of *M. betulae*-infected trees having these other cankers (DeSilva et al. submitted). There was also a significant relationship between the incidence of *M. betulae* foliar disease and crown dieback ($P < 0.0001$) and the severity of sunken cankers had a significant positive effect ($P < 0.0001$) on the severity of crown dieback (DeSilva et al. submitted).

4 DISCUSSION

This study has demonstrated that *M. betulae* is a more aggressive pathogen on silver birch than previously thought, causing sunken stem cankers and progressive shoot dieback when inoculated onto silver birch seedlings (Green – MacAskill 2007). Primary infections by *Marssonina* spp. tend to occur in spring shortly after the leaves emerge on the host, and are initiated by conidia from acervuli overwintering in lesions on shoots and fallen leaves (Sinclair et al. 1987). This study showed that young shoots of silver birch in early flush were most susceptible to infection by conidia of *M. betulae* with acervuli forming on lesions which developed at the inoculation site. It is not clear how *M. betulae* then spreads to cause secondary stem cankers. Conidia may be washed down the main stem during rainfall and collect at side shoot junctions, forming infection loci at these points. The fungus also causes lesions on young side shoots and may then grow down the side shoot to the main stem, causing cankers at these points (Green – MacAskill, 2007).

Marssonina betulae was also found to be a common pathogen on young birch trees at the nine planted sites surveyed in Scotland, causing foliar disease associated with sunken shoot

and stem cankers which result in crown dieback (DeSilva et al. submitted). This fungus is, therefore, a causal agent of crown dieback and is having a significant impact on the health of birch at these sites. Although *M. betulae* did not cause disease when inoculated onto downy birch seedlings, downy birch trees at field sites in Scotland were found to have the disease. It would appear that silver birch is the more susceptible species, although other factors such as provenance and site conditions may influence this.

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Sporulation and Identity of Tar Spot of Maple in Canada

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Abstract – Tar spot of maple has been increasing in incidence and severity in the Great Lakes region of eastern North America since the 1990's. The purpose of this work was to examine tar spot on native and imported maple species to determine the fungal species involved. By extracting and sequencing DNA from tar spot samples obtained from Europe and across Canada, we have found that giant tar spot on Norway maple (*Acer platanoides*) is caused by *Rhytisma acerinum*, the same fungus found in Europe, whereas native maple species in North America have the large tar spot caused by *R. americanum* (e.g. on silver maple, *A. saccharinum*) or the speckled tar spot caused by *R. punctatum* (on big-leaf maple, *A. macrophyllum*). We also found that ascospore release from tar spots on Norway maple in southern Ontario occurred over a three-week period, the start of which coincided with full leaf expansion in Norway maple.

Rhytisma* / fungi / *Acer* / *acerinum* / *americanum* / *punctatum

Kivonat – A juharlevél szurokfoltosság spóraszórása és azonossága Kanadában. A juharlevél szurokfoltosságának gyakorisága és súlyossága az 1990-es évek óta növekszik a Nagytavak vidékén, Észak-Amerika keleti részén. Munkánk célja volt megvizsgálni és meghatározni a szurokfoltosságot okozó gombafajt az őshonos és behozott juhar fajokon. Európából és Kanadából származó szurokfolt mintákból kivont DNS szekvenciák alapján megállapítottuk, hogy a korai juhar (*Acer platanoides*) nagy szurokfoltjait a *Rhytisma acerinum* okozza, ugyanaz a gomba, mint Európában, míg az észak amerikai őshonos juharok nagy szurokfoltjait a *R. americanum* (például az ezüst juharon, *A. saccharinum*), a pettyes szurokfoltokat pedig a *R. punctatum* (a nagylevelű juharon, *A. macrophyllum*) okozza. Továbbá megállapítottuk, hogy a korai juharon Dél-Ontarióban az aszkospórák szórása a szurokfoltokból három hétig tartott, kezdete a korai juhar teljes kilombosodásával esett egybe.

Rhytisma* / gombák / *Acer* / *acerinum* / *americanu* / *punctatum

1 INTRODUCTION

Tar spot of maple is caused by species of the ascomycete genus *Rhytisma,* and has a worldwide distribution wherever maples are found (Table 1). Tar spot was particularly abundant in 2006 across eastern North America with most leaves of Norway maple (*Acer platanoides*) bearing multiple black spots. There has been relatively little research done on tar spot in North America. The only scientific reports have come from Connecticut (Waterman 1941) and New York (Hudler et al. 1987 & 1998). The most recent research report is one from New York (Hudler et al. 1998), which found that the fungus *Rhytisma acerinum* is the

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cause of tar spot on Norway maple, both of which (host and pathogen) are immigrant species, while a native fungal species, *R. americanum*, occurs on the native red and silver maples (*A. rubrum and A. saccharinum*). This is probably the reason that a Norway maple may be heavily infected with tar spot while an adjacent red maple (*A. rubrum*) or silver maple (*A. saccharinum*) may have no spots. The purpose of this work was to examine the epidemiology of this disease, by gathering overwintered maple leaves from multiple locations in southern Ontario weekly from March through August 2006, and inspecting the asci for the presence of filiform ascospores, which initiate infections. Another objective of this research was to confirm the genetic identity of the organism causing giant tar spot on Norway maple in Ontario, as well as its relationship to tar spot on other European maples and North American maples.

Table 1. Maple species, their native range, and susceptibility to tar spot caused by Rhytisma species.

Common name	*Acer* species	Native range	*Rhytisma* species		
			R. acerinum	*R. americanum*	*R. punctatum*
Norway maple	*A. platanoides*	Europe	+		
Sycamore maple	*A. pseudoplatanus*	Europe	+		
Field maple	*A. campestre*	Europe	+		
Red maple	*A. rubrum*	Eastern N. America	?	+	
Sugar maple	*A. saccharum*	Great Lakes	?	+	
Silver maple	*A. saccharinum*	Eastern N. America	?	+	
Mountain maple	*A. spicatum*	Eastern N. America	?	+	
Big-leaf maple	*A. macrophyllum*	Western N. America			+
Vine maple	*A. circinatum*	Western N. America			+

2 METHODS

2.1 Sporulation

After snowmelt, overwintered leaves of Norway maple bearing tar spots caused by *Rhytisma acerinum* were collected from a copse of maples at the Guelph Turfgrass Institute, Guelph, Ontario every week from March through August in 2006. Samples were also taken from other locations in southern Ontario, such as the Niagara Dufferin Park (Niagara Falls), the Royal Botanical Gardens (Burlington) and the Queen's Royal Park (Niagara-on-the-Lake) from May to August, 2006. Diseased Norway maple leaves were soaked in distilled water for 24 h to allow the apothecia to open, and many spots were examined from each location, with several cross-sections per spot. The percent asci that were empty was estimated. Maple phenology and weather conditions were also recorded at each sampling.

2.2 Genetic identity

Many attempts were made to isolate the fungus from dried and fresh maple tissues. as well as from ascospores and conidia. Several putative isolates were subjected to DNA sequencing (methods below), but none proved to be *Rhytisma* species. Because we were unable to obtain pure cultures, we attempted to extract DNA directly from tar spot samples. We collected local samples of infected maple (*A. platanoides* and *A. saccharinum*), and obtained infected specimens of *A. pseudoplatanus* (courtesy of Dr. Roland Weber, Biotechnology, University of

Kaiserslautern, Kaiserslautern, Germany), and *A. macrophyllum* (courtesy of Dr. Brenda Callan, Canadian Forestry Service, Victoria, B.C., Canada). We also obtained a specimen of Amur maple (*A. ginnala*) as well as a specimen of tulip tree (*Liriodendron tulipfera*) with tar spot, but we were unable to extract *Rhytisma* from these old samples.

We used the Qiagen DNAeasy kit (Qiagen Inc., Mississauga, Ontario, Canada), to extract DNA. This DNA was then amplified with conserved ITS primers which target the internal transcribed spacer region of ribosomal DNA spanning the 3' end of the 18S gene to the 5' end of the 28S gene. The primer pair, ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were from White et al. (1991). The 12 5 ul reaction mixture for PCR amplification contained the following : 10 ng DNA, 1 DNA polymerase buffer, 0 5 ìm of each primer, and 1 U Tsg DNA polymerase (Biobasic, Scarborough, Ontario, Canada). Amplifications were performed in a GeneAmp PCR System 2400 (Perkin Elmer, Norwalk, CT, USA), with an initial denaturation step of 94 for 2 min, followed by 35 cycles of 94 for 30 s, 55 for 1 min, and 72 for 2 min, and a final extension at 72 for 7 min. These PCR reactions were diluted 10-fold in water, and sent for sequencing at the Laboratory Services Division, University of Guelph with both forward and reverse primers. At least two tar spot sequences from each maple species were used for analyses.

In addition to sequences downloaded from GenBank for *Lophodermium pinastri* (GenBank Accession AF462434), and *Rhytisma salicinum* (GenBank Accession AY465516), the sequences obtained from ITS sequencing mentioned above were subjected to phylogenetic analysis by multiple alignment of the sequences in CLUSTALX (Thompson et al. 1997), and generation of a dendrogram depicting relationships between the sequences.

3 RESULTS & DISCUSSION

In southern Ontario, the first symptoms of tar spot on Norway maple appeared in late June as small, round, light green, chlorotic spots, 2 mm across. Spots enlarged to 15 mm by mid-August, and developed small black tar-like raised structures on the adaxial surface with a yellow margin. Conidia, which are considered non-infective and possibly spermatizing, appeared as a shiny layer on the black stroma at this time. By early September, the individual spots merged into a circular black spot up to 2 cm across.

Overwintered Norway maple leaves collected in March 2006, had stroma, paraphyses and asci (56-80 µm × 8.5-10.6 µm), but no ascospores were visible. By the middle of April, the asci were still undifferentiated, but were found to contain globular vacuoles or bodies. The asci became swollen as spores developed, and filiform ascospores were first observed in early May, averaging 55 × 2.0 µm. By late May, after soaking in water, slits in the hysterothecia (modified apothecia) on the leaf surface opened, and contained a grayish milky substance. At this time, Norway maples were abundantly producing and shedding pollen, and small samaras were formed, with leaf sizes averaging 10 cm × 15 cm. By the end of May, a few partially filled or empty asci were observed (5.3%), with ascospore release through the tips, and paraphyses becoming curled beside asci after spore release. In early June, Norway maple leaves reached their full size (20 cm × 24 cm), and 10% of the asci had fully discharged their spores. By the end of June, nearly all the asci were empty. The practical implication is that fungicide protection against tar spot, if necessary, needs only to be applied during a very short period, which begins near the end of full leaf expansion in Norway maple.

Schweizer (1932) and Hudler et al. (1987) had earlier reported that isolation of *Rhytisma* from infected plant tissues was possible on common artificial media without special supplements. We were never able to isolate the fungus in pure culture, and in subsequent communications with Dr. George Hudler of Cornell University (Ithaca, NY, USA), he

indicated that he suspected what they isolated may have been *Aureobasidium*. He said that such cultures were commonly and easily obtained by scooping out the milky contents of overwintered leaves where the apothecia had been induced to open by wetting. We confirmed his suspicions by sequencing of isolates which turned out to be have the best match with *Aureobasidium pullulans* with 99% identity for both the ITS and partial 18S sequences.

Previous work by Hudler et al. (1998) to delineate *Rhytisma* species was based on morphology and RFLP of ribosomal DNA, but there was no sequence data. The only ITS sequence available for a *Rhytisma* species at the start of this project was one for *R. salicinum* (GenBank Accession AY465516), although there was an 18S and a 28S sequence for *R. acerinum* in GenBank (AF356695 and AF356696, respectively). In this study, we were able to obtain ITS sequence for fresh samples of maple with tar spot. Based on the phylogenetic analysis of ITS sequences from tar spot of Norway maple and silver maple from Ontario, sycamore maple from Europe, and big-leaf maple from British Columbia, we found that Norway maple tar spot specimens had sequences almost identical to those from sycamore maple, and conclude that tar spot of Norway maple in Ontario is caused by *R. acerinum*. The sequences from silver maple were quite different and considered to be *R. americanum*. The big-leaf maple tar spot yield a even more divergent sequence, and *R. punctatum* was not found on any Norway or silver maples even for spots which had a similar punctate phenotype. Both *R. americanum* and *R. acerinum* can pass through an initially punctate (speckled tar spot) stage when spots are just forming, where they might be easily confused with *R. punctatum.*

In summary, we have found that ascospore dispersal from overwintered Norway maple leaves occurred during a 3-week period in June. The practical implication of this result is that fungicide protection against tar spot, if necessary, needs only to be applied during a very short period, and this period coincides with the end of full leaf expansion in Norway maple in this region. We have also confirmed that the giant tar spot on Norway maple in Ontario is caused by *R. acerinum* which is the same as the fungus found in Europe, whereas native maple species in North American have the large tar spot caused by *R. americanum*. Although we were unable to grow the isolates in pure culture, we have obtained DNA that is similar to other species in the Rhystimataceae, and we plan to continue DNA sequencing to further resolve the relationships between *Rhytisma* species and closely related genera such as *Lophodermium.*

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Mycological Complex on the Leaves and Bark of *Salix* Species in Central Danube Basin

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Abstract – This work shows the results of the research on the microflora of willows (*Salix* spp.) in the area of Srednje Podunavlje. Dendro-material with disease and damage symptoms was collected in the field. The material was deposited in herbarium, the fungi were identified and isolated in laboratory on nutritive medium. During the research 36 fungal species were recorded on leaves and bark of willows. The most significant species causing economic damages and death of the plants were: *Colletotrichum gloeosporioides* Penz., *Glomerella miyabeana* (Fuk.) v. Arx, *Marssonina salicicola* (Bres.) Magn., *Sphaeropsis malorum* Peck., *Valsa salicina* Fr, *Phyllosticta salicicola* Thuem., *Fusarium oxysporum* Schlecht. and *Melampsora epitea* Thüm.

willow / *Salix* spp./ bark / fungi / Srednje Podunavlje

Kivonat – *Salix* fajok levél- és kéreggombái a Közép-Duna medencében. A munka a füzek (*Salix* spp.) mikroflóra kutatásának eredményeit mutatja be Srednje Podunavlje térségében. Betegség- és károsodási tüneteket mutató fás anyagot gyűjtöttünk a terepen. Az anyagot herbáriumba helyeztük, a gombákat meghatároztuk és táptalajra kitenyésztettük. A kutatás során a füzek leveléről és kérgéről 36 gombafajt azonosítottunk. A legjelentősebb, gazdasági károkat és a növények pusztulását okozó fajok a következők: *Colletotrichum gloeosporioides* Penz., *Glomerella miyabeana* (Fuk.) v. Arx, *Marssonina salicicola* (Bres.) Magn., *Sphaeropsis malorum* Peck., *Valsa salicina* Fr, *Phyllosticta salicicola* Thuem., *Fusarium oxysporum* Schlecht.és *Melampsora epitea* Thüm.

fűz / *Salix* fajok / kéreg / gombák / Srednje Podunavlje

1 INTRODUCTION

The restricted opportunities of wood production in natural forests and a rapid development of industrial processing of wood have brought about the decrease of timber supply and to the creation of the ever increasing deficit of wood as raw material. This problem has been somewhat mitigated by wood production in the plantations of fast growing tree species (primarily poplars and willows).

The fact that forests in Vojvodina are mainly concentrated along the rivers and that there are significant land areas that are not suitable for agricultural production increase the

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significance of establishment of willow plantations, which as a pioneer species occupies the lowest part of the microrelief.

Willow diseases, in contrast to poplar diseases, have not drawn any special attention. By the establishment of new willow clonal plantations, this problem has become outstanding and has made it necessary to undertake such investigations.

The aim of this paper was to identify the most significant parasitic and saprophytic fungi in willow natural stands and clonal plantations.

2 MATERIAL AND METHOD

The field research and collection of material was performed at several localities in willow plantations, natural populations and nurseries in the area of Central Danube Basin situated between $45^0 08' 18''$ and $45^0 42' 50''$ north latitude and $17^0 10' 10''$ and $17^0 58' 50''$ east longitude, at the altitude from 73 to 79 metres. The climate of this area is temperate continental with the characteristics of the Pannonian-steppe temperate-continental climate. The research was performed in the flooded area of the Danube Basin marshes, on the flat land intersected with micro-depressions and narrow land strips with the altitudinal difference between 1 and 6 metres. This is the case of the microrelief, which together with the level of underground water (which directly depends on the Danube water level), creates the special conditions for the development of some plant communities, and they are especially very favourable conditions for the development of willows.

The material collected in the field consisted of the diseased leaves, dead branches and branches with necroses and tumours, etc. All the material was kept in the herbaria and brought to the laboratory for the identification and isolation of fungi on the nutritive media. The determination of fungi was preceded by the preparation of the temporary microscopic preparations or by the cultivation of pure fungal cultures. The species determination was based on the appearance of the fruiting bodies, sporiferous organs, organs for reproduction, and the appearance of fungal mycelia.

3 RESULTS

During these researches, the following most frequent parasitic and saprophytic fungi which occur on willows were identified (*Table 1*).

Altogether 36 species of fungi were identified on willow foliage, on catkins and bark, of which on the foliage 7, on catkins 1, on bark 26, and on bark and foliage 2. One species was determined (*Darluca filum* (Biv.) Cast.), which behaves as a hiper-parasite on uredosori of the fungus *Melampsora epitea* Thüm. The above fungi were identified both on the willows in natural stands and on the willows in clonal plantations. Of all the identified species, the most significant are the following: *Phyllosticta salicicola*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Sphaeropsis malorum*, *Valsa salicina*, *Marssonina salicicola* and *Melampsora epitea.*

During the above research on willows, 22 parasitic and saprophytic species of fungi were identified for the first time, i.e. they have not been identified so far on willows on the territories of Serbia and Montenegro. Of the 22 new identified species of fungi, 19 species have never been identified in the study area, and three species were identified, but on other hosts (e.g. on poplars).

Table 1. The most frequent parasitic and saprophytic fungi on willow leaves, catkins and bark

Name of fungus	Sistematical place	The infect. part	The first time found in Serbia and Montenegro	The first time found on willow
Ascochyta salicicola Pass.	Subphylum *Deuteromycotina* Ord. *Sphaeropsidales* Fam. *Sphaerioidaceae*	Leaf	+	+
Botryosphaeria dothidea (Moug. ex Fr.) Ces. & de Not.	Phylum *Ascomycota* Ord. *Dothideales* Fam. *Botryosphaeriaceae*	Bark	-	+
Capnodium salicinum Mont	Phylum *Ascomycota* Ord. *Dothideales* Fam. *Capnodiaceae*	Leaf	-	-
Cladosporium herbarum (Pers.) Link ex SF. Gray	Subphylum *Deuteromycotina* Ord. *Hyphomycetales* Fam. *Dematiacae*	Bark	-	-
Colletotrichum gloeosporioides Penz.	Subphylum *Deuteromycotina* Ord. *Melanconiales* Fam. *Melanconiaceae*	Bark / Leaf	+	+
Cryptodiaporthe salicina (Pers.) Wehmeyer	Phylum *Ascomycota* Ord. *Diaporthales* Fam. *Valsaceae*	Bark	+	+
Crypthodiaporthe salicella (Fries) Petrak	Phylum *Ascomycota* Ord. *Diaporthales* Fam. *Valsaceae*	Bark	+	+
Cytospora ambiens Sacc.	Subphylum *Deuteromycotina* Ord. *Sphaeropsidales* Fam. *Sphaerioidaceae*	Bark	-	-
Cytospora chrysosperma (Pers.) Fr.			-	-
Cytospora fertilis Sacc.			+	+
Cytospora nivea Sacc.			-	-
Cytospora salicis Rab.			+	+
Cytospora translucens Sacc.			+	+
Darluca filum (Biv.) Cast.	Subphylum *Deuteromycotina* Ord. *Sphaeropsidales* Fam. *Sphaerioidaceae*	Hyper-parasite	-	-
Dasyscyphus pudibundus (Quelet) Sacc.	Phylum *Ascomycota* Ord. *Leotiales* Fam. Hyaloscyphaceae	Bark	+	+
Diatrype bullata (Hoffm.) Fr.	Phylum Ascomycota Ord. *Diatrypales* Fam. *Diatrypaceae*	Bark	+	+
Diplodina salicis Westd.	Subphylum *Deuteromycotina* Ord. *Sphaeropsidales* Fam. *Sphaerioidaceae*	Bark	+	+
Epicoccum purpurescens Ehrenb	Subphylum *Deuteromycotina* Ord. *Hyphomycetales* Fam. *Tubercolariaceae*	Bark	-	-
Fusarium oxysporum Schlecht.	Subphylum *Deuteromycotina* Ord. *Hyphomycetales* Fam. *Tubercolariaceae*	Bark	-	+

Table 1 cont. The most frequent parasitic and saprophytic fungi on willow leaves, catkins and bark

Name of fungus	Sistematical place	The infect. part	The first time found in Serbia end Montenegro	The first time found on willow
Glomerella miyabeana (Fuk.) v. Arx	Phylum *Ascomycota* Ord. *Phyllachoraceales* Fam. *Phyllachoraceae*	Bark	-	-
Leucostoma nivea (Persoon ex Fries) van Höhner	Phylum *Ascomycota* Ord. *Diaporthales* Fam. *Valsaceae*	Bark	-	-
Marssonina salicicola (Bres.) Magn.	Subphylum *Deuteromycotina* Ord. *Melanconiales* Fam. *Melanconiaceae*	Bark / Leaf	+	+
Melampsora epitea Thüm	Phylum *Basidiomycota* Ord. *Uredinales* Fam. *Melampsoraceae*	Leaf	-	-
Mollisia sp.	Phylum *Ascomycota* Ord. *Leotiales* Fam. *Dermataceae*	Bark	+	+
Monostichella salicis (Westd.) v.Arx.	Subphylum *Deuteromycotina* Ord. *Melanconiales* Fam. *Melanconiaceae*	Leaf	+	+
Mycosphaerella tassiana v. Arx.	Phylum *Ascomycota* Ord. *Mycosphaerellales* Fam. *Mycosphaerellaceae*	Bark	-	-
Nectria flavo-viridis (Fuckel) Wollenweder	Phylum *Ascomycota* Ord. *Hypocreales* Fam. *Hypocreaceae*	Bark	+	+
Phoma glyptica Cooke end Massel	Subphylum *Deuteromycotina* Ord. *Sphaeropsidales* Fam. *Sphaerioidaceae*	Bark	+	+
Phoma salicina Westol			+	+
Phomopsis salicina Died.			+	+
Phyllosticta salicicola Thuem	Subphylum *Deuteromycotina* Ord. *Sphaeropsidales* Fam. *Sphaerioidaceae*	Leaf	+	+
Sphaeropsis malorum Peck.	Subphylum *Deuteromycotina* Ord. *Sphaeropsidales* Fam. *Sphaerioidaceae*	Bark	+	+
Trichothecium rosae (Link)	Subphylum *Deuteromycotina* Ord. *Hyphomycetales* Fam. *Mucedinaceae*	Catkins	-	-
Uncinula salicis Wint.	Phylum Ascomycota Ord. *Erysiphales* Fam. *Erysiphaceae*	Leaf	-	-
Valsa salicina Fr.	Phylum Ascomycota Ord. *Diaporthales* Fam. *Valsaceae*	Bark	-	-
Venturia saliciperda Nüesch	Phylum *Ascomycota* Ord. *Dothideales* Fam. *Venturiaceae*	Bark	-	+

4 DISCUSSION

By the establishment of new clonal willow plantations in Serbia, the problem of willow diseases, which has so far not been paid much significance to (and therefore our literature on this subject is rather scarce), has become increasingly prominent. For this reason, it was necessary to research all harmful abiotic and biotic factors which occur both in willow natural stands and in clonal plantations, leading to tree decline and tree dying. Among the harmful biotic factors, special place is occupied by parasitic fungi.

In his capital work "Sylloge Fungorum", Sacardo (1898), was the first to describe more than 500 species of fungi on the material collected all over the world. Somewhat later, Grove (1935, 1937) described 42 species of fungi on willows, belonging to the genera *Sphaeropsidales Melanconiales*. Wilson and Henderson (1966) described 7 species of fungi of the genus *Melampsora* on willow leaves. Lanier et al. (1976) reported only the fungi which caused serious diseases on willows. Of altogether 33 species of fungi, 23 infest the leaves, and 10 species infest the bark. They point out especially the significance of the fungi *Marssonina salicicola* (teleomorph *Drepanopeziza sphaeroides)* and *Colletotrichum gloeosporioides,* which in addition to leaf spot on the leaves, also cause necrosis and canker on the shoots and branches. Karadžić (1979) emphasised the significance of the fungus *Diplodina salicina* which caused severe dying of branches on weeping willows (*Salix alba* var. *Vitalina* f. *Pendula*). During this research in the area of the Central Danube Basin, this fungus was not observed, but the fungus *Diplodina salicis* was identified on the diseased branches with similar symptoms. Ellis and Ellis (1985) described 69 species of parasitic fungi (on the leaves of 21 species, on catkins 1, and on bark and wood 47 species) on willows in Great Britain. In the Pacific north-west region of USA, Shaw (1980) reported 223 species fungi on willows. Butin (1989) in Germany reported that serious diseases on willows were caused by 11 species of fungi and 2 bacteria, with special reference to the significance of the fungi *Drepanopeziza sphaeroides* (anamorph *Marssonina salicicola*). In their study of leaf spot on forest tree species, Karadžić and Milijašević (2005) described three species on willows, of which the greatest significance was assigned to the species *Uncinula salicis*. If we compare the results of our research with those in the references, it can be concluded that it is generally agreed that the most important parasitic fungi are *Marssonina salicicola*, *Colletotrichum gloeosporioides* and "the rusts".

5 CONCLUSIONS

Based on the above research, we can conclude as follows:

- 36 species of fungi were identified on willow foliage, catkins and bark;
- of the identified species of fungi, 7 species were found on the foliage, 26 on bark, and 2 species synchronically on bark and foliage, 1 species of fungi was found on catkins;
- in contrast to the trees in natural stands, there were no fungal agents of wood decay on the trees in clonal plantations;
- 22 parasitic and saprophytic species of fungi were identified for the first time on willow leaves, catkins and bark, i.e. they have not been identified so far on willows on the territories of Serbia and Montenegro. Of 22 new identified species of fungi, 19 species have never been identified in the study area, and three species were identified, but on other hosts (e.g. on poplars).
- the fungus *Darluca filum* which behaves as a hyper-parasite on uredosori of the fungus *Melampsora epitea*, was also identified for the first time;

- of all the identified species, the most significant are the following *Phyllosticta salicicola*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Sphaeropsis malorum*, *Valsa salicina*, *Marssonina salicicola* and *Melampsora epitea.*

Based on the comparison of the mycoflora occurring on willow trees in natural stands and that occurring in plantations on clonal material, it was concluded that, on the clonal material, the dominant parasitic fungi are those which infest the leaves and bark, while on the old trees in natural stands, the dominant fungi are the agents of wood decay. To protect the juvenile willows in plantations (primarily against the parasites on the foliage) it is necessary to apply the chemical measures of protection, i.e. to apply the fungicides during the critical period for parasite infection.

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Efficacy of Some Fungicides in Parasite Suppression on Poplar Leaves (*Marssonina brunnea* (Ell. et Ev.) P. Magn. and *Melampsora* spp.

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Abstract – The efficacy of fungicides in suppression of diseases caused by *Marssonina brunnea* (Ell. et Ev.) P. Magn. and *Melampsora* spp. on poplar leaves is presented in this paper. The suppression was performed using three newer fungicides, which have not been studied in suppression of mentioned diseases in Serbia as yet (products Quadris, Equation Pro WG and Perfit). Also, the Copper lime 50 was used as test fungicide, whose efficacy was established previously by numerous authors. During biennial research in stoolbeds of clones *Pannonia* (*Populus x euramericana)* and S 6-36 (*Populus deltoides)*, satisfactory results were obtained for suppression of both fungus *Marssonina brunnea* and *Melampsora spp*. using Quadris and Copper lime 50, while fungicides Equation Pro WG and Perfit showed significantly lower activity. This conclusion was based on the average number of acervules, i.e. uredosoruses per cm² leaf surface, formed on treated plants. During both years, no significant differences were found between treated and untreated 1-year-old plants in diameter and height increment.

***Marssonina brunnea* / *Melampsora* spp. / poplar clones / chemical suppression / fungicides**

Kivonat – Fungicidek hatásossága nyárak levélkórokozóinak visszaszorításában (*Marssonina brunnea* (Ell. et Ev.) P. Magn. és *Melampsora* spp.). A dolgozat fungicidek hatásossági vizsgálatát tartalmazza a nyárak levelén kórokozó *Marssonina brunnea* és *Melampsora* fajok visszaszorításában. Három olyan új gombaölő szert használtunk, amelyeknek hatásosságát az említett betegségekre még nem vizsgálták Szerbiában (Quadris, Equation Pro WG és Perfit). Kontrollként a Copper lime 50 szert alkalmaztuk, amelynek hatásosságát előzőleg több szerző is megállapította. A kétéves kísérlet során Pannónia (*Populus x euramericana)* és S 6-36 (*Populus deltoides)* klónok anyatelepein a Quadris és a Copper lime 50 szerekkel mind a *Marssonina brunnea* mind a *Melampsora* fajok ellen kielégítő eredményeket értünk el, míg az Equation Pro WG és Perfit szerek szignifikánsan alacsonyabb aktivitást mutattak. A kiértékelést a kezelt növények cm²-nyi levélfelületén képződött acervuluszok, illetve uredo telepek átlagos száma alapján végeztük. A két év során nem találtunk szignifikáns különbséget a kezelt és kezeletlen egyéves növények átmérő- és magassági növedékében.

***Marssonina brunnea* / *Melampsora* spp. / nyár klónok / kémiai védekezés / fungicidek**

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1 INTRODUCTION

During the past decades, along with permanent attempts to define or create poplar genotypes less exposed to the leaf diseases attack (*Marssonina brunnea* and *Melampsora* spp.) and characterized by other desirable traits, possibilities of direct suppression of these fungi by chemical compounds were permanently studied. Considering the fact that poplar clones characterized by high susceptibility to these parasites are grown in our country, it is necessary to protect them using chemical products. According to numerous authors, a few fungicide treatments in nurseries and newly established plantations of poplars could provide satisfactory plant protection (Cellerino 1966, 1969, Castellani – Cellerino 1967, Gojković 1970, 1971, Avramović et al. 1991, 1997; Gojković-Avramović 1996, Keča 2003). Furthermore, chemical protection should be applied when favourable conditions for appearance and spreading of these fungi arise, when cultivating plants without optimal conditions, etc. In these cases, chemical protection is used in addition to basic (preventive) activities, related to selection of cultivars less susceptible to these diseases (Gojković – Avramović 1996). Applied in plantations, these chemical products could also suppress other diseases that occur on poplar plants. The absence of fungicide treatment during one year will not result in significant reduction of plant increment and plants decline, it will be accompanied with physiological weakening of plants, resulting in possible increase of other pathogens attack, such as *Dothichiza populea*. According to investigations of authors who studied the effects of these diseases upon plant development during two or more years (Castellani – Cellerino 1967; Avramović 1997, Keča 2003), only multiannual attacks of parasites on leaves lead to the reduction of plant height and diameter increment, resulting in decrease of total volume increment. Drying of individual trees in plantations occurs during intensive multiannual attacks.

The aim of the present study was to explore efficacy of some newer and one test fungicides in suppression of diseases on poplar leaves, caused by *Marssonina brunnea* (Ell. et Ev.) P. Magn. and *Melampsora spp.* There was an idea to protect leaves from these pathogens by several fungicide treatments, starting from the time of sprouting to the end of vegetative period. Also, differences in height and diameter increment between treated and untreated plants will be determined, due to plant height and diameter measurements.

2 MATERIALS AND METHODS

The possibility of parasites suppression on poplar leaves (*Marssonina brunnea* and *Melampsora* spp.) was tested in stoolbeds of poplars, established during year 2004 and 2005 at Experimental Estate of the Institute of Lowland Forestry and Environment (*Figure 1*). Two tested clones were present at experimental fields: clone *Pannonia* **(***P. x euramericana)* (*Figure 2*) and S 6-36 (*P. deltoides).*

The field trials were created in triplicate for each treatment and control (untreated plants), using complete random block design. Rows formed of twelve plants were used as replications. The spacing of planted cuttings in rows was 30 cm, while spacing between rows amounted 80 cm. Protective rows, formed of the I-214 clone, were established in order to prevent unwanted drift and deposition of fungicide on plants in individual treatments, as well as in control.



Figure 1. Experiment in 2005



Figure 2. Stoolbed of the Pannonia clone

The protection was performed using three newer fungicides, which have not been studied in restraint of mentioned diseases on poplars in Serbia as yet (products Quadris, Equation Pro WG and Perfit). Also, the Copper lime 50 was used as test fungicide, whose efficacy was established previously (*Table 1*). Application of active substances was done by backpack sprayer CP 3 (Cooper, Pegler and Co Ltd).

Table 1. Fungicides, their active substances and applied concentrations

Product	Active substance	Concentration of product
Copper lime 50	Copper oxichloride	0,5 %
Quadris	azoxistrobine	0,02 %
Equation Pro WG	famoxadone+cymoxanil	400 g/ha
Perfit	Hydrogen peroxide	0,4 %

Six fungicide treatments were performed in 2004: on June 1st and 22nd, July 8th and 28th, August 23rd and September 8th. Nine treatments were performed in 2005: on May 30th, June 14th and 24th, July 7th, 22nd and 30th, August 8th and 18th and September 7th. Number of treatments in studied years was determined according to reports and work of service for diagnostic and prognosis.

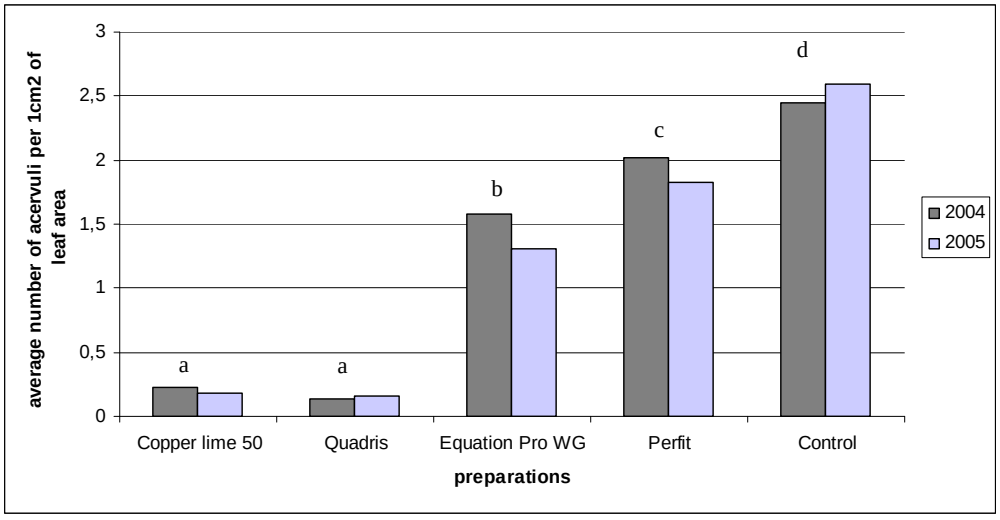
The final evaluation of applied fungicides efficacy in prevention of *Marssonina brunnea* and *Melampsora* spp. on plant leaves was done in the last decade of September, in both years. Five leaves from down, middle and above part of the crown were sampled from each seedling (15 in total). Acervules and uredosoruses were counted on each leaf, on five correctly arranged areas of 1 cm^2. During the data processing, sums were calculated for all five examined areas, and average number of fruiting bodies was expressed per cm^2 leaf area (Keča 2003). Also, the average number of fruiting bodies per cm^2 leaf area was calculated for each plant. Elements of plant growth (diameter and height) were measured during the overwinter period. Diameter of seedlings was measured at the plant base (accuracy 1 mm), while plant height with accuracy of 5 cm.

Results concerning efficacy of studied products were processed using the analysis of variance, and their relations are shown using Duncan´s test.

3 RESULTS

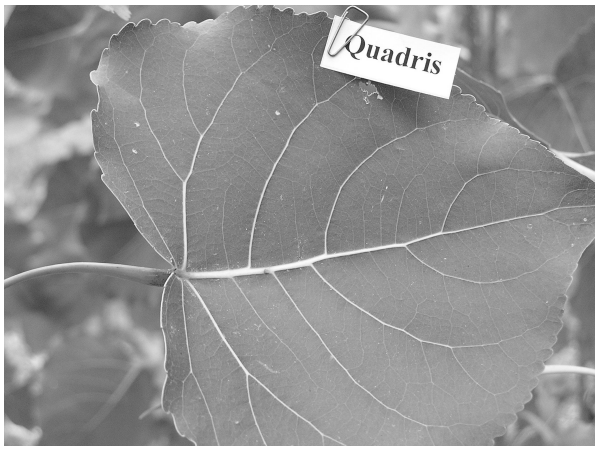
3.1 Fungicides efficacy in suppression of fungus *Marssonina brunnea*

On the leaves of *Pannonia* clone, all products showed significant activity in *M. brunnea* suppression, during both experimental years (*Figure 3*). Manifold higher activity to this pathogen showed Copper lime 50 and Quadris. Efficacy of these products was not significantly different, as well as the number of fruiting bodies between them, in both years. Lower level of efficacy was estimated for products Equation Pro WG and Perfit, mirrored in Duncan's test (*Figure 3 and 4*).



2004: F (for treatments)=140,6***, replications 0,10 ns;
2005: F (for treatments)=223,7***, replications 0,14 ns

Figure 3. Effect of fungicides upon intensity of M. brunnea emergence on the Pannonia clone leaves



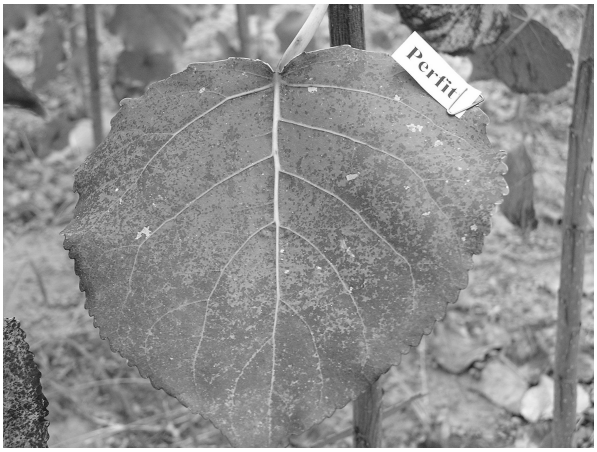
a) Quadris



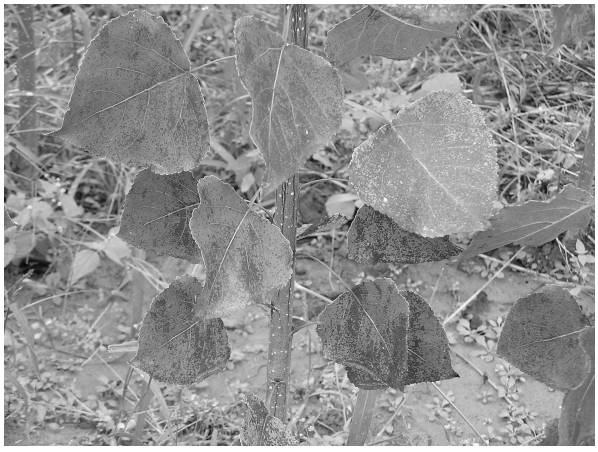
b) Copper lime 50



c) Equation Pro WG



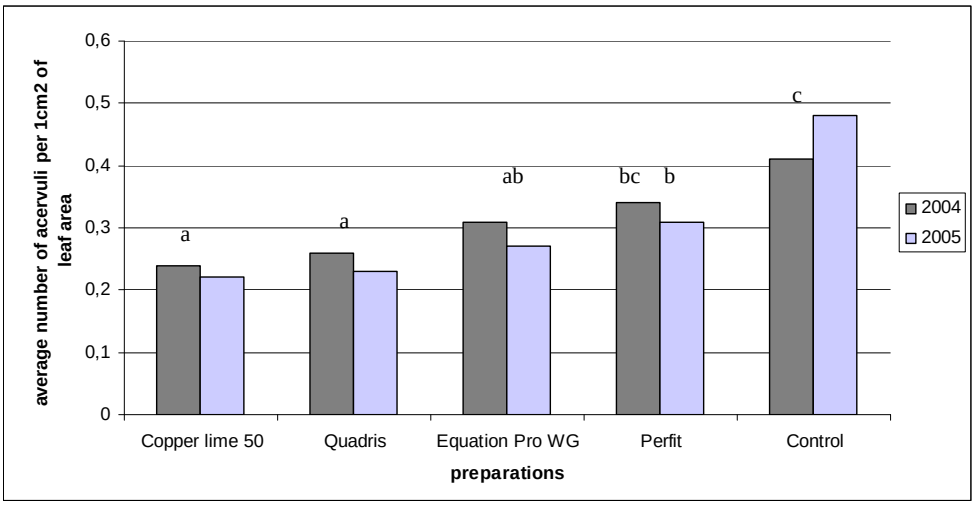
d) Perfit



e) Control

Figure 4a-e. Presence of M. brunnea acervules on the Pannonia clone leaves, in treated and control plants

Similar efficacy of fungicides in *M. brunnea* suppression was recorded on the leaves of S 6-36 clone. Therefore, the best protection of leaves was obtained with Copper lime 50 and Quadris. Lower efficacy was recorded for Equation Pro WG, while the lowest performance had Perfit, whose efficacy in 2004 was not significant, when compared with control plants (*Figure 5*).



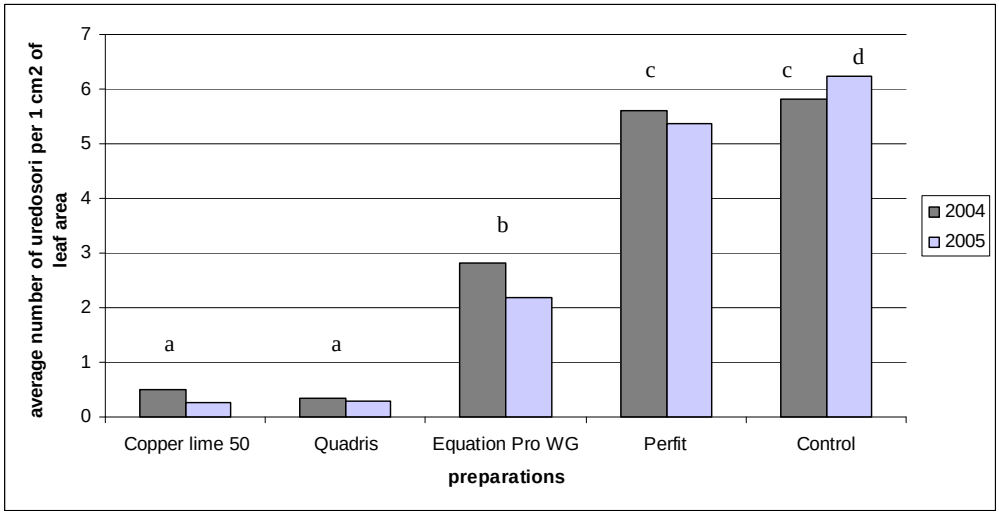
2004: F (for treatments) = 8,2***, replications 0,95 ns;
2005: F (for treatments) = 22,3***, replications 0,52 ns

Figure 5. Effect of fungicide treatments upon intensity of M. brunnea emergence on the S 6-36 clone leaves

3.2 Fungicides efficacy in suppression of fungus from the genus *Melampsora spp.*

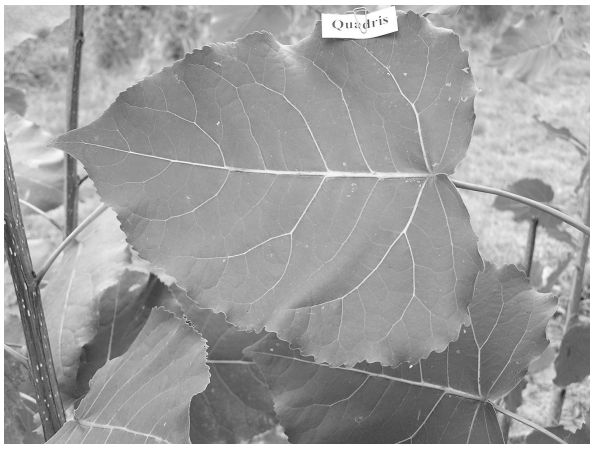
During two years, fungi from the genus *Melampsora* were not abundant on the *Pannonia* clone leaves. This is also confirmed by low average number of uredosoruses per 1cm² leaf area. During the final evaluation, uredosoruses were not found on seedlings treated with Copper lime 50 and Quadris in both years. At other treatments and the control uredosoruses occurred sporadically, so data were not shown in this report.

Products Copper lime 50 and Quadris exhibited very high efficacy in suppression fungi from the genus *Melampsora*, which is confirmed by low number of uredosoruses on the S 6-36 clone leaves in both years (*Figure 6 and 7*). Product Equation Pro WG had significantly lower efficacy, while the lowest activity showed Perfit. Inefficiency of Perfit was more pronounced in 2004, when significant differences were not found between treated and control plants, due to intensity of attack, i.e. number of fruiting bodies.



2004: F (for treatments) = 393,8***, replications 0,35 ns;
2005: F (for treatments) = 921,7***, replications 0,29 ns

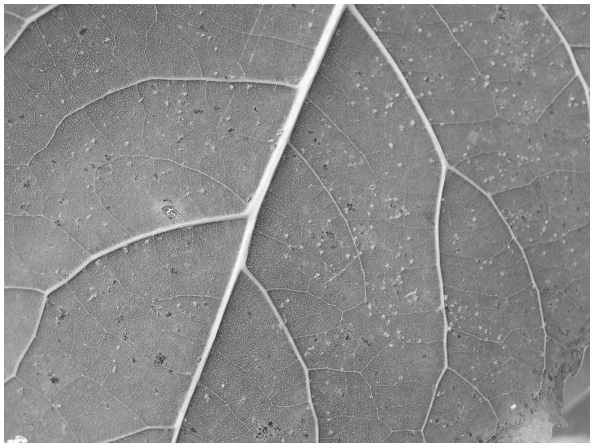
Figure 6. Effect of fungicides upon intensity of Melampsora spp. emergence on the S 6-36 clone leaves



a) Quadris



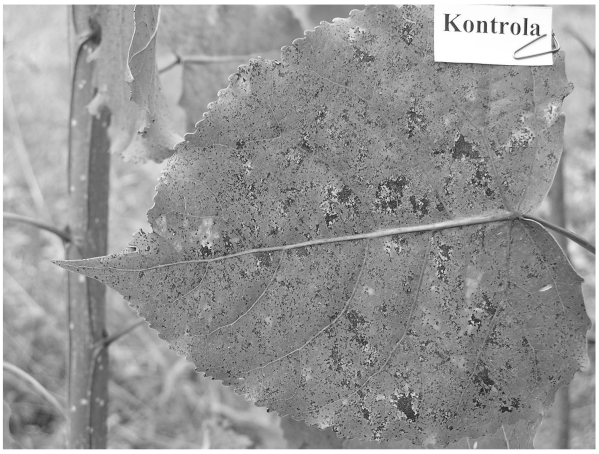
b) Copper lime 50



c) Equation Pro WG



d) Perfit



e) Control

Figure 7. Presence of Melampsora spp. uredosoruses on the S 6-36 clone leaves in treated and control plants

Results presented in figures indicated the same efficacy of Copper lime 50 and Quadris in suppression of both leaf pathogens. Also, obtained differences between these two products were not significant. Also, it should be emphasized that in all cases considerable differences between replications were not found.

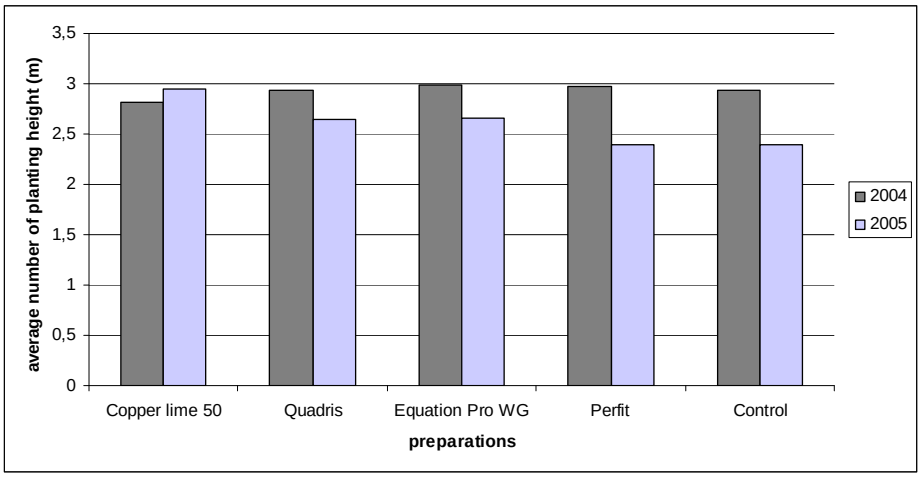
Furthermore, it was clearly shown that average numbers of fruiting bodies of both pathogens were lower on leaves of treated plants during 2005. Leaves protection was better in

2005 than in 2004, due to more abundant fungicide treatments in 2005, with shorter time intervals between them. Taking into consideration meteorological conditions, especially amount of precipitation, it may be concluded that these conditions were favorable for leaf diseases in both years. But, a greater amounts of precipitations during vegetative period in year 2005 (51 mm more than in 2004) probably facilitated appearance of a greater number of fruiting bodies on leaves of untreated plants (*Table 2*, *Figures 3, 5 and 6*).

Table 2. Amounts of precipitations (mm) in the period from April to September in year 2004 and 2005

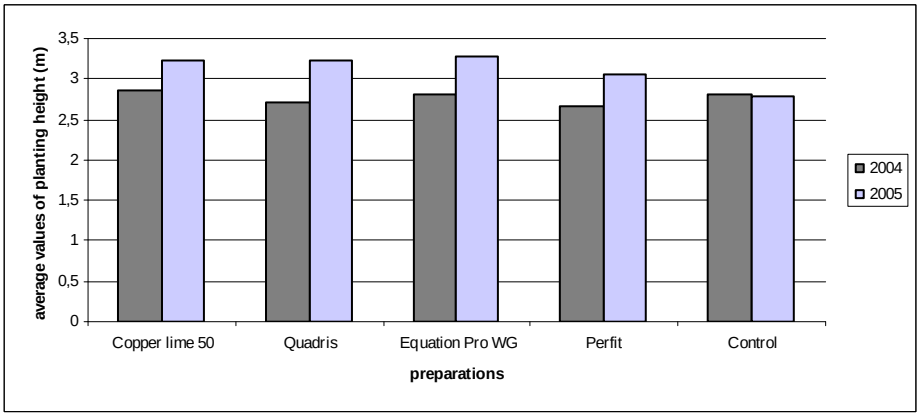
Year	Month						Total
	April	May	June	July	August	September	
2004	112	89	97	63	39	42	442
2005	65	67	68	94	138	61	493

Results of seedlings height measurements at the end of both experimental years indicated some differences between average values obtained for individual treatments. Analysis of variance showed no statistically significant differences, neither between individual fungicides, nor between control and treated plants (*Figures 8 and 9*).



2004: F (for treatments) = 1,33 ns, replications 0,24 ns;
2005: F (for treatments) = 0,59 ns, replications 0,08ns

Figure. 8 Average height of treated and untreated seedlings of Pannonia clone



2004: F (for treatments) = 0,41 ns, replications 0,61 ns;
2005: F (for treatments) = 1,74 ns, replications 0,57 ns

Figure 9. Average height of treated and untreated seedlings of S6-36 clone

Besides plant height, in order to accomplish the most complete impression about fungicide treatment upon plant development, plant diameter measurements were also used. According to analysis of variance, there were no significant differences between control and treated plants (data not shown).

4 DISCUSSION

According to numerous literature data, parasites on poplar leaves (*Marssonina brunnea* and *Melampsora* spp.) belong to the group of economic important pathogens. The long-term problems, caused by appearance of these diseases on poplar leaves, directed attention of scientists to explore possibilities of their suppression using chemical compounds.

During experiment planning, four fungicides of different chemical composition were selected. The Copper lime 50 (product from the group of copper compounds) is used because of its stability and efficacy in managing a wide range of fungal diseases. Quadris is a fungicide from the group of strobilurins, and it is effective against numerous diseases in both agriculture and silviculture. But, the resistance occurrence presents a serious problem in the use of strobilurines. Since 1998, several pathogens have developed resistance to strobilurins in the field (Kuck – Mehl 2003). Equation Pro WG is a fungicide that contains two active substances (famoxadone+cymoxanil), primarily labeled for managing pathogens that cause leaf rust on vegetable crops and grapevine. Hydrogen peroxide is formulated as a domestic product Perfit. It is used for soil, tools and equipment disinfection, in order to suppress pathogens responsible for flattening of sprouts.

Considering the importance of the problem, many authors have been interested in suppression poplar leaf diseases by fungicides.

During the sixties, numerous products were tested in various concentrations in poplars protection from the *Melampsora* species (Gojković – Vujić 1966). The best results in experiments concerning chemical suppression of leaf rusts, lasting for three years (1964-1966) in nursery production, were obtained with Copper lime 25, while somewhat lower efficacy had Maneb. Results with other fungicides were not satisfactory. A very high efficacy of the Copper oxichloride in treating fungi from the genus *Melampsora* on the leaves of sensitive clone S 6-36 was confirmed in our experiment.

Chemical suppression of *M. brunnea* has been examined in details during the sixties, in Italy (Cellerino 1969). Great complexes of poplar plantations were treated from both ground and air, in order to investigate efficacy and duration of protection, considering number and timing of treatments. The best results were obtained with products Ditan M-45 and Maneb. Furthermore, author reported that plantations of moderately sensitive clones needed two to three treatments per year in strictly defined time intervals, while sensitive clones required additional treatment.

In the late sixties, chemical protection of poplars from fungus *M. bunnea* has been studied by Gojković (1970). Considering previous results and his own three year long experiments in nursery plantations, he concluded that the most effective protection was achieved with Ditan 45, Maneb and Copper lime. Author especially emphasized importance of prevention, and in particular, early spring treatments. Experiments revealed acceptable results in plants protection in nurseries, using three or more applications. In comparison with other products used, Quadris and Copper lime showed considerably higher degree of efficacy in treating *M. brunnea* in our experiments.

Avramović et al. (1991) evaluated efficacy of nineteen fungicide compounds and their combinations in treating fungus *M. brunnea*. Experiments were conducted in nurseries situated at different localities, during the period from 1983 to 1986. According to their results,

bitertanol provided the best protection of leaves, while satisfactory effects were obtained for fungicides containing triphorin, copper oxichloride, benomyl and carbedazim, as well as following combinations: triphorin+propineb, benomyl+propineb and tiophanate+propineb.

According to two year long researches, various efficacies of fungicides in managing *M. brunnea* and *Melampsora* spp. were reported by Keča (2003). During the year 2000, which was centenary extreme considering low rainfall and high daily mean temperatures, the best results were recorded for Benomyl WP-50. The most effective product in the rainy year 2001 was Copper lime, due to its longevity and steadiness on leaves. Along with high efficacy of Copper oxichloride, its steadiness was also evident in our study, considering that climate events during 2004 and 2005 were similar to those in 2001.

Statistically significant differences were not found in height and diameter increment between treated and control plants, although leaves of untreated plants were exposed to intensive attack of studied fungi. Similar results were reported by Avramović (1997) and Keča (2003), when crowns of poplar plants in newly established plantations were repeatedly treated with different fungicides during two years. These authors approved that juvenile plants, exposed to moderate attack of these fungi, do not exhibit significant reduction of height and diameter increment. It may be concluded that these leaf diseases show cumulative effects, while consequences become evident in the following years, when trees are older (Castellani – Cellerino 1967, Cellerino 1969). In our experiment, considerable variations of growth elements were not found, probably due to a strong influence of other factors (the juvenile phase of plants, favourable climate conditions, clones tolerance to these diseases, etc.). These factors affected plants stronger than parasites.

Taking into consideration our Institute's results related to clones susceptibility to *M. brunnea* and *Melampsora spp.*, collected during several years (Avramović et al. 1998, Pap et al. 2006), clones used in our experiments exhibited different sensitivity to the major leaf diseases of poplars. According to these authors, clone *Pannonia* belongs to the group of moderately sensitive clones to *M. brunnea* and insignificantly sensitive to fungi from the genus *Melampsora spp.* Clone S 6-36 is labeled as very sensitive to the rust and a little sensitive to fungus *M. brunnea*. Sensitivity of clones obtained in our study is in accordance with results of authors mentioned above.

Number of treatments, necessary for safe plant protection in nurseries, was not investigated. According to obtained results, it could be concluded that at least 6-8 fungicide treatments, applied in appropriate time intervals, are necessary for sensitive clones under favorable climate conditions for development and spreading of these diseases. Complete plant protection could probably be achieved even with fewer treatments, but the main periods of spores dispersion must be precisely determined for both pathogens. Plants protection must be simultaneous with the major period of leaves infection, in order to accomplish satisfactory protection of nursery with low number of treatments.

5 CONCLUSIONS

Following conclusions could be drawn from biennial research:

In stoolbeds of poplar clones *Pannonia* and S6-36, satisfactory suppression of both *M. brunnea* and fungi from the genus *Melampsora spp*. were performed with Quadris and Copper lime 50, while lower efficacy was obtained with fungicides Equation Pro WG and Perfit.

Products Quadris and Copper lime 50 are recommended for treating *M. brunnea* and *Melampsora* spp. in nurseries and newly established plantations, while poorly effective fungicides Equation Pro WG and Perfit should be avoided.

Successive protection of plants from studied pathogens in nurseries could be achieved by 6-8 fungicide treatments during the vegetative period.

Statistically significant differences were not found between treated and control one-year-old plants in relation to height and diameter increment in both experimental years.

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Shoot blights

Sphaeropsis sapinea Dyko & Sutton Associated with Shoot Blight on *Pinus brutia* Ten. in Southwestern Turkey

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Abstract – This study was conducted in order to determine the agents of shoot blight and dieback of Calabrian pines at Aşağı Gökdere, Isparta province, in 2005 and 2006. Ninety trees were selected systematically in the stand. One dead branch from each tree was cut and the shoots were investigated under the stereomicroscope for the presence of fungal structures. *Sphaeropsis sapinea* Dyko & Sutton and *Truncatella hartigii* (Tubeuf) Steyaert were common fungi, with the observation frequencies of 21.1% and 46.7%, respectively. Pathogenicity of two fungi was investigated by winter and spring inoculations on Calabrian and Crimean pine. After eight months incubation period, the lesion sizes were measured. *S. sapinea* was found to be quite aggressive and large lesions formed on both hosts while lesions caused by *T. hartigii* did not differ significantly from the control.

Calabrian pine / Crimean pine / dieback / pathogenicity

Kivonat – A *Sphaeropsis sapinea* Dyko & Sutton és a *Pinus brutia* Ten. hajtáspusztulása Délnyugat-Törökországban. E tanulmány célja a kalábriai fenyők hajtáspusztulásáért és száradásáért felelős kórokozók meghatározása Aşağı Gökdere térségében, Isparta vidékén 2005-ben és 2006-ban. Az állományban rendszeresen kilencven fát választottunk ki. Minden fáról levágtunk egy-egy elhalt ágat és sztereómikroszkóp alatt megvizsgáltuk a gombaszervezetek jelenlétét a hajtásokon. A *Sphaeropsis sapinea* Dyko & Sutton és a *Truncatella hartigii* (Tubeuf) Steyaert gombák esetében 21,1%-os, illetve 46,7%-os gyakoriságot állapítottunk meg. A két gomba patogenitását téli és tavaszi mesterséges fertőzéssel vizsgáltuk a kalábriai és a krimi fenyőn. Nyolc hónap inkubációs idő elteltével megmértük a nekrózisok nagyságát. A *S. sapinea* meglehetősen agresszívnek bizonyult, mindkét gazdán nagyméretű nekrózisokat okozott. A *T. hartigii* okozta nekrózisok a kontrolltól szignifikánsan nem különböztek.

kalábriai fenyő / krimi fenyő / pusztulás / patogenitás

1 INTRODUCTION

Mediterranean region of Turkey exhibit a rich diversity of forests ranging from coniferous to deciduous trees belonging to this mild climatic zone. There are single-species forests of softwood and hardwood trees, and also mixed forest formations. The most frequently occurring coniferous forest in the Mediterranean belt consists of *Pinus brutia* Ten., *Pinus*

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nigra Arn. (Lamb.) Holmboe, *Abies cilicia* Carr. and *Cedrus libani* A. Rich. The highway from Isparta to Antalya where the Calabrian pine plantation was managed is attractive and famous for domestic and international guests with its special mountainous landscape, *Liquidambar orientalis* L. protection area and also for many other natural beauties.

Shoot dieback of coniferous trees caused by fungi have economic importance when it causes tree to loose its vitality (Butin 1995). *Sirococcus conigenus* (DC.) P. Cannon & Minter (syn. *S. strobilinus* G. Preuss) (Hartig 1893, Klein 1987, Minerbi 1987, Wulf – Maschning 1992, Neumüller 1994, Anglberger – Halmschlager 2003), *Sphaeropsis sapinea* Dyko& Sutton (syn. *Diplodia pinea*) (Brookhouser – Peterson 1971, Peterson 1977), and *Gremmeniella abietina* (Lagerberg) Morelet. (Yokota et al. 1974, Setliff et al. 1975, Gibbs 1984, Laflamme 1993) are among most common agents of the disease all over the world.

Shoot dieback of *P. brutia* has devastating effects from the beginning of 2004 in western part of Taurus Mountains located in Mediterranean site of Turkey. Calabrian pine plantation (app. 16 352 ha) in Aşağı Gökdere, Isparta province exhibits unhealthy appearance, specifically characterised with branches carrying dead shoots.

The objective of this study was to find out the causal agent of the serious damage observed on Calabrian pine plantation at Aşağı Gökdere.

2 MATERIAL AND METHOD

A field study was conducted in July 2005 at Aşağı Gökdere. A Calabrian pine stand, which was as homogenous as possible regarding to tree size and shoot blight symptoms, was chosen for the investigation. Ninety trees were selected systematically within the stand. One dead branch from each tree was cut and the shoots were investigated under the stereomicroscope for the presence of fungal structures. The fungi were identified based on conidial and pycnidial morphology (Sutton 1980). Fungi were also isolated from the shoots having dieback symptoms and kept as PDA slants for further studies.

Virulence of the fungal isolates was investigated in 2005-2006, by inoculating branches of *P. brutia* and *P. nigra.* Pathogenicity tests *were* carried out in two different inoculation trials using one isolate of each fungus with ten replicates.

Healthy looking shoots of 15 years old *P. brutia* trees were inoculated in December 2005 and April 2006 (as winter and spring inoculations) in the same area where the field study was performed. In addition, shoots of 15-year-old *P. nigra* were inoculated in April 2006 near the main campus of Süleyman Demirel University.

Inoculations were performed on a healtly branch (about ∅ 15 mm) of the trees. In each tree outer bark was removed with a cork borer (∅ 5 mm), and the hole was filled with a plug of actively growing mycelium of each fungus cultured a week on PDA at 25°C. The hole was covered with parafilm to retard desiccation. After an eight month incubation period, branches were cut and the length of the cancers was measured. Fungi were re-isolated from the cankers.

Data were statistically analysed by using SPSS software programme and means were compared by Duncan's multiple range test ($p = 0.05$).

3 RESULTS AND DISCUSSION

As a result of the field work, *Sphaeropsis sapinea* Dyko & Sutton (syn. *Diplodia pinea*) and *Truncatella hartigii* (Tubeuf) Steyaert were commonly found on the shoots of 90 collected branches, with the observation frequencies of 21.1% and 46.7%, respectively. Similarly, it was reported that *T. hartigii* was one of the most common fungi occurring on Austrian pines

infected with *S. sapinea* (Diminiae 1994, Jurc 2005, Milijasevic- Karadzic 2007). *S. sapinea* and *T. hartigii* were also found to be the most common fungi isolated from pine cones and seeds (Vujanovic et al. 2000).

S. sapinea is a latent fungal endophyte of coniferous trees occurring throughout the world (Eldridge 1961, Punithalingam - Waterston 1970, Nicholls et al. 1977, Sutton 1980, Swart - Wingfield 1991 a, b, Hausner et al. 1999, Flowers 2001). Although the existence of the fungus was reported in Marmara Region (Unligil - Aytekin 1993), its damage, pathogenicity and distribution have not been studied for Turkey. The fungus causes devastating effects in plantation sites, especially where susceptible *Pinus* spp. are extensively planted. As it is an opportunistic pathogen, predisposition due to a variety of biotic and abiotic stress factors can also result in this naturally being fungus causing substantial deaths in pine plantations (Bega et al. 1978, Swart et al. 1987, Nicholls - Ostry 1990, Palmer 1991, Swart - Wingfield 1991, Stanosz - Cummings-Carlson 1996). Poor sites, draught, hail, snow and insects are among stress factors (Chou 1987). Our plantation site is surrounded with mountains located in a large valley, which has high summer temperatures causing draught stress for the trees. Pine Stem Borer, Pine Sawfly and Pine Engraver Beetle are the common insects in this location. Apart from the site properties, origin of the seeds or seedlings is known among important factors for the adaptation and healthy growth of the trees in the plantation sites. Reliable data confirming the origin of the planting material does not always exist in Turkish forestry practice. Appropriate silvicultural treatments such as pruning and thinning also affect future health and survival of the plantation.

In the pathogenicity test, winter inoculations on *P. brutia* and spring inoculations on *P. brutia* and *P. nigra* with both fungi resulted in lesions with different sizes. Lesion sizes caused by *T. hartigii* did not differ significantly from that on the control shoots, but *S. sapinea* was found to be quite aggressive and cause larger lesions *(Table 1* and *Figure 1).* Results of Milijasevic and Karadzic (2007) who mentioned that *T. hartigii* occurred as a weak parasite or saprophyte on shoots colonised by *S. sapinea*, supported our findings.

Table 1. Lesion lengths on P. brutia and P. nigra branches caused by S. sapinea and T. hartigii inoculations

Treatments	Lesion length (mm)		
	P. brutia		*P. nigra*
	Winter inoculation	Spring inoculation	Spring inoculation
S. sapinea	47,6 a*	96,9 a	44,5 a
T. hartigii	9,2 b	17,1 b	7,4 b
Control	5,4 b	5,8 b	7,0 b
Means	B	A	
		A	B

* Means in the same column followed by the same lowercase letter and means in the same row shown by the same uppercase letter were not significantly different from each other according to Duncan's Multiple range test (P=0.05)

Spring inoculations with *S. sapinea* caused bigger lesions than winter inoculations on *P. brutia.* It can be due to higher temperatures, causing rapid fungal growth and predisposing the trees. Between two host species, *P. brutia* seems more succeptible against both fungi than *P. nigra*. But climatical conditions of two sites may be the main factor causing the significant difference between lesion lengths on the trees. *P. nigra* plantation area has lower temperature and humidity than Aşağı Gökdere. Extensive pycnidia formation was observed under the barks of *S. sapinea* inoculated shoots.



Figure 1. Lesion inoculated with S. sapinea (right hand) and control treatment (left)

In conclusion, *S. sapinea* was found to be the main agent of the shoot blight disease of Calabrian pines in the Mediterranean region of Turkey. This is the first report of the fungus for this part of our country. Further research is needed on the biology, host range and economical importance of the pathogen.

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The Effect of Fertilisation on the Severity of Sirococcus Shoot Blight in a Mature Norway Spruce (*Picea abies* [L.] Karst.) Stand

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Abstract – The paper reports on the effect of fertilisation on the severity of Sirococcus shoot blight in a mature Norway spruce stand. Trees with severe symptoms of Sirococcus shoot blight were characterised by insufficient Mg and Ca supply and enhanced N/Mg and N/Ca-ratios in the current-year and 3-year-old needles at the start of the project. Application of appropriate fertilisers in 2001 mitigated disease severity of the fertilised trees and promoted tree recovery. Best results were achieved by fertilisation with a water soluble Ca- and Mg-fertiliser (gypsum + kieserite-variant) which resulted in an 18.9 % decrease of disease severity in the period 2001 – 2006. While dolomitic liming also promoted tree recovery **(**decrease in disease severity was 11.8 %), in the unfertilised control variant a 3.5 % increase was observed in the same period.

***Sirococcus conigenus* / disease severity / liming / application of gypsum and kieserite**

Kivonat – Tápanyag-utánpótlás hatása a Sirococcus hajtáspusztulásra egy érett lucfenyő (*Picea abies* [L.] Karst.) állományban. A dolgozat témája a tápanyag-utánpótlás hatásának vizsgálata a Sirococcus hajtáspusztulás súlyosságára egy érett lucfenyő állományban. A Sirococcus hajtáspusztulás által súlyosan érintett fákra a kísérlet kezdetén jellemző volt a Mg és Ca elégtelenség és a megnövekedett N/Mg és N/Ca arány a folyó évi és a három éves tűkben. Megfelelő tápanyagok 2001-ben történt kijuttatása enyhítette a betegség súlyosságát és elősegítette a fák gyógyulását. Legjobb eredményt egy vízben oldódó Ca és Mg tápszerrel értünk el (gipsz + kieserite változat), amely a 2001-2006 időszakban a betegség súlyosságát 18,9%-kal csökkentette. A dolomitos mész szintén elősegítette a gyógyulást (a betegség súlyosságának csökkenése 11,8% volt). A kezeletlen kontroll változatnál a betegség mértéke ezalatt 3,5%-kal növekedett.

***Sirococcus conigenus* / betegség súlyossága / meszezés / gipsz és kieserite alkalmazás**

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1 INTRODUCTION

Sirococcus conigenus (DC.) P. Cannon & Minter (syn. *S. strobilinus* G. Preuss, *Ascochyta piniperda* Lindau) is an asexually reproducing fungus causing shoot blight and seedling death of many conifer hosts throughout much of the northern hemisphere (Smith 1973, Shahin – Claflin 1978, Sutherland 1987, Sanderson – Worf 1986, Farr et al. 1989, Halmschlager et al. 2000). Although the fungus was described already in 1796 from cone scales of Norway spruce (*P. abies* [L.] Karst.) by Persoon (Cannon – Minter, 1983), the disease was first reported in 1890 from Germany on current-year shoots of Norway spruce by Hartig. Sirococcus shoot blight has subsequently been reported on a wide range of conifer hosts in Europe and North America, where it mainly affects *Picea* and *Pinus* spp. but also attacks *Abies*, *Cedrus*, *Larix*, and *Pseudotsuga* (Peace 1962, Sutherland 1987, Butin 1995, Danti – Capretti 1998, Bronson et al. 2003, Smith et al. 2003, Sinclair – Lyon 2005). There is also one report from *Pinus halepensis* Mill. in North Africa (Morelet 1972) and most recently the disease also has been found on *Picea spinulosa* (Griff.) Henry in Bhutan (Kirisits et al. 2007).

In Central Europe the pathogen mainly occurs on Norway spruce (Hartig 1893, Rudolph 1898, Klein 1987, Minerbi 1987, Wulf – Maschning 1992, Neumüller 1994, Anglberger 1998, Anglberger – Halmschlager 2003, Stetter et al. 2004). According to recent estimates about 6500 ha of spruce forests are affected by *Sirococcus* shoot blight in the county of Upper Austria (Reisenberger 2007). In two forest districts (Freyung, Passau) in the neighboring Eastern Bavaria 850 ha are affected and on 240 ha stands decline and need to be harvested long before they reach the prescribed rotation age (Stetter al. 2004).

Disease severity was found to be highest in secondary spruce forests on poor and acidified soils and west exposed upper slopes as well as on hilltops, where *Sirococcus* shoot blight has become a major destabilizing factor (Anglberger – Halmschlager 2003, Stetter et al. 2004). Furthermore, the study of Anglberger et al. (2003) revealed insufficient Mg and Ca supply and enhanced N/Mg and N/Ca ratios in the needles of severely affected trees, whereas in healthy trees all needle element contents were above the threshold for deficient supply. Although healthy trees also yielded the fungus, no symptoms developed, which indicates latent infections. Thus, a close relation between supply of base cations, in particular magnesium, and severity of Sirococcus shoot blight of Norway spruce was hypothesized (Anglberger et al. 2003). To test the hypothesis that improved nutrient supply will have an impact upon Sirococcus shoot blight and promote recovery of diseased trees, a single-tree fertilisation experiment was established at a site already investigated by Anglberger et al. (2003).

2 MATERIAL AND METHODS

2.1 Study site and experimental design

The fertilisation experiment was established in autumn 2000 in a 90-year-old Norway spruce stand severely affected by Sirococcus shoot blight *(Table 1)*. The stand is situated in the Kobernausser Wald, Upper Austria (48°04´42´´ N, 13°14´19´´E) and has already been investigated prior to fertilisation by Anglberger et al. (2003). A total of 144 dominant or co-dominant trees, randomly distributed within an 8.2 ha area in the investigated stand, were selected. Half of the trees were severely affected by Sirococcus shoot blight ("Sirococcus +"), whereas the other trees were apparently healthy and vigorous ("Sirococcus -"). A tree was counted as 'healthy' when less than 5% of the current year shoots were affected by Sirococcus shoot blight.

A randomised block design with the factors "slope section" (lower slope versus upper slope) and "Sirococcus shoot blight" (severely affected versus healthy trees) was used. Within these blocks sample trees were randomly assigned to one of the three treatments (dolomitic liming, application of gypsum and kieserite, unfertilised control) (*Table 2, Figure 1*). In order to characterise the current nutritional and health status of sample trees and to derive treatments, soil analyses and needle analyses as well as an evaluation of disease severity had been carried out prior to fertilisation. The nutrient status of diseased and healthy trees prior to fertilisation has already been assessed on 72 out of the 144 sample trees in a previous study (Anglberger et al. 2003).

Table 1. Site and stand characteristics of the experimental site

Site characteristics	
Location	48°04´42´´ N, 13°14´19´´E
Elevation (m)	600
Aspect	W
Bedrock	Tertiary gravels
Slope (°)	15
Soil types	Dystric cambisols and podzols
Humus type	Moder
Soil pH ($CaCl_2$)	Organic layer: 3.00, A horizon: 2.99
Base saturation (%)	Mineral horizons: 4.62 – 14.48

Stand characteristics	
Tree age (a)	90
Number of trees per hectare	432
Basal area (m^2ha^{-1})	37.7
Breast height diameter (cm) of the mean basal area stem	33.3
Volume per hectare(m^3ha^{-1})	463
Stocking degree	0.79

Table 2. Experimental design

	Upper slope (dbh ≥ 33cm)*		Lower slope (dbh ≥ 30cm)*		
Treatments	Severely affected trees	Healthy trees	Severely affected trees	Healthy trees	
Dolomitic lime	12 (6)	12 (6)	12 (6)	12 (6)	Σ = 48 (24)
Gypsum & kieserite	12 (6)	12 (6)	12 (6)	12 (6)	Σ = 48 (24)
Unfertilised control	12 (6)	12 (6)	12 (6)	12 (6)	Σ = 48 (24)
Σ	36 (18)	36 (18)	36 (18)	36 (18)	
Σ	72 (36)		72 (36)		
Σ	144 (72)				

Numbers in parentheses refer to the number of trees subjected to needle analyses in the previous study of Anglberger et al. (2003); one-third (= 48) of the sample trees were felled in June 2004. *) Minimum diameter of trees included in the study (dominant or co-dominant trees) – it differed between upper and lower slope due to the lower number of trees per hectare on the upper slope which implied a higher mean tree diameter on this slope section.

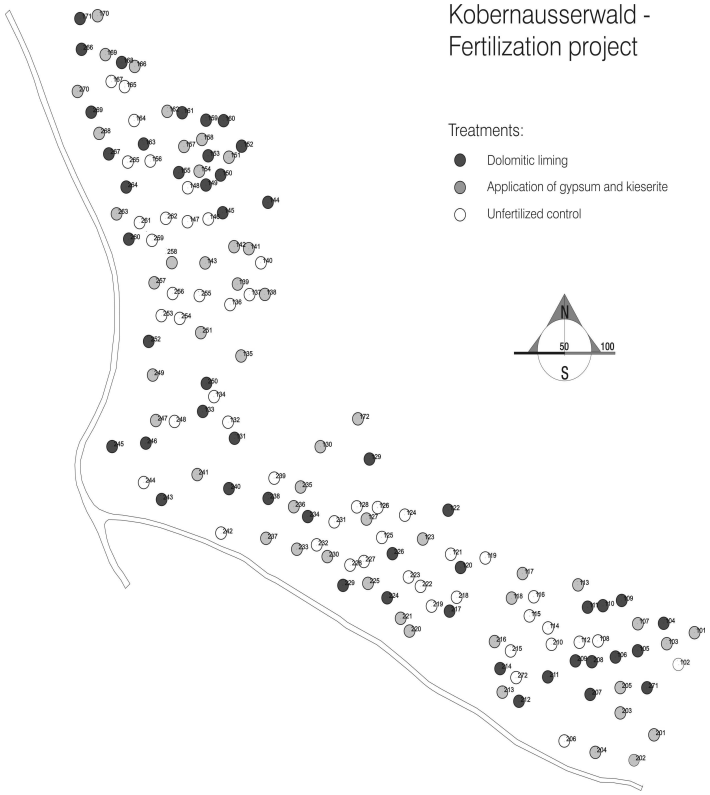


Figure 1. Distribution of sample trees randomly assigned to one of the three treatments (see legend), the dots indicate the area that was fertilised or left untreated; Upper Slope: Tree Nos. starting with 101; Lower slope: Tree Nos. starting with 201; ÖBF district management Traun- & Innviertel, forest district 'Frauschereck', subcompartment 237b$_1$

2.2 Treatments

Because results of needle analyses prior to fertilisation (Anglberger et al. 2003) revealed insufficient Mg and Ca supply especially on severely affected trees, Ca- and Mg-fertilisers were applied in this study to promote recovery of diseased trees. Two different fertiliser treatments (dolomitic liming, combined application of gypsum and kieserite) and an unfertilised control variant were used in this study. Short term amelioration should be achieved by fertilisation with a water soluble Ca- and Mg-fertiliser (gypsum and kieserite variant: 500 kg ha^{-1} gypsum, Co. Moldan, Kuchl and 400 kg ha^{-1} kieserite, Co. Kali & Salz GmbH via Co. Danufert, Krems). As a medium-term amelioration measure slow-release dolomitic liming (3000 kg ha^{-1} dolomitic lime, Co. Bodenkalk, Graz) was applied, aiming at both improving Ca- and Mg-supply, enhancing soil pH and accelerating litter turnover.

The granular fertilisers were spread manually in a circular area around the individual trees (within a horizontal radius of 4 m from the tree base) before bud break in late April 2001. According to the applied amounts of fertilisers the following quantities (kg ha^{-1}) of Ca and Mg were applied in the two fertiliser treatments (*Table 3*).

Table 3. Quantities (kg ha^{-1}) of Ca and Mg applied in the two fertiliser treatments

	Ca	Mg
Dolomitic liming	974	282
Application of gypsum and kieserite	90	60

2.3 Disease severity of individual trees

Ratings of disease severity of individual trees were carried out yearly from 2001 until the end of the project in summer 2006 by estimating the percentage of diseased previous year shoots in April (before the emergence of new shoots), using a pair of binoculars. If there was an uniform distribution of *Sirococcus* shoot blight in the crown, disease severity (DS) was assessed for the whole crown, whereas in the case of a patchy distribution, DS was estimated separately for each part of the crown (crown halves or crown thirds) and ratings were afterwards weighted with the respective crown mantle area to calculate DS_{total} as follows (*Figure 2*):

Total DS calculated from estimates of crown halves (a):

$DS_{total} = 0{,}32\ DS_{upper} + 0{,}68\ DS_{lower\ part\ of\ crown}$

Total DS calculated from estimates of crown thirds (b):

$DS_{total} = 0{,}14\ DS_{upper} + 0{,}43\ DS_{middle} + 0{,}43\ DS_{lower\ part\ of\ crown}$

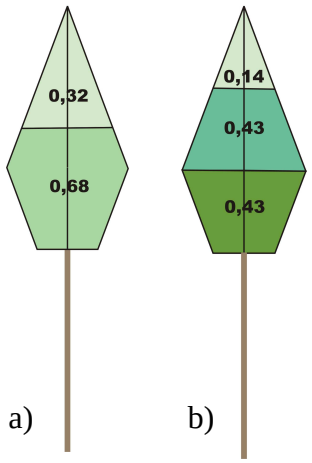


Figure 2. Calculation of disease severity (DS) on trees with patchy distribution of Sirococcus shoot blight

2.4 Statistical analyses

The effects of treatment, tree health status and slope position and the interaction between tree health status and treatment on disease severity (statistical significance of the differences between disease severity of the respective years and disease severity prior to fertilisation) were tested by univariate analyses of variance, using GLM procedure of SPSS.

3 RESULTS

3.1 Effects of treatment, tree health status and slope position

In the period 2001-2002 fertilisation had no effect on disease severity yet, whereas in the 2-year period 2001-2003 a significant effect of treatment occurred for the first time (*Table 4*). This significant effect of fertiliser treatments on disease severity was found in all following periods until the end of the project in 2006.

The affiliation of sample trees to the class of the severely diseased or that of the healthy trees (tree health status) was significant in the periods 2001-2002, 2001-2003 and again in 2001-2006. In the latter period as well as in 2001-2003 and 2001-2005 there was also a

significant effect of the interaction between tree health status and treatment. A significant effect of slope position only occurred in the periods 2001-2003 and 2001-2005.

Table 4. Effects of treatment, tree health status and slope position on disease severity (univariate ANOVA)

Factors	Significant probability				
	2001-2002	2001-2003	2001-2004	2001-2005	2001-2006
Treatment	n.s.	*	*	(*)	**
Tree health status (healthy / diseased)	***	**	n.s.	n.s.	***
Interact.: Tree health status x treatment	n.s.	(*)	n.s.	(*)	**
Slope position	n.s.	**	n.s.	**	n.s.

Stars indicate error probability levels obtained by F-tests (n.s. = not significant):

(*) $0.10 \geq P > 0.05$ * $0.05 \geq P > 0.01$ ** $0.01 \geq P > 0.001$ *** $P \leq 0.001$

3.2 Comparison between treatments due to tree health status prior to fertilisation

Although a significant effect of treatment was already detected in the period 2001-2003, a reduction of disease severity could only be achieved by the gypsum + kieserite -treatment in this period. In the unfertilised control variant disease severity increased between 2001-2003 by 6.3%, and in the liming-treatment by 12.5%. In the periods 2001-2004, 2001-2005 and 2001-2006 differences between treatments became more pronounced: From 2004 onwards disease severity was always lower for the fertilised treatments compared to the unfertilised control variant. Until the end of this investigation in 2006, disease severity decreased on the severely affected trees by 18.9% in the "combined gypsum and kieserite"-treatment and by 11.8% in the „liming"-treatment, whereas a 3.5% increase was observed in the control variant. In summary, results indicate that the improvement of the nutritional status of single trees by application of appropriate fertilisers mitigated disease severity of the fertilised trees and promoted tree recovery.

Table 5. Mean decrease (-) or increase (+) of disease severity [in %] for the healthy and the severely affected trees due to treatments and tree health status (in relation to disease severity prior to fertilisation)

Periods	Tree health status	Treatments		
		Dolomitic liming	Gypsum and kieserite	Unfertilised control
2001-2003	Healthy	-0.1	-0.8	0.0
	Severely affected	+12.5	-0.6	+6.3
2001-2004	Healthy	-0.3	-0.8	+0.9
	Severely affected	-1.9	-8.8	+3.5
2001-2005	Healthy	-1.3	0.0	0.0
	Severely affected	+0.4	-9.2	+4.0
2001-2006	Healthy	-1.0	0.0	-0.4
	Severely affected	-11.8	-18.9	+3.5

3 DISCUSSION

Stands suffering from Sirococcus shoot blight are mostly growing on poor sites with soils characterized by low base saturation (Klein 1987, Neumüller 1992, Anglberger – Halmschlager 2000, Halmschlager et al. 2000, Jandl et al. 2000, Stetter et al. 2004). Furthermore, the majority of Central European forest ecosystems were additionally impoverished by litter raking in the past (Glatzel 1991, Katzensteiner – Glatzel 1997), which was also a common form of historic land use in the investigation area (Reinisch 1873, Jenner 1979).

Consequently, affected stands are characterized by poor supply of soil Mg and Ca which causes poor status of these nutrients in foliage. Berger – Katzensteiner (1994) found high input rates of air pollutants, in particular nitrogen, in the research area, further deteriorating base cation supply. The input of nitrogen compounds by atmospheric deposition is most likely to induce nutritional imbalances (Glatzel et al. 1987, Schulze 1989, Katzensteiner et al. 1992) and is probably the cause for enhanced N/Ca and N/Mg ratios in the foliage. Poor supply of base cations and nutritional imbalances are suggested to increase susceptibility of Norway spruce to *Sirococcus* shoot blight (Anglberger et al. 2003). The nutritional status of the stands may worsen in the following decades as long as measures of amelioration are lacking. However, fertilisation of forest stands remains a controversially discussed topic until today, because amelioration fertilization often caused a change in resistance of trees to fungal diseases and insect pests (e.g. Dimitri 1977, Marschner 1995, Kytö et al. 1996, Piri 1998).

In the present study, however, site-specific compensatory fertilisation with Ca- and Mg-fertilisers (dolomitic liming, application of gypsum and kieserite) resulted in a significant decrease of disease severity of the severely affected trees and promoted tree recovery. The lower level of significance observed in 2005 ($P \leq 0.10$) may be due to the reduced sample size, because one-third of sample trees were felled in June 2004 for a growth study. Results correspond well with the findings of Anglberger - Halmschlager (2000) and Jandl et al. (2000) in earlier studies, comparing the severity of Sirococcus shoot blight on fertilised and unfertilised plots in degraded Norway spruce stands on poor podzolic soils over old silicate bedrock or tertiary gravels. In both studies the application of magnesium rich carbonate fertilisers harmonised tree nutrition and mitigated severity of Sirococcus shoot blight at the investigated sites, whereas the application of fertilisers with relatively high N-contents and only small portions of Mg did not achieve similar results. However, in contrast to the present study the health status of trees prior to fertilisation could not be assessed in these earlier studies. Furthermore, comparison was carried out for trees on adjacent plots whereas in the present study sample trees were randomly assigned to one of the three treatments within the given blocks on the study site.

The applied quantities of Ca and Mg in the two fertiliser treatments correspond to the amounts that were used in other vitality fertilisation studies (Kilian et al. 1995, Kytö et al. 1996). Due to the slow release of Ca and Mg from dolomitic lime, amounts of applied Ca and Mg were five (Mg) to ten times higher (Ca) in the liming variant compared to the gypsum and kieserite variant.

In the gypsum and kieserite variant a significant effect of treatment was already detected 2 years after fertilisation, indicating that short term recovery of trees can be achieved with this water soluble fertiliser. This effect proceeded till the end of the project in 2006, when disease severity decreased on the severely affected trees by 18.9%. The rapid response of fertilised trees can be explained by the quick plant availability of Ca and Mg in this treatment. In the dolomitic liming slight differences compared to the control occurred already after 3 years, but a considerable decrease in disease severity was not found until 5 years after fertilisation (-11.8%). Thus, liming has a medium term effect due to the slow release of Ca and Mg, by increasing soil pH and by acceleration of litter-turnover. In the unfertilised control variant

disease severity of the severely affected trees increased by 6.3% in the period 2001-2003 and then was constant about 3.5 – 4% above the value obtained in 2001. Thus, no recovery of trees was observed in the control variant. On the other hand, disease severity did not further increase over years.

As it was clearly shown, application of appropriate fertilisers diminished disease severity in both fertiliser treatments and promoted tree recovery. It is therefore suggested that microsite differences and/or genetic variation may contribute to differences in disease incidence and severity of individual trees.

Increased host susceptibility due to nutritional imbalances has already been reported from other fungi, causing shoot blight and canker on conifers. Ylimartimo (1991) demonstrated that high N/K and N/Mg ratios reduced resistance of Scots pine seedlings to *G. abietina* infections and Roelofs et al. (1985) reported that foliar K and Mg levels were lower in *Pinus nigra* var. *maritima* trees infected by *Gremmeniella abietina* (Lagerb.) Morelet and/or *Sphaeropsis sapinea* (Fr.) Dyko & Sutton, compared with uninfected trees, whereas availability of N was increased in the damaged trees. However, there were no studies up to now to our knowledge that aimed to reduce disease severity and to promote tree recovery by application of site-specific compensatory fertilisers.

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Detection of *Diplodia pinea* in Asymptomatic Pine Shoots

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Abstract – *Diplodia pinea* is a fungal pathogen that causes tip blight shoot on several conifers trees. During the last few years the occurrence of this fungus in symptomless pine shoots, has been investigated on Austrian pine (*Pinus nigra* A.) pinewoods located in Central and Northern Italy. Fungal detection from apparently healthy samples was performed by using both isolation of pine tissue on agarized media but also by the real-time PCR. Differences between two sampled pinewoods were found showing a different behaviour of the fungus in the host.

Conifers / fungi / latent phase / real-time PCR / *Sphaeropsis*

Kivonat – A *Diplodia pinea* kimutatása tünetmentes *Pinus* hajtásokban. A *Diplodia pinea* kórokozó gomba a fenyőfélék hajtáspusztulását okozza. Az utóbbi években e gomba tünetmentes előfordulását vizsgáltuk feketefenyő (*Pinus nigra* A.) erdőkben, Közép- és Észak-Olaszországban. A látszólag egészséges mintákban a gombát a fenyők szöveteiből való kitenyésztéssel és real time PCR módszerrel mutattuk ki. A gomba eltérő viselkedését mutató különbségeket találtunk két megmintázott fenyőerdő között.

Fenyők / gombák / latens fázis / real-time PCR / *Sphaeropsis*

1 INTRODUCTION

Diplodia pinea (= *Sphaeropsis sapinea* (Fr.:Fr.) Dikko & Sutton) is a fungus with a world-wide distribution (Stanosz et al.1996) responsible of shoot dying of pines. Two *S. sapinea* morphotypes, initially designated A and B, were indicated as two different species: *Diplodia pinea* for the A morphotype and *Diplodia scrobiculata* for the B morphotype (de Wet et al. 2003). They basically differ for conidia and colony morphology and aggressiveness against host plants (Smith-Stanosz 1995, Hauser et al. 1999, de Wet et al. 2002). The two species can be differentiated more clearly using molecular techniques (Smith – Stanosz 2006, Zhou – Stanosz 2001).

In Italy *D. pinea* occurs on some Mediterranean species of *Pinus* along the peninsula, like *P. halepensis*, *P. pinaster*, *P. pinea*, and *P. nigra*. In this country the main damages are recorded on *Pinus nigra* plantations, especially those present in Northern Italy where the fungus is particularly injurious, causing tip blight and progressive dying of trees (Maresi et al. 2002). On pine the fungus, occasionally vectored by insects (*Tomicus* sp.), causes also blue stain of wood

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(Sabbatini Peverieri et al. 2006). Other *D. pinea* disease occurs on cones, related with the cone bug, *Gastrodes grossipes* (Feci et al. 2002). Along the Tyrrhenian coast of peninsula, the main damages on cones have also an economic impact on edible seeds production on *P. pinea* (Vagniluca et al. 1995).

Studies on the environmental conditions enhancing the outbreak of the disease on pine plantations underlined the influence of water stress on the host susceptibility (Stanosz et al. 2001, Paoletti et al. 2001). *D. pinea* is able to live for long time inside the host tissue in latent phase, without any visible symptoms, until climatic and environmental factors induce the fungus to invade host cells and cause death of the tissues (Stanosz et al. 2001).

Massive presence of latent infections may explain the ineffectiveness of pruning and spraying in controlling the disease (Flowers et al. 2001). Is not known how long *D. pinea* might persist in symptomless trees, but was observed that the fungus can rapidly become pathogenic after water stress condition for host (Stanosz et al. 2001).

The occurrence of *D. pinea* in host tissue have been described by using classical approaches, such as plating pine samples on agar media (Stanosz et al. 2001; Flowers et al. 2001), but also with molecular techniques (Flowers et al. 2003; Luchi et al. 2005) that revealed sensitive tools for the detection of this fungus.

2 MATERIALS AND METHODS

The study was carried out in two Austrian pine (*Pinus nigra* Arn.) plantations located in Tuscany (Montesenario, Florence; 700 m a.s.l.) and South Tyrol (Val D'Adige, Trento; 600 m a.s.l.). Eighteen trees were selected from each pinewood. For each tree one symptomless shoot was cut, from the lower part of the crown and the apical portion was used to detect *D. pinea* in host tissue. Each sample was longitudinally split in two portions and the fungal occurrence was detected by isolation on agarized media (as number of fragments colonized by *D. pinea*) and by using real-time PCR (Maresi et al. 2007). For each sampling sites 10 needles per shoot were processed for fungal detection: 5 were used for isolation and 5 for real-time PCR, according to the method already used by Maresi et al. (2007). The fungal presence was evaluated as percentage of fragments colonized by *D. pinea*, and colonies were identified according to Luchi et al., (2007). After using real-time PCR, the fungal picograms were expressed as pg DNA *D. pinea*/ μg total DNA.

3 RESULTS

The occurrence of *D. pinea* was detected in symptomless pine shoots in Trentino and Tuscany pinewoods with significant differences ($p=0.014$) between two sites.

Molecular approach showed a total of 17 out of 18 positively colonized shoots in Montesenario (*D. pinea* DNA rangedg from 0.01 to 2.5 pg), and 14 out of 18 (fungal DNA ranged from 0.02 to 1.4×10^3 pg) in Val d'Adige.

Results of isolation showed that the number of colonised shoots was lower than those obtained by real-time PCR. After plating method only 3 samples out of 18 collected in Montesenario (1 fragment for each one) and 10 out of 18 from Val d'Adige (mean: 9 fragments per shoot), resulted colonised by the fungus.

Some relationship between amount of fungal DNA colony presence after plating was also recorded. No fragments were colonized when the amount of *D. pinea* DNA was lower than 3 pg. This data was recorded in 94% from shoots collected in Montesenario and 44.4% from Val D'Adige. Occurrence of *D. pinea* was always negative both after isolation or real-time PCR.

4 DISCUSSION

In this study the use of both cultural and molecular methods for fungal detection, showed the presence of *D. pinea* in symptomless Austrian pine shoots, with differences between the two sampling areas. In the place of Trentino the amount of *D. pinea* was significantly higher than in the site from Tuscany.

Although is known that the fungus can rapidly become pathogenic after water stress condition that affect the host (Stanosz et al. 2001), is still unknown how long *D. pinea* might persist in the shoots before to produce symptoms.

In a recent work Maresi et al. (2007) showed that the frequency of *D. pinea* in symptomless pine tissue was positively correlated with Normalized Insolation index (NIi), considered as the solar radiation that trees receive every years.

Results from this study may explain further aspects of fungal behaviour. In Tuscany the occurrence of the fungus detected both in terms of colonized fragments but also as fungal DNA, was high as frequency on samples but low in term of quantity, indicating that the latent phase was prevalent. On the contrary, the bigger amount of fungal DNA detected in Trentino give the idea that the fungus although still on asymptomatic shoots, is moving from latent phase, to the parasitic phase.

For the last aspect the use of a sensitive tool, able to detect the latent phase in symptomless tissue, and their relationship with environmental parameters may be useful to predict the outbreak of *Diplodia* blight infection in pine forests.

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Differences in Occurrence and Co-occurrence of *Sirococcus conigenus* and *Diplodia pinea* on Blighted Red Pine Shoots

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Abstract – Blighted red pine shoots, either having symptoms only on the most recent year's growth or having symptoms present both on most recent and previous year's growth, were examined for pycnidia of *Sirococcus conigenus*, *Diplodia pinea*, or both. On shoots having symptoms only on the most recent year's growth, *S. conigenus* was detected more frequently than *D. pinea*. On shoots having symptoms on both the most recent and the previous year's growth, however, *D. pinea* was detected more frequently than *S. conigenus* on the most recent year's growth. In addition, on those shoots having symptoms on both the most recent and the previous year's growth, *D. pinea* was detected almost eight times more frequently on the previous year's growth than the most recent year's growth. Whether only one pathogen was detected or the two pathogens were detected co-occurring on a single year's growth also varied with shoot condition and shoot year of growth, but the two fungi co-occurred on shoots of each condition.

Pinus resinosa* / *Sirococcus strobilinus* / *Sphaeropsis sapinea

Kivonat – Eltérések a *Sirococcus conigenus* és a *Diplodia pinea* előfordulása és együttes előfordulása között *Pinus resinosa* pusztuló hajtásain. Vagy csak a folyó évi, vagy a folyó és előző évi növedékeken is tünetet viselő, pusztuló *Pinus resinosa* hajtásokon vizsgáltunk a *Sirococcus conigenus* és/vagy *Diplodia pinea* piknídiumok jelenlétét. A csak folyó évi tünetes hajtásokon a *S. conigenus* gyakoribb volt, mint a *D. pinea*. A folyó, és előző évi növedéken is tünetes hajtásokon viszont a legújabb növedéken *a D. pinea* gyakoribb volt, mint a *S. conigenus*. Ráadásul a legújabb és az előző évi növedéken is tüneteket hordozó hajtások esetében az előző évi növedéken a *D. pinea* csaknem nyolcszor gyakoribbnak bizonyult, mint a folyó évi növedéken. Csak az egyik, vagy mindkét kórokozó együttes előfordulása az egyes növedékeken a hajtás állapotától és a növedék évétől függően változott, de a két gomba minden állapotú hajtáson együttesen fordult elő.

Pinus resinosa* / *Sirococcus strobilinus* / *Sphaeropsis sapinea

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1 INTRODUCTION

Red pine (*Pinus resinosa*) is an ecologically and commercially important tree species of the northern United States and southern Canada, where it extends from the Atlantic coast westward through the Great Lakes region. Rudolf (1990) reviewed the silvical characteristics of red pine, which may exist naturally in relatively pure stands or in mixtures. Associated species on the coarser soils on which red pine is most common include jack pine (*Pinus banksiana*), eastern white pine (*P. strobus*), and scrubby oak species (e.g., *Quercus ellipsoidalis*). Natural regeneration is sporadic, apparently dependent on an abundant seed crop and a coincidence of local conditions that may include a preceding fire, opening of the overstory canopy, reduction of seed predators, favorable weather providing moisture for seedling establishment, and subsequent absence of fire for several decades. Because silvicultural techniques that might allow consistent achievement of adequate natural regeneration on most sites have not yet been mastered (Farnsworth 2002), planting is the primary means of stand replacement following commercial clear cut harvests. For example, almost 250 million red pine seedlings have been produced in state-operated nurseries during the last 25 years in Wisconsin (personal communication, Gregory Edge, Wisconsin Department of Natural Resources), and red pine plantations in Wisconsin occupy approximately 213,000 ha (almost three-fourths of the total area of planted forest in this state) (Schmidt 1997). Mechanical and/or chemical treatments to reduce herbaceous and woody competition with this relatively intolerant species may be applied both before and after planting. Seedlings may require 4-10 years to reach breast height (1.37 m), with height growth thereafter usually ranging from 0.3-0.5 m per year. Three to four thinnings beginning at age 30-40 years until a final harvest at age 60-80 years are common in stands commercially managed for pulpwood, poles, and small sawtimber. Left to grow, however, red pines may be long-lived and exceed 300 years of age.

Among the diseases that can severely damage red pines is shoot blight caused by the fungus *Sirococcus conigenus* (DC.) P. Cannon and Minter (syn. *Sirococcus strobilinus* G. Preuss). This pathogen occurs widely in Europe and both the eastern and western United States and Canada, attacking a variety of conifers, especially spruces and pines (Farr et al.1989, Smith et al. 2003, Sutton 1980). In northern Wisconsin and the upper peninsula of Michigan, a disease often characterized by drooping and retention of needles on diseased shoot tips was first noticed in 1959, but over a decade passed before *S. conigenus* (as *S. strobilinus*) was identified as the causal agent (O'Brien 1973). Pycnidia of the pathogen develop on killed shoots, especially at the bases of necrotic red pine needles. Ostry et al. (1990) trapped conidia dispersed from diseased red pines during periods of wet weather in spring and early summer when they are carried by rainfall to young, susceptible shoots. Small red pine seedlings may be killed within one growing season, while growth of saplings is reduced or they are killed from cumulative effects of infection in successive years, especially in the understory of infested stands (Bronson - Stanosz 2006, Ostry et al. 1990). The "die up" of lower branches to result in progressive crown reduction and the probability of mortality of large, overstory red pine trees was noted by O'Brien (1973).

Red pine also is among the forest tree species most severely damaged by a second shoot blight pathogen, *Diplodia pinea* (Desmaz.) J. Kickx. fil. (syn. *Sphaeropsis sapinea* (Fr.:Fr.) Dyko and Sutton). This fungus reportedly attacks over 30 pine species, and less commonly other conifers, both in their native ranges and where introduced in the southern hemisphere (Farr et al. 1989, Gibson 1979, Punithalingam – Waterston 1970). In the northcentral United States *D. pinea* has been particularly damaging to red pine seedlings in nurseries with infested red pine windbreaks providing inoculum (Palmer – Nicholls 1985, Palmer et al. 1988, Stanosz et al. 2005). Conidia are disseminated from pycnidia borne in necrotic needles, stems, and

cones during periods of rain (Palmer et al. 1988). Infection of red pines has been achieved experimentally through wounds and by conidial application to nonwounded, young, expanding shoot tips (Blodgett – Stanosz 1997). Virulent strains of this pathogen persist on or in red pines in nurseries and in the forest in the absence of symptoms (Stanosz et al. 1997, Stanosz et al. 2001, Stanosz et al. 2005). The potential for *D. pinea* to subsequently proliferate and rapidly cause disease, including mortality, under conditions that induce host stress is supported by greenhouse experiments (Stanosz et al. 2001). Epidemics characterized by high frequencies of mortality of red pine seedlings and saplings, and branch dieback, stem cankers, and top-kill on larger trees have been associated with drought (Nicholls – Ostry 1990, Palmer 1991, Stanosz – Cummings Carlson 1996). The role of water stress in enhancing colonization of red pine shoots by water stress was demonstrated using potted seedlings and established plantation trees (Blodgett et al. 1997a, Blodgett et al. 1997b).

2 MATERIALS AND METHODS

Red pine shoots were collected in mid-winter from four sites (replicates) in the Northern Highland-American Legion (NHAL) State Forest in Vilas County of northern Wisconsin (46°05'N, 89°40'W). Planted red pine saplings were present at each site and scattered red pine overstory trees had been deliberately retained (for aesthetic reasons) during the previous harvest. The soils are nutrient-poor sands on glacial outwash. Preliminary observations revealed shoot blight symptoms and indicated presence of both *S. conigenus* and *D. pinea* at each site.

Shoots were obtained at five sampling points located at intervals along transects across each site. At each sampling point, five shoots of each of two different shoot conditions were collected, either having symptoms present only on the most recent year's growth or having symptoms present both on most recent year's and previous year's growth (25 shoots of each condition at each of the four sites, or 200 shoots total). Shoots were individually bagged, carried to the laboratory, and refrigerated until they were examined further.

A dissecting microscope was used to inspect needles and stems of the symptomatic most recent year's growth and symptomatic previous year's growth for pycnidia. If pycnidia were present, spores were examined at up to 400x using a light microscope. The shoot blight pathogens *S. conigenus* and *D. pinea* were identified based on presence of characteristic spores obtained from pycnidia (Sutton 1980). Although not used during this study, molecular methods for identification of each of these fungi (as differentiated from closely related species) (Smith – Stanosz 2006, Smith – Stanosz submitted) have been employed on multiple samples obtained on other occasions in the vicinity of the collection sites.

Occurrence of each pathogen was recorded for shoots of each of the two conditions by shoot year of growth (most recent or previous year's growth). The means and standard errors of the frequencies of occurrence of pycnidia of each pathogen for each shoot condition-year of growth combination were calculated. Comparisons were made using the Kruskal-Wallace test of equality of medians. Analyses were performed using Minitab for Windows version 14 (Minitab Inc., State College, PA).

3 RESULTS

Blighted shoots exhibited symptoms that have been considered characteristic of Sirococcus shoot blight or Diplodia shoot blight on red pine (Palmer - Nicholls 1985, Ostry et al. 1990). These symptoms included brown to gray discoloration of needles, retention of dead needles

on dead stems, death of shoots either before or after full needle elongation, curl or crook of the stem of the most recent year's growth on shoots killed during expansion (a symptom commonly associated with Diplodia shoot blight), and droop of killed, fully expanded needles (considered characteristic of Sirococcus shoot blight). The particular symptoms were not, however, always indicative of whether *S. conigenus* or *D. pinea* was detected on a particular shoot. For example, *S. conigenus* was detected on crooked or curled dead shoots, and *D. pinea* was detected on killed shoots with drooped, killed fully-expanded needles (*Figure 1*).

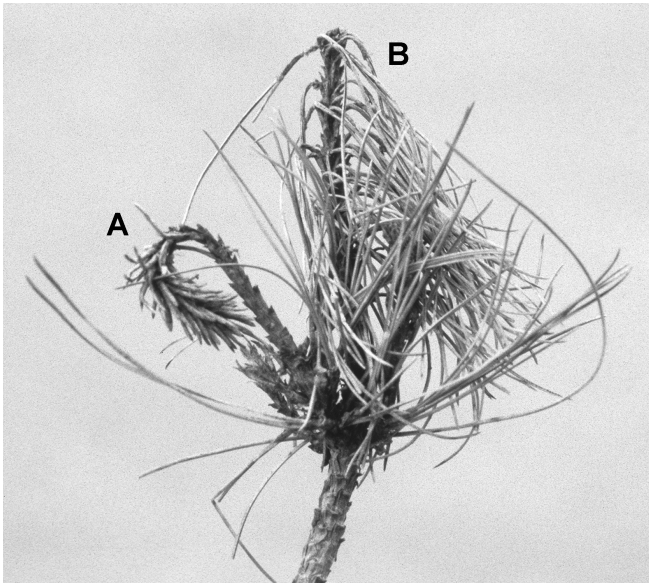


Figure 1. Blighted red pine shoots exhibiting: (A) curl of the stem of a shoot killed during expansion (a symptom commonly associated with Diplodia shoot blight); and (B) droop of dead fully-expanded needles (considered characteristic of Sirococcus shoot blight). However, pycnidia with conidia of S. conigenus were found on the shoot at (A), and pycnidia with conidia of D. pinea were found on the shoot at (B)

Although either pathogen (at least one of these two fungi) was detected on the majority of the shoots collected at each site, the frequency of detection of each pathogen varied with shoot condition and shoot year of growth (*Table 1a, b*). *Sirococcus conigenus* or *D. pinea* were detected on 77/100 of the shoots having symptoms present only on the most recent year's growth and 91/100 of the shoots having symptoms present both on most recent year's and previous year's growth. On shoots having symptoms present only on the most recent year's growth, *S. conigenus* was detected more frequently than *D. pinea*. On shoots on which symptoms were present on both the most recent year's growth and the previous year's growth, however, *D. pinea* was detected more frequently than *S. conigenus* on the most recent year's growth. In addition, on shoots on which symptoms were present on both the most recent and the previous year's growth, *D. pinea* was detected almost eight times more frequently on the previous year's growth than the most recent year's growth.

Table 1a. Total occurrence of pycnidia of Sirococcus conigenus (Sc) or Diplodia pinea (Dp) on blighted most recent and previous years' growth of red pine shoots. Values are means ± standard error of the mean for four sites (replicates); 25 shoots of each condition at each site were examined. Uppercase letter under each value identifies that cell for pairwise comparison with other cells (see Table 1b).

Shoot condition	Year of growth of needles or stems on which the indicated pathogens were detected	Total no. of shoots (out of 25) on which the indicated pathogens were detected	
		Sc	Dp
Symptoms present only on most recent year's growth	Most recent	15.75 ± 1.49 A	9.25 ± 2.29 D
	Previous	NA	NA
Symptoms present both on most recent year's and previous year's growth	Most recent	13.75 ± 1.11 B	18.75 ± 1.80 E
	Previous	1.25 ± 0.63 C	9.75 ± 1.94 F

Table 1b. Values of p for pairwise comparisons using the Kruskal-Wallis test of equality of medians. Upper case letters correspond to those in cells in Table 1a. Comparisons that were not of interest were omitted (-).

	A	B	C	D	E
B	0.31				
C	0.02	0.02			
D	0.06	-	-		
E	-	0.03	-	0.02	
F	-	-	0.02	1.00	0.02

Whether one pathogen was detected and not the other, or whether the two pathogens were detected co-occurring on a single year's growth of a shoot also varied with shoot condition and shoot year of growth (*Table 2a, b*). On shoots having symptoms present only on the most recent year's growth, *S. conigenus* only tended to be detected more frequently than *D. pinea* only or both pathogens together. On the most recent year's growth of shoots on which symptoms were present on both the most recent year's growth and the previous year's growth, *D. pinea* only or both pathogens together were detected more frequently than *S. conigenus* only. On the previous year's growth of shoots on which symptoms were present on both the most recent and previous year's growth, *D. pinea* only was much more frequently detected than *S. conigenus* only or both pathogens together.

Table 2a. Occurrence of pycnidia of Sirococcus conigenus (Sc) only, Diplodia pinea (Dp) only, or both on blighted most recent and previous years' growth of red pine shoots. Values are means ± standard error of the mean for four sites (replicates); 25 shoots of each condition at each site were examined. Uppercase letter under each value identifies that cell for pairwise comparison with other cells (see Table 2b).

Shoot condition	Year of growth of needles or stems on which the indicated pathogens were detected	No. of shoots (out of 25) on which the indicated pathogens were detected		
		Sc only	Dp only	Both
Symptoms present only on most recent year's growth	Most recent	10.00 ± 2.48 A	3.50 ± 0.50 D	5.75 ± 1.93 G
	Previous	NA	NA	NA
Symptoms present both on most recent year's and previous year's growth	Most recent	3.50 ± 1.19 B	8.50 ± 1.94 E	10.25 ± 0.25 H
	Previous	0.25 ± 0.25 C	8.50 ± 2.06 F	1.00 ± 0.48 I

Table 2b. Values of p for pairwise comparisons using the Kruskal-Wallis test of equality of medians. Upper case letters correspond to those in cells in Table 2a. Comparisons that were not of interest were omitted (-).

	A	B	C	D	E	F	G	H
B	0.08							
C	0.02	0.03						
D	0.05	-	-					
E	-	0.06	-	0.02				
F	-	-	0.02	0.04	0.88			
G	0.19	-	-	0.35	-	-		
H	-	0.02	-	-	0.24	-	0.18	
I	-	-	0.16	-	-	0.02	0.03	0.02

4 DISCUSSION

Plant disease is often studied in the growth chamber, greenhouse, or the field as the product of a single pathogen with its host. For woody plants in complex ecosystems such as forests, however, there are likely to be many situations in which fungi (including plant pathogens) could exist in communities with other microorganisms within the same trees and parts of trees. Recognition that *S. conigenus* and *D. pinea* may co-occur, not just in the same location and tree but on the same shoot, has implications for surveys to assess incidence and severity of disease and also for further studies of their potential interactions in shoot blight disease cycles.

Reliance on symptoms and even the utilization of fruiting structures as indicators of occurrence of a fungus have great limitations. Our experience indicates a lack of specificity in blight symptoms, or at least that a symptom considered characteristic of one pathogen (e.g., droop of red pine needles caused by *S. conigenus*) does not exclude the possibility of other pathogens also being present. Therefore, symptom-based surveys that fail to substantiate

presence of particular pathogens must be viewed with skepticism. For example, a visual survey of red pines for shoot blight damage attributed to *S. conigenus* was conducted in the Northern Highland-American Legion State forest in 1995 (Prey et al. 1995). "Level of infection" was categorized as heavy or light at each of 49 widely distributed sites, but no information was presented regarding frequency of identification of *S. conigenus* (or other pathogens) on even a subset of shoots. Conversely, while fruiting structures can substantiate presence of a fungus of interest, a lack of pycnidia with conidia of *S. conigenus* or *D. pinea* does not support the conclusion that they are absent. Sporulation may not always occur even on symptomatic shoots. And because sporulation of these pathogens occurs only on necrotic tissues, fruiting structures are not useful indicators of their association with asymptomatic needles or stems. Therefore, our data present a very conservative estimate of the frequency of the co-occurrence of *S. conigenus* and *D. pinea*. Future studies might employ both species-specific molecular methods (Smith – Stanosz 2006; Smith – Stanosz submitted) and cultural techniques to detect and confirm viability of *S. conigenus* and *D. pinea* in a variety of host organs prior to, during, and after disease development.

Co-occurrence of *S. conigenus* and *D. pinea* on shoots examined in this study does validate two previous observations by our group of the co-occurrence of *S. conigenus* and Diplodia shoot blight fungi on red pine shoots. Smith and Stanosz (submitted) attempted to culture *S. conigenus* from blighted red pine shoots on which pycnidia with conidia of this pathogen (i.e., *S. conigenus*) had been observed. Fungi identified as either *D. pinea* or the similar fungus *D. scrobiculata* (De Wet et al. 2003) on the basis of characteristics of pycnidia with spores that were produced in culture, were obtained in 43 of 180 attempts from needles, bark, or wood of these shoots. Bronson and Stanosz (2006) found pycnidia and spores identified as *S. conigenus* and *D. pinea* occurring together on red pine seedlings that became blighted after being planted in the understory of a mature red pine plantation. After just one summer, fruiting of both pathogens on current year's shoot growth was observed on as many as 31% of the seedlings that had become blighted, and fruiting of both pathogens on previous year's shoot growth was observed on as many as 10% of the seedlings that had become blighted. As in our current report, Bronson and Stanosz (2006) found *S. conigenus* (alone or with *D. pinea*) more frequently on the most recent year's shoot growth, and found *D. pinea* (alone or with *S. conigenus*) more frequently on the previous year's shoot growth.

The natural co-occurrence of *S. conigenus* and *D. pinea* on red pine shoots may stimulate further studies of their possible interactions before, during, and after development of disease. The potential for each fungus to inhibit or stimulate spore germination, infection, or colonization by the other is unknown. Similarly, each could influence pycnidial production and sporulation, or survival of the other fungus in shoots in which they compete for substrate. The relative prevalence of *S. conigenus* on the most recent year's shoot growth and *D. pinea* on previous year's growth suggest the potential for resource partitioning. These differences reflect the relative invasiveness of these fungi and differences in progression of the diseases they cause. Damage by *S. conigenus* to pines is associated with invasion of succulent new growth on branch tips, although it may extend into 1-year-old twigs (Smith 1973, Nicholls – Robbins 1984, Sinclair – Lyon 2005). In contrast, although *D. pinea* readily infects expanding needles and shoots, it is much more invasive, colonizing bark and sapwood of large branches and main stems of pines, including red pine (Nicholls – Ostry 1990, Palmer 1991).

Prevalence of *D. pinea* on blighted shoots of red pine collected in the Northern Highland-American Legion State Forest also supports the hypothesis that *D. pinea* is an invasive pathogen that has greatly expanded its range and activity in the northcentral United States in the last 30 to 40 years. A single instance of a fungus referred to as *Sphaeropsis ellisii* was reported from eastern white pine (*P. strobus*) in Wisconsin in the 1930's (Crandall 1938), but the identity of this fungus cannot be confirmed. However, reports of economic damage did

not soon follow, and *D. pinea* (or synonyms) was not included on comprehensive lists of fungi occurring on Wisconsin's native pines for several decades (Greene 1965). Further, during extensive studies of Sirococcus shoot blight in the Northern Highland-American Legion State Forest in the 1970's that were described by Ostry et al. (1990), *D. pinea* was not encountered (personal communication Michael Ostry, USDA Forest Service; Thomas Nicholls, USDA Forest Service, retired). Reports of economic damage to jack and red pines in seedling nurseries and plantations in Wisconsin began to appear only during the 1970's (Renlund 1977, Renlund 1979). The probable extension of the geographic range of *D. pinea* and its current prevalence at sites in the Northern Highland-American Legion State Forest today is not surprising, however, given knowledge of the persistence of virulent strains of *D. pinea* on asymptomatic red pine seedlings in nurseries and the many years of planting and subsequent disease of seedlings from infested nurseries (Stanosz – Cummings Carlson 1996, Stanosz et al. 1997, Stanosz et al. 2001, Stanosz et al. 2005).

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Scleroderris canker

Spatial and Temporal Variation in the Occurrence of *Gremmeniella abietina* in Scots Pine in Finland

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Abstract – Spatial and temporal patterns in the occurrence of the infections caused by *Gremmeniella abietina* in Scots pine stands in Finland during the years 1985-2005 were described in this paper The study utilized data from forest health monitoring plots (Forest Focus Level I plots) and permanent and temporal plots of the National Forest Inventories. The National Forest Inventories showed that the disease was far more common in southern than in the northern parts of the country. The disease was also clearly spatially clustered. The proportion of diseased stands decreased between the 8th NFI (1986-1994) and 9th NFI (1996-2003). The forest health monitoring revealed a heavy outbreak of *Gremmeniella* in 1988-89, and smaller peaks in 1997 and 2001. Temporal and spatial distributions of the disease were obtained using the level I data. The usability of various datasets were also compared with each other.

forest health monitoring / national forest inventory / forest diseases / *Pinus sylvestris* / *Gremmeniella abietina*

Kivonat – A *Gremmeniella abietina* előfordulásának térbeli és időbeli változása az erdeifenyőn Finnországban. A dolgozat a *Gremmeniella abietina* előfordulásának térbeli és időbeli mintázatát ismerteti a finnországi erdeifenyő állományokban, az 1985-2005 időszakban. Felhasználtuk az erdők egészségi állapotfelmérő mintaterületeinek adatait (Forest Focus I mintaterületek) és a Nemzeti Erdőleltár állandó és időszakos mintaterületeit. A Nemzeti Erdőleltár adatai szerint az ország déli részén a betegség messze gyakoribb volt, mint északon. A 8. Nemzeti Erdőleltár (1986-1994) és a 9. Nemzeti Erdőleltár (1996-2003) közötti időszakban a beteg állományok aránya csökkent. Az erdők egészségi állapotfelvétele a *Gremmeniella* erős kitörését jelezte 1988-89-ben és kisebb csúcsokat 1997-ben és 2001-ben. A betegség időbeni és térbeni eloszlását az I. szint adatainak felhasználásával állapítottuk meg. A különböző adatsorok felhasználhatóságát összehasonlítottuk.

erdők egészségi állapotfelvétele / nemzeti erdőleltár / erdőbetegségek / *Pinus sylvestris*/ *Gremmeniella abietina*

1 INTRODUCTION

The forest environments are changing rapidly. Concern about large-scale decline in forest vitality in central Europe in the late 1970's and early 1980's led Finland, as many other European countries, to initiate an extensive national survey of forest condition. The changes in forestry practices and the climate change in particular, can potentially increase the

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preconditions for biotic diseases. The changes may be expressed as the modifications in the frequencies of known pathogens, as changes in their host spectrum, but also as introduction of completely new pathogens.

However, the literature on the effects of global warming on forest diseases, for instance, is relatively scarce (see Boland et al. 2004, Garrett et al. 2006).

These factors set a need for comprehensive, systematical and statistically representative monitoring of forest condition, including biotic diseases. In some cases, the rapid deterioration in the vitality of forests has been attributed to abiotic or biotic damage (Innes and Schwyzer 1994, Keane et al. 1989). A large-scale increase in defoliation occurred in many parts of Europe after the severe drought in 2003 (see Fischer et al. 2005, for instance). It has even been proposed that the condition of trees merely reflects the fluctuating effects of biotic or abiotic agents or site conditions (Skelly and Innes 1994).

The area of forest land in Finland is over 20 million hectares. The Finnish National Forest Inventory (NFI) has produced information on forest resources over this area for more than 70 years. The first inventory dates back to 1921-1924. Different forms of damage in statistically representative samples have been registered since the 7th inventory (1977-1984). In the 7th NFI, however, the only identified causes of damage were wind, snow, moose (*Alces alces* L.) and pine- twisting rust (*Melampsora pinitorqua* (Braun) Rostr.). The Eighth NFI (1986-1994) was the first to include more detailed information on health of forests, including diseases and pests. The Ninth NFI started in 1996. Satellite images and digital maps have been routinely exploited since the Eight NFI, to produce up-to-date information of forests, and for smaller areas than before. The main aim of the first NFIs of Finland has been to estimate the volume and growth of growing stock and the cutting potential. Other aims, like forest health, multiple use of forests and biodiversity have become more and more important in the recent inventories.

A network of 3009 permanent sample plots was established in the 8th NFI in 1985-86, covering the whole country systematically. A systematic subsample of this network (see below) has been used for forest health monitoring since 1985. This subsample has been a part of a large-scale, extensive level I monitoring network covering the whole of Europe.

Gremmeniella abietina (Lagerb.) Morelet var. abietina is the causative agent of Scleroderris. canker on coniferous trees. In southern Finland, it is the most common fungal disease in forests dominated by Scots pine *(Pinus sylvestris* L.). The disease is epidemic in nature. An epidemic requires that the host trees, here Scots pines, are predisposed. If the trees are of local origin, meteorological factors and site conditions determine the degree of predisposition. Epidemics are often said to be common after cold and rainy growing seasons (Aalto-Kallonen - Kurkela 1985, Uotila 1988). Frost damage is an important cause for the differences in susceptibility (Dietrichson 1968). The susceptibility of pines is increased in shaded or dense stands (Read 1968, Niemelä et al. 1992, Nevalainen 1999). It has even been proposed that air pollution can be one of the factors that promotes the disease (Bragg – Manion 1984).

The purpose of this paper was to describe the spatial and temporal variation in the occurrence of the disease caused by *Gremmeniella abietina*, based on nationwide monitoring.

2 MATERIAL AND METHODS

This study utilized forest damage data from tree different sources a) permanent plots of the 8th National Forest Inventory , from the years 1990 and 1995 (standwise damage assessments) b) data from forest health monitoring plots (Forest Focus Level I plots (treewise assessments) and c) temporary plots of the 8th and 9th National Forest Inventories (stand damage data).

In permanent plots of the 8th NFI the sampling units were four-plot clusters in a 16 x 16 km grid with a 400-m distance between fixed- sized circle plots (0,1-0,3 ha each) in southern Finland, and three plot clusters in a 32 x 24 km grid in northern Finland (plots 600 m apart). These plots have been measured three times, 1985/86, 1990 and 1995. Unfortunately *Gremmeniella* was not recorded as a causal agent in 1985.The damage recording system was similar than in forest health surveys (see below).

A systematic subsample was taken from the permanent plots of the 8th NFI, e.g. the first plot of the tract in mineral soils was chosen, rejecting every tenth tract. These plots were used in national forest health monitoring (1986 onwards) for annual assessment of forest vitality (defoliation, discolouration) and biotic and abiotic injuries. All the dominant or co-dominant coniferous or birch trees were used as sample trees. The present network includes 499 sample plots on mineral soil and 110 on peatland. The number of Scots pine observation trees has ranged from 2002 (in 1990) trees to 6450 trees (in 2005) (for details, see Lindgren et al. 2006). In the forest health monitoring, a national system for describing the symptom, apparent severity (degree of damage) and the cause, as well as the age of the damage, was used prior to 2004. An example of the variables and codes used in the national forest health survey can be found e.g. in Nevalainen (1999). After 2004, the ICP-Forests manual of damage causes (Assessment of …) (referred to as the Biotic manual) has been adopted in Finland. Climatic data for these plots was produced with the models of Ojansuu and Henttonen (1983).

The sampling units of the normal NFI were temporary sample plots located systematically in clusters. Survey designs have been somewhat variable in different inventories and in different parts of the country. The distance between tracts increases from south to north, and is 7 x 7 km in southern and mid- Finland in the Ninth NFI. The tracts comprise of 14-18 relascope plots, with a 250- or 300- m distance between plots. The stand in which the centre point of the field plot was located is referred to as the centre-point stand. The area that one plot (actually, a centre point) theoretically represented, varied from 266 to 6726 hectares in the 8th NFI, and from 135 to 2285 hectares in the 9th NFI . The recent NFIs of Finland have been regional inventories, i.e. the field work has been done district wise. About 150 variables were assessed or measured in the 8th and 9th NFI at the stand, tally tree or sample tree levels. The field inventory also contained data on forest injuries, e.g. damage symptoms, their causes and apparent severities (degrees of damage), as well as an estimation of the time of the damage. Only standwise damage data from these two NFIs was used in this study. Damage has been recorded similarly, in principle, than in the permanent plots and in the forest health monitoring. Codes for registering damage symptoms, degrees of damage and causal agents of damage in the 8th NFI can be found in Nevalainen 1999 b). The description of the damaging agents was somewhat more detailed in the 9th NFI. 30 codes were used for causal agents. Moreover, two causes, instead of one, could be recorded for each stand. The codes for the degree of damage at the stand level were 0) slight damage, symptoms observed, but the damage does not reduce the silvicultural quality of the stand 1) moderate, the silvicultural quality of the stand is reduced by one class 2) severe, the stand quality is reduced by more than one class 3) complete, artificial regeneration is required. More information of the 9th NFI, for instance, can be found at http://www.metla.fi/ohjelma/vmi/vmi-historia-en.htm.

3 RESULTS

3.1 The permanent plots of the 8th NFI (1990 and 1995)

The overall occurrence if the disease had slightly decreased on the permanent plots of the 8th NFI from 1990 to 1995 (from 8.4 % to 5.6 % of Scots-pine dominated stands, respectively). A great portion of the slight damage observed in1990 was not recorded anymore in the very

same plots in 1995. The disease was not re-recorded in 128 stands (*Table 1*). Most of such stands (84) were mineral soil plots. In 75 plots the disease had increased between the two dates. These changes were not spatially clustered in any part of the country, however. The lambda statistics indicates that knowing the presence of the disease in 1990 did not help in predicting the presence of the disease in 1995.

Table 1. *The occurrence of Gremmeniella in Scots-pine dominated permanent plots of the 8th NFI in 1990 and 1995.*

		Number of pine-dominated stands In 1995		
		Absent	Present	Total
In 1990	Absent	1627	75	1702
	Present	128	33	**161**
	Total	1755	**108**	**1863**

Pearson Chi-Square = 69.731 p= 0,000
Lambda (Ga 1995 dependent) = 0,000

3.2 The forest health monitoring data (1985-2005)

The proportion of symptomless trees increased rather than decreased during the 21-year period (1985-2005). As a grand mean, fungal damage occurred in 10,4 % of the Scots pine observation trees during this period (*Table 2*). The proportion of the trees infected with Gremmeniella was 8,2 % over the whole period. Most of the damage to the observation trees was slight, i.e. did not affect the vitality of the trees.

Table 2. *The mean incidence of causal agent groups in the sample trees on the Level I plots in Finland during 1985-2005. The group 'other' mostly consists of competition.*

Causal agent group	% of trees			
	Scots pine	Norway spruce	Broadleaves	Total
No damage	58.3	58.0	58.9	58.3
Game and grazing	.8	.1	1.5	.7
Insects	10.3	.3	4.4	6.3
Fungi	10.4	9.5	7.3	9.6
Abiotic	3.6	7.8	6.5	5.3
Direct action of man	1.8	3.3	2.1	2.3
Other	11.5	12.5	13.1	12.1
Unknown	3.4	8.6	6.2	5.5
Total %	100.0	100.0	100.0	100.0
Number of trees	76527	45238	22114	143879

However, there were considerable changes in the occurrence of the agent groups and specified agents over the years. The most notable of the changes was a heavy outbreak of *Gremmeniella* observed in 1988-89. Smaller peaks were observed in 1997 and 2001 (*Figure 1*). Apart from competition, *Gremmeniella* was the most important identified factors that had increased needle loss (defoliation) in Scots pine. Coarse temporal patterns of the most common causes of damage, including *Gremmeniella*, were obtained on the basis of the annual Level I data (*Figure 2*).



Figure 1. The annual occurrence of Gremmeniella abietina infection in Scots pine trees on the Level I plots in Finland. All and new infections are shown.

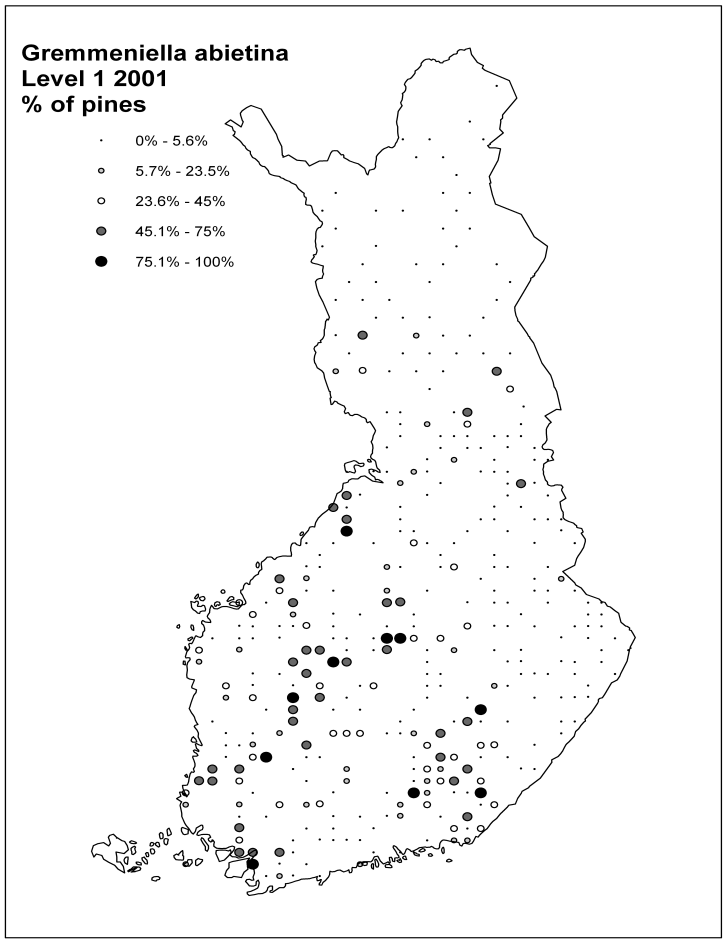
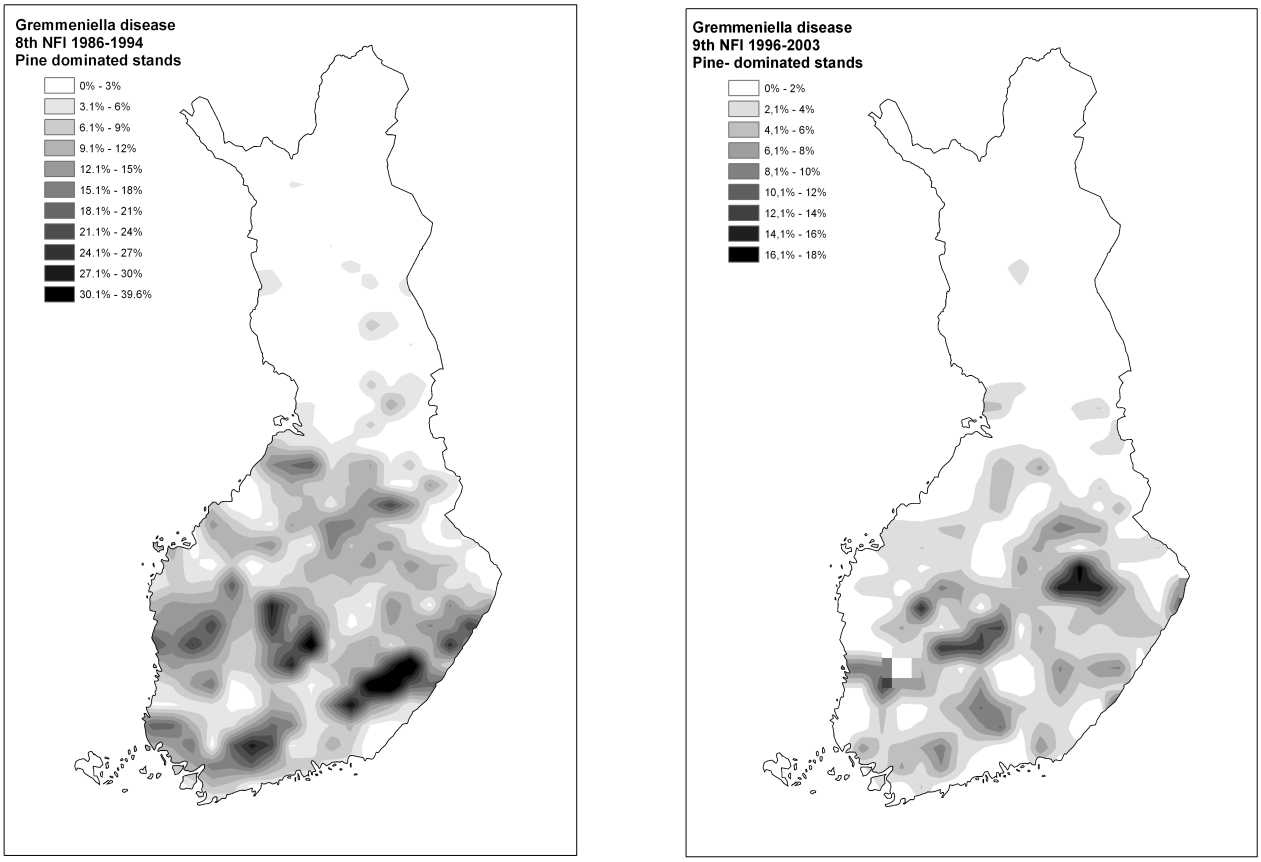


Figure 2. Example of the spatial (point) distributions of biotic damage on the Level I plots in Finland.: Gremmeniella abietina damage in 2001. The size and shading of the points shows the incidence of the disease (% of the pine trees in the plot).

It proved very difficult to model the changes in the disease occurrence in this data. In the regression models, in which two or more weather-variables explained the annual changes of the disease, the coefficients of the models were not coherent in differed parts of the country or on different years. For instance, the epidemics of 1988-89 were preceded by a rainy summer. This was however not true for the other peak years. One reason for the failure in modelling was that the annual changes were small on the average compared with the differences between the sample plots and to the number of the sample plots. The weather variables also correlated with each other. In the within years analysis, multiple regression models explained at most 10% of the differences between the changes of the disease of the sample plots in one year. Most of the weather variables were significant at least once, but the estimates of the effects were not coherent in different years. The same was true with modelling attempts using recursive partitioning or decision tree techniques (CART software): most of the variables were significant at least in some years, but the final decision tree was very complex containing 56 terminal nodes.

3.3 8th and 9th National Forest Inventories (1986-1994 and 1996-2003)

The National Forest Inventories showed that the disease was far more common in southern than in the northern parts of the country. The disease was also clearly spatially clustered (*Figure 3*).



A: B.

Figure 3. The spatial occurrence of Gremmeniella abietina in pine dominated plots in A) 8th National Forest Inventory 1986-1992 and B) B) 9th National Forest Inventory 1996-2003.

Maps were produced by kriging in ArcGis Spatial Analyst.

Most of the *Gremmeniella* damage observed in the NFIs was slight, i.e. did not decrease the silvicultural quality of the stand. The estimated area of Scots-pine dominated stands infected by *Gremmeniella* diminished in the 9th NFI (1996-2003) as compared to 8th NFI (1986- 1994. The proportion of diseased stands decreased from 6,8 % to 2,6 %, respectively. The diseased area had decreased by 4946 km^2. The standard errors of the estimated area infected by the disease and disease degrees were computed for the 9th NFI with the method presented by Matern (1960) (see also Ranneby 1981) (*Table 3*). Assuming that the sampling error is similar in the 8th NFI, the statistical significance and confidence intervals for the change in the estimated area between two inventories can be estimated. In the case of independent samples, the standard error of the change is $\sqrt{(s1^2+s2^2)}$, where s1 and s2 are the standard errors in the two inventories, respectively. The t- values were computed by dividing the difference in the area estimation with the standard error of change. All the p- values obtained for the change were all very significant, except for the disease degree 'complete' (*Table 4*).

Table 3. The occurrence of Gremmeniella abietina in two National Inventories (NFI's). N= number of pine-dominated plots, %= estimated percentage of pine-dominated stands, km^2 =estimated area of diseased stands.

Part of the country	Degree of *Gremmeniella* damage	8th NFI 1986-1994		9th NFI 1996-2004	
		%	km^2	%	km^2
Southern Finland	Slight	7,9	5178	3,9	2436
	Moderate	2,3	1538	1,0	612
	Severe	,2	138	,1	60
	Complete	,00	7	,0	9
	All damage	10,5	6881	4,9	3116
Northern Finland	Slight	2,0	1306	,6	446
	Moderate	,8	517	,3	236
	Severe	,1	66	,0	25
	Complete	0,0	0	,0	0
	All damage	2,9	1888	1,0	707
Whole country	Slight	5,0	6483	2,2	2882
	Moderate	1,6	2055	0,6	848
	Severe	,2	204	,1	84
	Complete	,0	27	,0	9
	All damage	6,8	8769	2,9	3823
Number of pine-dominated plots		37 243		39 049	

Table 4. Change in the estimated area of disease degrees in different parts of Finland, the sampling error and its statistical significange.

Part of the country	Disease degree	Change in the estimated area, km^2	Sampling error in 9th NFI	t- value	Significance of the change
Southern Finland	Slight	2742	94	20,628	,000
	Moderate	926	47	13,932	,000
	Severe	78	12	4,618	,000
	Complete	18	7	1,778	,075
	All damage	3765	109	24,426	,000
Northern Finland	Slight	859	47	12,924	,000
	Moderate	281	39	5,096	,000
	Severe	401	12	2,409	,016
	Complete	0	-	,000	,500
	All damage	1181	70	11,930	,000
Whole country	Slight	3601	61	41,746	,000
	Moderate	1207	105	8,129	,000
	Severe	120	17	5,002	,000
	Complete	18	7	1,778	,075
	All damage	4946	130	26,904	,000

Compared with level I data, it was evident, for instance, that the *Gremmeniella* epidemics in 1988-89 and 2001, was partly missed by NFI surveys, due to the fact that the inventory was being carried out in different parts of the country during the worst years.

4 DISCUSSION

The three sources of data used in this study complement each other quite well. The two yet unanswered questions for the research in future could be: Why the disease is more common in some years then in the others?. Why some stands are more susceptible than the others? This study at least failed to prove the assumption that epidemics usually begin after cold and rainy growing seasons (see Uotila 1988, for instance). The other question will be studied by applying risk models.

The weaknesses in the NFI- data can related to sampling error and detection error, in other words: i) spatial representativeness of field plots ii) reliability of the field survey, including observers ability to identify the causes and iii) epidemic nature of some damage. Identification of the causal agent is not an easy task in NFI field data collection because most of the damages are already old at the time of the observation and field work is done throughout the field season. The proportion of unidentified damage has remained at about the same level since the 8th NFI (see Yli-Kojola – Nevalainen 2006).

The field team leaders could reliably distinguish at least injuries caused by *Gremmeniella abietina* from other symptoms (Nevalainen 1999), but on the other hand, in the routine inventory slight infections are easily overlooked. However, the most important stand damage (in the economic sense) are recorded reliably in the routine NFI's.

National Forest Inventories are statistically representative samples of Finland's forests. The NFIs of Finland (before 2004) were regional inventories, i.e. the field work was carried out districtwise. The design of the 10th NFI, which started in 2004, was changed into a continuous inventory, i.e. field plots are measured throughout the whole country each year,

and results at the nation level can therefore be achieved annually or bi-annually. Also, a new network of permanent plots was established during the 9th NFI: every fourth cluster is marked as permanent, and these permanent plots will be reassessed in the 10th NFI.

The Level I network is not a representative sample of the forests in Finland, due to the rather sparse network (especially in northern Finland), as well as to the fact that the sample is restricted to dominant and co-dominant trees. The Level I network provides an annual picture of large-scale trends in crown condition (defoliation, discoloration, abiotic and biotic damage) at the European level. It also offers the possibility to investigate relationships between stress factors and forest condition. Although the most widespread epidemics are revealed in the level I network (seealso e.g. Nevalainen – Heinonen 2000), the forest health monitoring data is not suitable for the monitoring of the changes in the frequency of individual causes. There are also other sources of information of forest damage, such as questionnaires and voluntary reports among foresters and forest owners, but the results are very general. Therefore, despite of its shortcomings, the national forest inventory is the only statistically representative and extensive way to monitor the changes in the forest health in Finland.

In the future, a system for evaluation monitoring (see Smith et al. 2004, for instance) is urgently needed. When major changes or trends in forest health are detected, the extent, severity, and causes of undesirable changes should be determined, associations between forest health and forest stress indicators should be identified, and management consequences and alternatives for reducing the effects of forest stress should be defined.

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Gremmeniella abietina in North-western Spain: Distribution and Associated Mycoflora

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Abstract – *Gremmeniella abietina*, in its conidial state (*Brunchorstia pinea*), was recently reported in Palencia (north-west Spain) on *Pinus halepensis* in 1999. For that reason, the main aim of the present study was to determine the distribution of *G. abietina* in areas next to that where it was first recorded in order to evaluate there the current spread of the pathogen. Fungal mycoflora occurring in trees showing symptoms of *G. abietina* was also recorded with the goal of discussing the possible role it plays in the disease expression observed in the field. The isolation method consisted of finding fruitbodies on plant tissues after incubating them in wet and warm conditions. *G. abietina* was found in five out of a total of 40 stands that were examined. Furthermore, in another 25 stands, trees showing symptoms similar to those caused by *G. abietina* were also recorded. In addition to that, another 22 fungal species were isolated from plant fragments. *Thyriopsis halepensis*, *Sclerophoma pythiophila* and *Cenangium ferruginosum* were frequently isolated from injured plant fragments and were recovered from many stands (up to 70% of the total stands). These fungal species could play a role in the disease symptoms expression observed in the field, which were initially attributed exclusively to *G. abietina*. *Lophodermium pinastri*, *Naemacyclus niveus* and *Pestalotia stevensonii*, previously reported to be secondary pathogens on pine, were also occasionally recovered.

Brunchorstia pinea* / *Cenangium ferruginosum* / *Pinus halepensis* / *Sclerophoma pythiophila* / *Thyriopsis halepensis

Kivonat – A *Gremmeniella abietina* Északnyugat-Spanyolországban: elterjedése és a kapcsolódó mikoflóra. A *Gremmeniella abietina* konídiumos alakját (*Brunchorstia pinea*) Palencia-ban (Északnyugat-Spanyolország) *Pinus halepensis*-en jelezték először 1999-ben. Jelen tanulmány fő célkitűzése a *G. abietina* előfordulásának meghatározása az első megtaláláshoz közeli területeken, a kórokozó jelenlegi elterjedésének felmérése érdekében. Felvettük a *G. abietina* tüneteit mutató fák mikoflóráját is, a betegség terepi megnyilvánulásában játszott esetleges szerepének megismerése érdekében. A kitenyésztést nedves és meleg körülmények közötti inkubáció során a szövetekben kifejlődő termőtestekből végeztük. A *G. abietina* a vizsgált 40 állomány közül ötben fordult elő. További 25 állományban a *G. abietina* tüneteihez hasonlókat találtunk. A növényi részekből 22 gombafajt tenyésztettünk ki. A *Thyriopsis halepensis*, *Sclerophoma pythiophila* és *Cenangium ferruginosum* fajokat gyakran izoláltuk károsodott növényi részekből (az összes állomány 70%-ában). Ezek a gombafajok részt vehetnek az eredetileg csak a *G. abietina*-nak tulajdonított terepi tünetek kialakulásában. A korábban a *Pinus*-ok másodlagos kórokozóiként ismert *Lophodermium pinastri*, *Naemacyclus niveus* és *Pestalotia stevensonii* fajokat is alkalmanként megtaláltuk.

Brunchorstia pinea* / *Cenangium ferruginosum* / *Pinus halepensis* / *Sclerophoma pythiophila* / *Thyriopsis halepensis

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1 INTRODUCTION

Gremmeniella abietina (anamorph *Brunchorstia pinea*) is an ascomycete fungus that causes stem canker and shoot blight on many conifer species (Donaubauer 1972). The pathogen has been responsible for the destruction of many plantations in North and Central Europe, North America and East Asia in recent decades (Yokota et al. 1974, Dorworth 1979, Kaitera – Jalkanen 1992, 1994). In Spain *G. abietina* was first reported on *Pinus pinaster* causing seedling mortality in 1929 (Martinez 1933). It was not recorded again until 1999, when dieback caused by *G. abietina* was seen on *Pinus halepensis* (Santamaría et al. 2003), tree species that is currently undergoing a severe decline in Spain.

Since then, several studies about this fungus have been conducted in Spain: in both physiological and morphological experiments (Santamaría et al. 2004), it was determined that Spanish isolates of *G. abietina* developed well on malt agar added with pine needle extract at 15 ºC. The length and the width of those isolates ranged between 10.7-44.8 μm and 1.5-4.4 μm, and most of them had 3 septa. The results also suggested that the isolates from Spain do not belong to the Alpine biotype, and the disease symptoms caused by these isolates resembled those of the European biotype. The isolates from Spain were also genetically characterized (Santamaría et al. 2005) using RAPD markers and comparison of RAPD patterns for Spanish isolates and those originating from different regions of Europe and North America. The results showed that the Spanish isolates represent the European race of *Gremmeniella abietina* var. *abietina* and not the Alpine or Northern biotype. Spanish isolates appeared to be clearly separated from all other biotypes within the EU race and preliminary data suggested that Spanish isolates have low genetic variability.

In inoculation tests (Santamaría et al. 2006) performed on the more representative conifer species from Spain, Spanish isolates of *G. abietina* were shown to be pathogen on seedlings of all the pine species tested. *P. halepensis* were consistently the most susceptible one, although it is important to take into account that all the isolates used in the experiments were isolated from *P. halepensis*, suggesting a certain host specificity of *G. abietina*. The susceptibility of the other pine species was regarded with the age of the seedlings. In a preliminary study, it was observed that fungi *Sclerophoma pythiophila* and *Cenangium ferruginosum* were frequently recovered from diseased twigs by *G. abietina*; then, it was decided to study the interactions between *Gremmeniella abietina* and both fungi (Santamaría et al. 2007) in order to evaluate the role that each fungus plays in the symptom expression observed in the field. The results of that study suggested that, even though it can be considered as a weak pathogen, *S. pythiophila* might be involved in disease symptoms caused by *G. abietina* on pine trees in Spain, since it was able to cause damage on *P. halepensis* seedlings and, more importantly, it was able to increase the damage severity caused by *G. abietina* on plants. On the contrary, *C. ferruginosum*, which was not able to damage healthy seedlings, showed antagonism against *G. abietina*.

Finally, several control strategies, including fungicides, fungal endophytes and fungal filtrates, were evaluated *in vitro* (Santamaría et al. in press) as a first step to develop a management control programme against the pathogen before a potential spread of the disease. In that study, chlorothalonil and chlorothalonil-carbendazim would be the most suitable fungicides at low doses to reduce growth of *G. abietina* isolates from Spain. Results also indicated that four of the endophytes tested *in vitro* showed a strong antagonistic activity against *G. abietina* which deserve further testing *in vivo*.

Since the pathogen was observed in 1999 in Palencia (North-west Spain), new records of *G. abietina* have not been made in another areas of Spain yet, therefore the actual distribution of the pathogen in Spain is unknown. In that way, to evaluate the actual spread level of the pathogen, the main aim of the present study was to determine the distribution of *G. abietina* in areas next to that where it was first recorded. Fungi occurring in plant fragments collected

from trees showing symptoms of *G. abietina* was also recorded with the goal of discussing the possible influence they could play in the disease expression observed in the field.

2 MATERIAL AND METHODS

2.1 Sampling

Samples were collected at forty *P. halepensis* stands in the north-west of Spain at altitudes between 800-900 m in transitional areas, where both evergreen sclerophyll broad-leaf and coniferous forest occur within the temperate zone, and the soil is less than 50 cm thick containing limestone with basic pH (> 7). Both hot and dry summers, and a lot of frost days in winter (about 60 per year) are common, but snow is rare in these areas. The localization, altitude and some topographic characteristics of the forty sampling stands are given in *Table 1*. Stands were sampled twice, in spring and summer 2001, and samples were collected from a total of 160 trees (4 trees per stand). Within each stand, trees were chosen among those showing typical symptoms of *G. abietina*: drying up of needles and branches with some distortion of terminal twigs, and dieback. From each tree, 2- to 3-yr-old recently diseased twigs, located at 3-4 m above the ground, were collected from the periphery of the canopy. The samples were brought to the laboratory, stored at 4ºC and processed within 24 h.

2.2 Fungal isolation and identification

From each tree, six twig segments (0.5 cm diam., 0.5-1 cm thick, including bark) and six needles were randomly selected and processed according to the moist chamber method, as it has been suggested in previous mycoflora studies (Santamaría - Diez 2005). The method consisted of finding fruitbodies on plant tissues (twigs and needles) after incubating them, within Petri dishes with wet paper, at room temperature (22ºC ± 2ºC) in diffused daylight until fruitbody production. The samples used in this method were not surface sterilised in order to find endophytes as well as fungal epiphytes. Cultures were identified according to morphological characteristics.

2.3 Statistical data analyses

The comparison between the species distribution of each sampling stand was made using a cluster analysis, with the statistical package STATISTICA´ 99, STATSOFT®, Ink. (Tulsa, OK. USA). To construct the dendogram, levels of similarity between the stands were calculated by using Dice coefficient (Dice 1945) and the cluster analysis of similarity matrices was calculated with the unweighted pairgroup method with arithmetic averages (UPGMA). For the comparison among the fungal species recovered in each stand a correspondence analysis was applied by means of 'corresp procedure' of SAS statistical package (Anonymous 1989).

3 RESULTS

The fungal species isolated from *Pinus halepensis*, as well as the stands where they were recovered, are shown in Table 2. *G. abietina*, in its anamorphic state *Brunchorstia pinea*, was recorded in five out of a total of 40 stands that were examined, although in another 25 stands, similar symptoms to those caused by *G. abietina* were observed (*Table 1*). In addition to that, another 22 fungal species were isolated from plant fragments. From the total, four taxa, *Thyriopsis halepensis*, *Sclerophoma pythiophila*, *Alternaria* sp. and *Cenangium ferruginosum* were very frequently isolated (in more than 70% of the stands) whilst seven fungal species,

Dichomera sp., *Fusarium* sp., *Hendersonia acicola*, *Hysterographium elongatum*, *Pithomyces chartarum*, *Rhizopus stolonifer*, and *Trichoderma viride* (*Table 2*) were isolated in a quite low frequency (lower than 10% of the stands). Another species, like *Cladosporium* sp., *Lophodermium pinastri* (it was recorded in both teleomorphic and anamorphic state *Leptostroma pinastri*) and *Trichothecium roseum*, were also frequently isolated.

Table 1. Sampling stands location and description

Stand	Location	***UTM coordinates***		***Altitude (m)***	Slope	Cardinal direction	*G. abietina* symptoms
		X	Y				
00-VAL	Valle del Cerrato	4640475	386450	880	high	South	20
01-MEL	Melgar de Yuso	4676700	393775	850	medium	West	5
02-SAN	Santoyo	4674300	390450	810	medium	North	15
03-AST	Astudillo	4673075	398625	790	high	West	5
04-AST	Astudillo	4670250	391025	810	medium	South-East	0
05-AST	Astudillo	4668950	392100	860	high	East	5
06-AST	Astudillo	4671775	393550	818	high	North	15
07-PAL	Palencia	4655800	373500	810	medium	South- West	15
08-PAL	Palencia	4649575	368725	825	high	South-East	5
09-AST	Astudillo	4647465	390225	845	medium	North-East	10
10-AMU	Amusco	4665625	381900	860	medium	South-East	0
13-VIL	Villamediana	4655075	385075	840	Low	South-West	0
14-VAL	Valdeolmillos	4655925	382975	825	medium	West	10
15-VLB	Villalobón	4653775	377825	835	high	West	15
16-TOR	Torquemada	4653525	397425	820	high	South-West	0
18-VLH	Villahán	4657800	406650	825	medium	West	10
19-TAB	Tabanera de Cerrato	4652625	409675	855	high	South	10
20-VLM	Villamuriel de Cerrato	4647375	372175	856	high	East	0
21-TOR	Torremormojón	4644175	352700	820	high	West	10
22-AMP	Ampudia	4643425	356100	845	high	West	15
23-VLM	Villamuriel de Cerrato	4642700	371075	865	flat	North-East	0
24-AMP	Ampudia	4642475	354650	830	high	North-East	15
27-REI	Reinoso de Cerrato	4647725	385750	824	high	North-West	5
28-BAL	Baltanás	4646850	395275	874	high	North-East	15
29-BAL	Baltanás	4643075	398075	820	high	South-West	10
30-TAR	Tariego de Cerrato	4638675	379600	861	high	South-West	0
31-CEV	Cevico de la Torre	4637375	386475	835	high	South	0
32-VAL	Valle del Cerrato	4638450	388075	845	medium	South	5
33-CAS	Castrillo de Onielo	4635825	393100	885	high	South-West	0
34-VER	Vertavillo	4633650	389175	800	low	North-East	15
35-TAB	Tabanera de Cerrato	4649450	406325	887	high	South-West	20
36-CUB	Cubillas de Cerrato	4630850	378225	840	high	South-West	15
37-POB	Población de Cerrato	4630475	382125	857	high	South	15
38-ALB	Alba de Cerrato	4628600	387675	838	high	South-West	5
40-VER	Vertavillo	4630750	397100	830	high	South	15
41-HER	Hérmedes de Cerrato	4631200	399325	860	high	South	5
42-POB	Población de Cerrato	4626675	381825	835	high	South-West	10
44-HER	Hérmedes de Cerrato	4630775	403225	880	high	South	0
45-CAS	Castrillo de Don Juan	4627575	410800	905	high	South-West	20
out1	Hontoria de Cerrato	4639825	382900	790	high	South-West	15

Stand.- Code to designate each stand.
Location.- Village the stand is located in.
UTM coordinates.- Universal Transverse Mercator, UTM, coordinates (in meters).
A.- Altitude, in meters above sea level, of each sampling site.
G. abietina symptoms.- Approx. percentage of the total stand area showing symptoms similar to those caused by *G. abietina* (drying up of needles and branches with some distortion of terminal twigs, and dieback).

In all the stands where *G. abietina* was recovered, three species, *T. halepensis*, *S. pythiophila* and *C. ferruginosum*, were consistently isolated too (*Table 2*). This fact was confirmed by the correspondence analysis (*Figure 1*). The plot representing the first two dimensions of the model (which explain 17.03% and 12.36% respectively of the total

variance), showed the scores corresponding to those four species (*G. abietina*, *T. halepensis*, *S. pythiophila* and *C. ferruginosum*) to be very related in some way, since all of them were found in the negative quadrant of both dimensions.

Table 2. Fungal species recovered from twigs and needles of Pinus halepensis *and stands where they occurred.*

Fungi	N[1]	Stands where fungus was recovered
Alternaria complex.	34	01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 06-AST, 07-PAL, 08-PAL, 10-AMU, 13-VIL, 14-VAL, 15-VLB, 18-VLH, 20-VLM, 21-TOR, 22-AMP, 24-AMP, 27-REI, 28-BAL, 29-BAL, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 40-VER, 41-HER, 42-POB, 45-CAS, OUT1
Brunchorstia pinea (Karst.) Höhn.	5	00-VAL, 09-AST, 15-VLB, 24-AMP, 41-HER,
Camarosporium propinquum (Sacc.) Sacc.	10	01-MEL, 07-PAL, 14-VAL, 20-VLM, 21-TOR, 24-AMP, 28-BAL, 34-VER, 36-CUB, 37-POB.
Cenangium ferruginosum Fr.: Fr.	28	00-VAL, 01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 07-PAL, 08-PAL, 09-AST, 10-AMU, 14-VAL, 15-VLB, 18-VLH, 20-VLM, 21-TOR, 24-AMP, 28-BAL, 29-BAL, 31-CEV, 32-VAL, 34-VER, 35-TAB, 36-CUB, 37-POB, 40-VER, 41-HER, 45-CAS, OUT1
Cladosporium sp.	19	01-MEL, 02-SAN, 03-AST, 06-AST, 07-PAL, 14-VAL, 15-VLB, 18-VLH, 20-VLM, 21-TOR, 22-AMP, 24-AMP, 27-REI, 32-VAL, 34-VER, 36-CUB, 37-POB, 38-ALB, 42-POB
Cytospora sp.	14	00-VAL, 03-AST, 06-AST, 15-VLB, 18-VLH, 20-VLM, 28-BAL, 31-CEV, 32-VAL, 33-CAS, 36-CUB, 37-POB, 38-ALB, 42-POB
Dichomera sp.	2	13-VIL, 15-VLB
Epicoccum nigrum Link	5	01-MEL, 14-VAL, 24-AMP, 36-CUB, 37-POB
Fusarium sp.	1	14-VAL
Gonatobotrys sp.	9	04-AST, 06-AST, 13-VIL, 14-VAL, 27-REI, 32-VAL, 36-CUB, 37-POB, 42-POB
Hendersonia acicola Münch et Tub.	4	00-VAL, 01-MEL, 02-SAN, 04-AST
Hysterographium elongatum (Wahl.) Corda	2	13-VIL, 38-ALB,
Leptostroma pinastri (Desm.)	19	00-VAL, 02-SAN, 03-AST, 05-AST, 06-AST, 13-VIL, 14-VAL, 18-VLH, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 42-POB, OUT1
Lophodermium pinastri (Schard. ex Hook.) Chev.	19	00-VAL, 02-SAN, 03-AST, 05-AST, 06-AST, 13-VIL, 14-VAL, 18-VLH, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 42-POB, OUT1
Naemacyclus niveus (Pers. ex Fr.) Fuck. ex Sacc.	5	03-AST, 14-VAL, 15-VLB, 35-TAB, 36-CUB
Penicillium sp.	14	00-VAL, 01-MEL, 02-SAN, 03-AST, 05-AST, 06-AST, 10-AMU, 15-VLB, 30-TAR, 31-CEV, 32-VAL, 34-VER, 38-ALB, 45-CAS
Pestalotia stevensonii Peck	5	00-VAL, 03-AST, 06-AST, 13-VIL, 32-VAL
Pithomyces chartarum (Berk. & Curt) M. B. Ellis	1	06-AST
Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.	1	22-AMP
Sclerophoma pythiophila (Corda)	35	00-VAL, 01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 06-AST, 07-PAL, 09-AST, 10-AMU, 13-VIL, 14-VAL, 15-VLB, 18-VLH, 19-TAB, 21-TOR, 22-AMP, 24-AMP, 27-REI, 28-BAL, 29-BAL, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 40-VER, 41-HER, 42-POB, 45-CAS, OUT1
Thyriopsis halepensis (Ck.) Theiss y Syd.	40	00-VAL, 01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 06-AST, 07-PAL, 08-PAL, 09-AST, 10-AMU, 13-VIL, 14-VAL, 15-VLB, 16-TOR, 18-VLH, 19-TAB, 20-VLM, 21-TOR, 22-AMP, 23-VLM, 24-AMP, 27-REI, 28-BAL, 29-BAL, 30-TAR, 31-CEV, 32-VAL, 33-CAS, 34-VER, 35-TAB, 36-CUB, 37-POB, 38-ALB, 40-VER, 41-HER, 42-POB, 44-HER, 45-CAS, OUT1
Trichoderma viride Pers.: Fr.	3	36-CUB, 37-POB, 38-ALB
Trichothecium roseum (Pers.: Fr.) Link	20	00-VAL, 01-MEL, 02-SAN, 03-AST, 04-AST, 05-AST, 06-AST, 08-PAL, 10-AMU, 13-VIL, 15-VLB, 18-VLH, 22-AMP, 27-REI, 32-VAL, 34-VER, 36-CUB, 37-POB, 38-ALB, 42-POB

[1] - Number of stands where the fungus was recovered

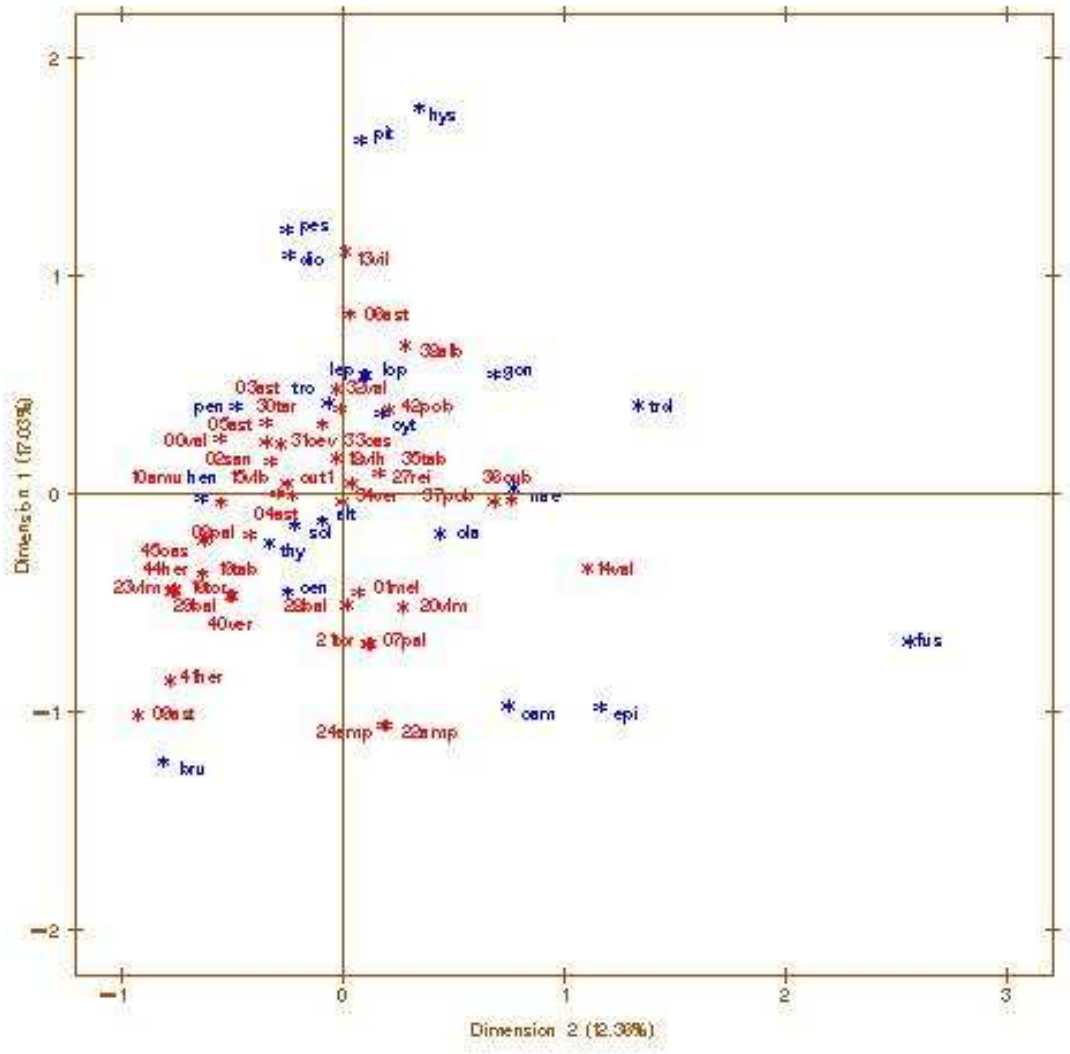


Figure 1. Plot of the first and second dimension scores from a correspondence analysis based on species distribution from each stand.
Fungi are designed in the plot as the three first letters of the species latin name

The number of fungal species recovered per stand ranged between 1 (in 16-TOR, 23-VLM and 44-HER) and 14 (in 36-CUB stand). In 32.5% of the total stands were recovered more than nine fungal species, whilst in 20% only were found less than five taxa. The stands, where a lower number of species was recovered, corresponded to those showing good forest health and grouped together in the cluster analysis (*Figure 2*). Only *T. halepensis* and *S. pythiophila* were found in such stands. However, in general terms, no correlation was found between species richness and cluster grouping, as well as no correlation was observed between topographic conditions of the stands and the fungal species distribution recovered. The stands where *G. abietina* was recovered are not very related among them with regard species distribution, as it is shown in dendrogram (*Figure 2*). Nevertheless, in the correspondence analysis, scores corresponding those stands were located in the negative part of the dimension 2.

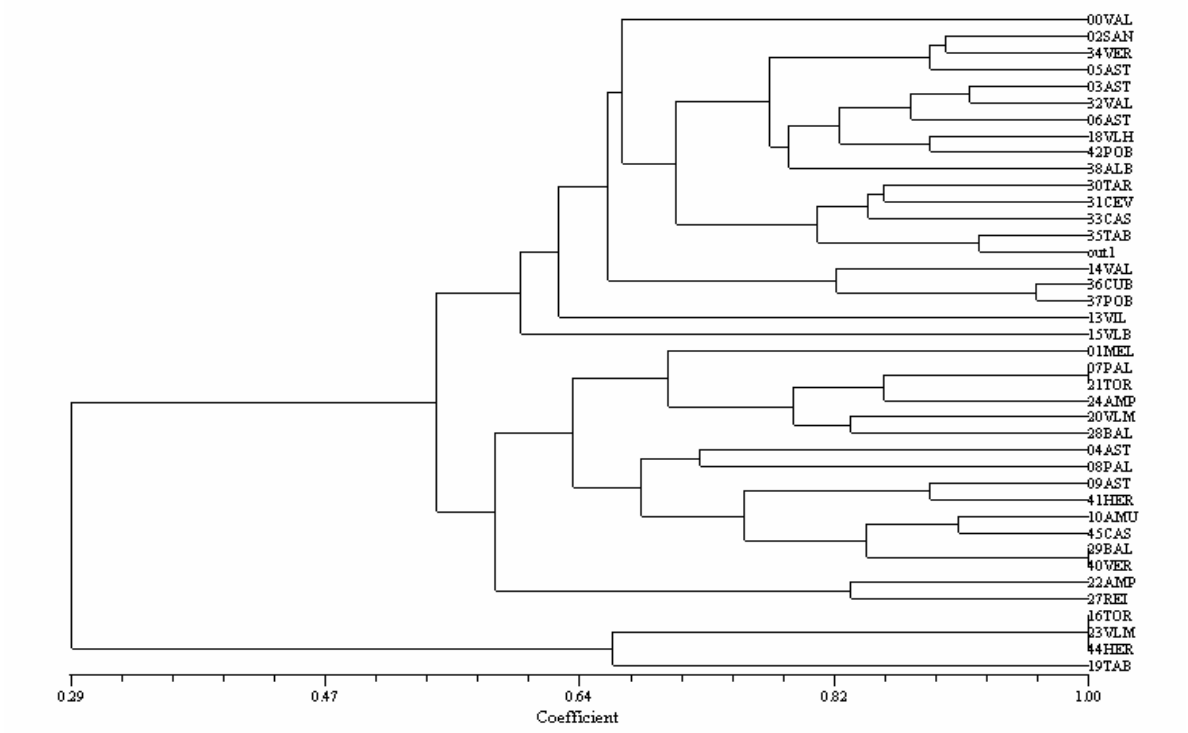


Figure 2. Dendrogram showing the similarity among the stands regarding to the species distribution

4 DISCUSSION

Thirty stands showed symptoms similar to those described in previous works for the European race of *G. abietina* (Uotila 1983, 1993, Virtanen et al. 1997, Santamaría et al. 2006); however, *G. abietina* only was recovered from five out of these 30 stands. To explain this fact, two possibilities are suggested: the first one might consist of that *G. abietina* was indeed the primary pathogen that caused the disease observed in the field, but later, either secondary pathogens or opportunist fungi colonized the necrotic tissues making it difficult to isolate the primary agent. The other possibility might be that symptoms observed in the field were not as specific to *G. abietina* as initially thought and they were caused by another fungal pathogen alone or by a combination of several fungi (including *G. abietina*).

In this sense, the only fungi recovered in almost all of the stands showing those symptoms were *T. halepensis*, *S. pythiophila*, *Alternaria* and *C. ferruginosum*. Among those, *Alternaria* complex is known as saprobe and ubiquitous; therefore, it is not very probably its implication in the symptoms expression. *T. halepensis* has previously shown a pathogenic behaviour on pine needles (Muñoz et al. 2003), so it could play a certain role on the defoliation observed in diseased trees. The other two fungi, *S. pythiophila* and *C. ferruginosum*, have been shown to be generally weak pathogens on several conifers (Brener et al. 1974, Phillips – Burdekin 1992), although *C. ferruginosum* has also been reported to cause severe damage on pine species (Koiwa et al. 1997). Furthermore, they have been found associated to *G. abietina* infections so frequently (Dorworth 1971, Barklund 1989, Duda – Sierota 1997) that *C. ferruginosum* was even thought to be the teleomorphic state of *Brunchorstia pinea* early last century (Dorworth 1971).

In addition to that, in a previous work (Santamaría et al. 2007), it was observed that *S. pythiophila* was able to cause damage on healthy seedlings but inoculated by wounding and, what is more, it was able to increase the damage severity caused by *G. abietina* on plants

of *P. halepensis*. This is in agreement with results obtained in the present study, as it is observed in the correspondence analysis plot where scores, representing these fungi, are grouped together. Therefore, these four fungi, *T. halepensis*, *S. pythiophila*, *G. abietina* and *C. ferruginosum*, could be involved in some way in disease symptoms observed in the field and initially attributed exclusively to *G. abietina.*

From the rest of fungal species listed in *Table 2*, several of them are well-known pine pathogens and therefore they could cause damage on *P. halepensis* if climatic conditions turn adverse to the plant and increase their populations. *Lophodermium pinastri*, which has been previously reported as a pine needle pathogen (Lanier et al. 1978, Phillips – Burdekin 1992), was frequently isolated in many stands. *Naemacyclus niveus* and *Pestalotia stevensonii* have been shown to be secondary pathogen on pine needles (Lanier et al. 1978, Phillips – Burdekin 1992), although, they were isolated at a low level.

Differences in the species richness among stands could be explained by the different forest health conditions among them, as it has been widely stated by other authors (Petrini et al. 1989, Bettucci et al. 1999, Frohlich et al. 2000) that dead or dying tissues can be usually colonised by a highest number of fungal species than the healthy or slightly damaged ones. In addition to that, the effect that the local environmental conditions have on the species richness, has been already stated on diverse tree species (Elamo et al. 1999, Ragazzi et al. 2003).

5 CONCLUSION

In conclusion, *Thyriopsis halepensis*, *Sclerophoma pythiophila* and *Cenangium ferruginosum*, which were very frequently recovered from almost all the stands, could be involved, in adittion to *G. abietina*, in the disease symptoms expression observed in the field, which were initially attributed exclusively to *G. abietina*.

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How Do the Epidemics of *Gremmeniella abietina* Start?

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Abstract – *Gremmeniella abietina* type A caused two widespread epidemics in Nordic countries: in 1982 in Finland and in 2001 in Sweden. The reasons for these epidemics are discussed based on the inoculation experiments in Finland and the literature. The fungus has been inoculated by putting a piece of mycelium in the phloem or by spraying the conidia or ascospores on pine shoots. Mycelial inoculations cause cankers in dormant period inoculations, but not in summer inoculations. During the dormant period, pine cannot actively defend against the fungus. Spore inoculations are successful in summer, which is also the natural spreading time of the fungus. The reason for infection seems to be poor structural resistance in infected shoots. Firstly the fungus infects the bract and during the dormant period it grows to the phloem through poorly developed cork layers between the dead bract and living phloem. A serious epidemic needs a rainy and cloudy summer and also the same kind of summer two years before. A mild winter enhances the growth in cankers, but a mild winter alone cannot cause the epidemics. *Gremmeniella abietina* damage is controlled by using local or a little bit of northern provenances.

inoculation/ Finland/ Sweden/ bract

Kivonat – Hogyan indulnak a *Gremmeniella abietina* járványok? A *Gremmeniella abietina* A típusa két nagykiterjedésű járványt okozott az északi országokban: 1982-ben Finnországban és 2001-ben Svédországban. Finnországi inokulációs kísérletek és a szakirodalom alapján elemezzük a járványok okait. A mesterséges fertőzéseket micelium darabkák háncsba helyezésével, illetve konídium, vagy aszkospóra szuszpenzió hajtásokra történő permetezésével végeztük. Miceliummal a nyugalmi időszakban végzett fertőzések nekrózist okoztak, de a nyári fetőzések nem. A nyugalmi időszakban a fa nem képes aktívan védekezni a gomba ellen. A spórával végzett fertőzések nyáron sikeresek, ami a gomba természetes terejdésének időszaka is. A fertőzés sikere a megfertőzött hajtások gyenge szerkezeti ellenállásával magyarázható. Kezdetben a gomba a fedőpikkelyeket fertőzi, majd a nyugalmi időszakban a gyengén fejlett kéregrétegeken keresztül az elhalt fedőpikkelyek és az élő háncs között behatol a háncsba. Egy komoly járvány esős és felhős nyarat igényel, és ugyanilyen nyarat az előző két évben is. Az enyhe tél elősegíti a nekrózisok növekedését, de egymagában nem okoz járványt. A *Gremmeniella abietina* okozta károk ellen a helyi, vagy kissé északibb származások alkalmazásával védekezünk.

Inokuláció / Finnország / Svédország / fedőpikkely

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1 INTRODUCTION

Gremmeniella abietina (Morelet) type A has caused serious damage in pole stage and bigger *Pinus sylvestris* L. trees. In Finland the worst epidemic was in 1982 and in Sweden in 2001 (Uotila 1988, Wulff et al. 2006). The previous weather conditions have been assumed to the main causes of *Gremmeniella* epidemics (Uotila 1988). The conidia and ascospores spread in the summer and so the rainy summer enhances the infections (Petäistö – Heinonen 2003, Nevalainen 1986, Uotila 1985). Also the spores germinate only in moist conditions (Dorworth 1972). It is obvious that the resistance of the diseased shoots has been weakened. In provenance experiments, southern origins suffer more than local and northern provenances (Uotila 1985). The summer frosts and shading are also important factors affecting epidemics (Petäistö – Repo 1990, Petäistö & Kurkela 1993, Read 1968, Uotila 1988, Sairanen 1990). The life cycle of the fungus lasts two years (Uotila 1985, Hellgren – Barklund 1992), so it is enough for a fungus to have good conditions for infections every second year. The infection happens after a latent period probably through the stomata of bracts on the base of needle fascicles (Patton et al. 1984). The same kind of infection process has been described on *Diplodia pinea* (Flowers et. al. 2006). After a first year infection the necrophylactic periderm can protect the surrounding phloem tissues from canker spreading. *Gremmeniella* infection needs enough so-called conducive days during the dormant period (temperature +5°C – -5°C) (Marosy et al. 1989). In Finland and Sweden we have enough conducive days every year and so the number of conducive days or dormant period weather cannot fully explain the variation in yearly disease level. Here we will synthesise the factors affecting the *Gremmeniella* epidemics based on the results of several inoculation experiments and the literature.

2 INOCULATION EXPERIMENTS

Several inoculation experiments have been done in Finland (Kurkela –& Norokorpi 1979, Petäistö – Kurkela 1993, Petäistö 1999, Petäistö and Laine 1999, Petäistö 1995, Petäistö et al. 2005, Uotila 1983, 1990, 1991). Type A and B isolates have been used in these inoculations. Most often the type is known. The mycelium inoculations in phloem have been done over the year (*Figure 1*). So we know that *Gremmeniella* mycelium causes the canker always if it is inoculated during the dormant period. In August type A cause more often cankers than type B, which is showing that type A is more aggressive pathogen (Terho – Uotila 1999). In the summer the pine can resist mycelial inoculations in phloem and no big cankers are formed. The biggest cankers are formed in October inoculations (Uotila 1990). This is logical because the fungus has then more time to grow without the active defence of the tree. In spring it seems that the defence activities begin in April, so the canker is mainly grown in late autumn and early spring. The mycelium can grow slowly, when the temperature is below zero. At zero degrees, the growth is enough to cause serious cankers (Petäistö 1993). In spore inoculation experiments the delayed start of growth in the spring has increased infections (Petäistö – Laine 1999).

The spore inoculations with conidia or ascospores have been made over the year in spite of the period from January to April. It is interesting that these inoculations have been successful in the same time as the spores are spreading in nature (*Figure 1*). The successful period of spore inoculations is just opposite than that of the mycelial inoculations. At first this sounds confusing, but this fact gives a good opportunity to understand epidemics. The infection happens in the cases when the pine has not developed structural resistance against the fungus, which is waiting latent in the bract. This sounds too simple. We need find more facts to support this theory. The first-year nursery seedlings are most susceptible to spore inoculations during late summer simultaneously with bud development (Petäistö 1999, Petäistö 2005). This difference is noticed in container

seedlings and in bareroot seedlings. The needles of these first-year seedlings are primary needles. There is no structural resistance between the primary needle and the shoot. Primary needles are not developed as needles after the first summer, but they form a bract at the base of the needle fascicle. The bract initials are already in the bud in the first growing season. Older seedlings have been more susceptible to early summer inoculations. The infection of these seedlings probably happens via bracts (Patton et al. 1985) or via the scales of the long shoot (Siepmann 1976). Why does not the infection happen via the base of the needles? Is latent infection possible in the needles? In diseased nursery seedlings pycnidia are common on primary needles. In nature pycnidia are not common in the needles. The reason for this could be that the diseased needles drop down before pycnidia develop or that the infection really occurs via the bracts and the *Gremmeniella* mycelium is not so much grown in the needles. The typical first symptom of *Gremmeniella* infection is that the needle bases turn brown in the spring. This happens only in those needles which are connected to infected phloem. The tip of needle is still green which probably means that the fungus has not originally penetrated into the needle.

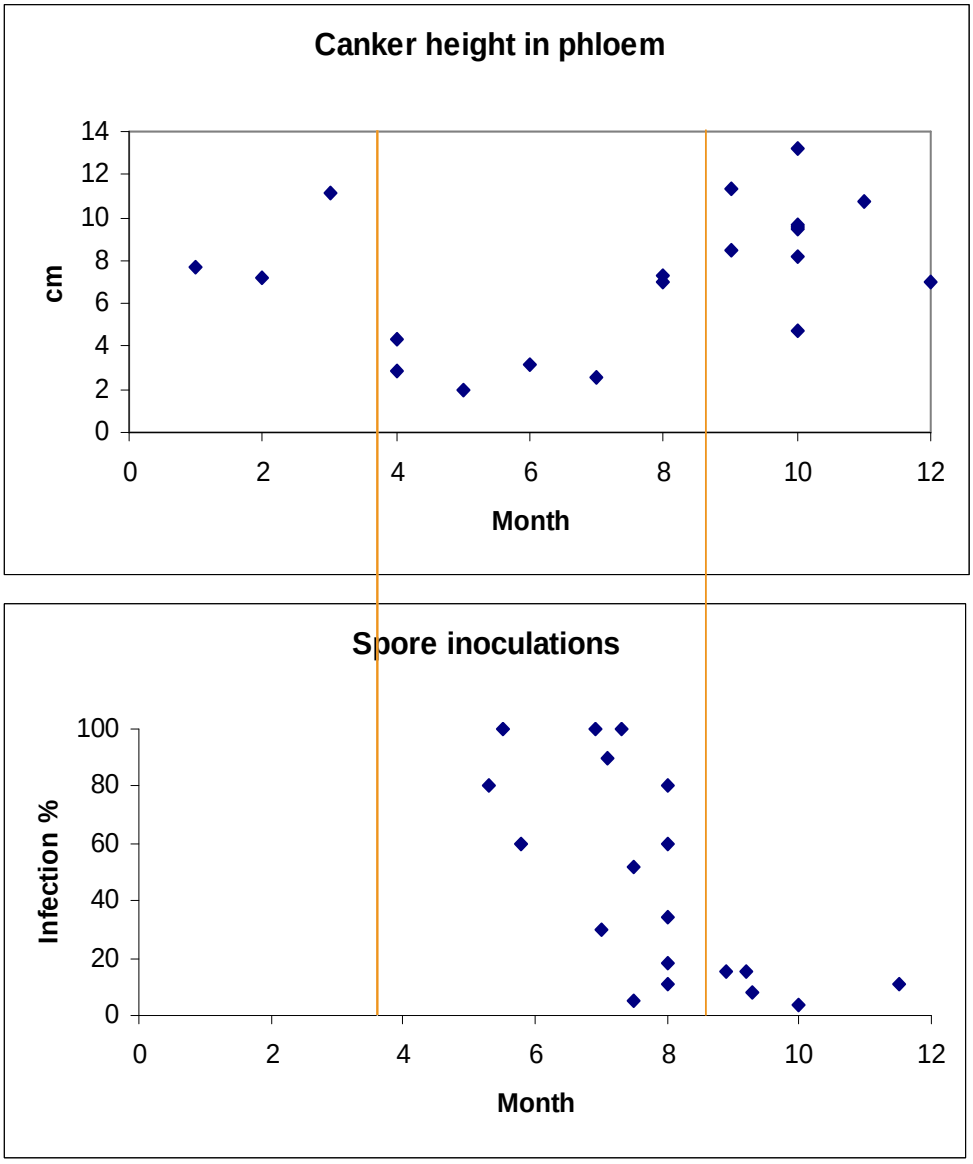


Figure 1. The shown results originate from several experiments in different years by the authors. In the upper picture the results of mycelium inoculations in phloem are shown and in lower picture are the results of spore inoculations sprayed on shoots

3 WEATHER CONDITIONS

The following weather conditions were common for major Nordic epidemics: a big rainfall during the previous summer and low solar radiation during the growing season. The winter of 1981-1982 in Finland was cold and the winter of 2000-2001 in Sweden was mild. So we can conclude that the epidemics can occur in spite of the winter conditions. The damage in Sweden was very severe and it is possible that the fungus causes larger cankers during a mild winter compared to the cankers in a cold winter. The winter conditions are not the main factors, because *Gremmeniella* spores spread in summer and the structural resistance of pine is developed during the summer. Type B Gremmeniella damage is a little bit different because type B Gremmeniella typically grows in perennial cankers below the snow.

In winter storage experiments the seedlings have been infected even more in cold winter temperatures (Petäistö – Laine 1999). The cold winter temperatures delay the start of growth in spring and so the fungus has time to invade the host tissues. This corresponds with the late appearance of visible symptoms.

Single stands were destroyed in the areas where most stands were not severely infected. This is clearly caused by the seed origin coming from too southern conditions or nitrogen fertilisation (Kallio et al. 1985, Aalto-Kallonen – Kurkela 1985). In both epidemics the fungus was present almost everywhere in the lower branches and the pine understory. So the inoculum was everywhere, but the surviving shoots had developed their structural resistance to the disease.

4 CONCLUSIONS

The main cause of *Gremmeniella* epidemics is the rainy weather during the summer in conditions where there is a big inoculum potential due an epidemic two years before. If the rainy season occurs just occurs at the same time as shoot lengthening the *Gremmeniella* infections will increase. Late summer frosts increase also the pine susceptibility. We can control the future damage by using local or north to south transferred provenances. The altitude is also important. A new risk is that foresters react to global warming by planting southern provenances since these provenances are still susceptible to *Gremmeniella abietina.*

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Canker diseases

Environmental Condition and Cypress Canker Disease

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Abstract – Cypress canker by *Seiridium cardinale* (Wag.) Sutt. & Gibson is a disease particularly harmful for cypress trees in Tuscany where the landscape value of this species is very important. The paper reports on the relationship among *S. cardinale* and environmental factors that may influence the spreading of the fungus using GIS technology. The study conduced in the neighbourhood of Florence recording the occurrence of the fungus on a sample of about 6900 trees, demonstrate that the damage by *S. cardinale* varied according to the environmental conditions of the area. The study showed that the fungus affects the trees under relatively high humidity and mild temperature that in Tuscany occur during the growing season and enhance Cypress susceptibility to the disease.

***Seiridium* / spreading / elevation / exposure / GIS**

Kivonat – Környezeti feltétel és a ciprusok kéregrákja. A ciprusok *Seiridium cardinale* (Wag.) Sutt. & Gibson okozta kéregrákja különösen ártalmas Toscana-ban, ahol e fafaj tájképi szerepének nagy jelentősége van. A dolgozat a *S. cardinale* gomba és a terjedését befolyásoló környezeti tényezők közötti kapcsolat vizsgálatát tartalmazza GIS technológia alkalmazásával. Firenze környékén, a gomba előfordulását mintegy 6900 faegyedet tartalmazó mintán felvevő tanulmány bizonyítja, hogy a *S. cardinale* okozta károk a terület környezeti feltételei szerint változnak. A vizsgálat kimutatta, hogy a gomba viszonylag magas nedvesség és enyhe hőmérséklet esetén támadja a fákat, e feltételek Toscana-ban a vegetációs idő alatt előfordulnak, és növelik a ciprusok fogékonyságát a betegségre.

***Seiridium* / terjedés / tengerszint fölötti magasság / kitettség / GIS**

1 INTRODUCTION

Cypress canker by *Seiridium cardinale* (Wag.) Sutt. & Gibson is a serious disease of *Cupressus sempervirens* that in Italy develops quite slowly and it is often related to environmental factors that may influence compartmentalization of infected tree tissue (Moriondo 1972, 1999). *S. cardinale* was recognised for the first time in 1927 in Monterrey, California on *Cupressus macrocarpa* and then it was spread in lots of part of the world (New Zealand, South America, Africa, France, Greece, Spain, Turkey and Portugal) probably by economical human activities (Moriondo 1972, 1999, Panconesi – Intini 1977, Panconesi – Ongaro 1982, Graniti 1998). In Tuscany *C. sempervirens* it's a very important landscape plant (about 17,500 ha) and it's a kind of symbol of this region. Its presence in the landscape and his health are two important goal for public administration that in the last ten years ago had financed and planned a Regional monitoring programme META (Monitoraggio Estensivo dei

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boschi della ToscanA a fini fitosanitari, www.arsia.toscana.it/meta) to observe the occurrence of some important forestry disease (ARSIA 2003).

S. cardinale is a mitosporic fungus responsible of the Cypress bark canker disease which generally spread by conidia transported by the wind or vectored by bark beetles as *Phloesinus spp.* and *Orsillus spp.* that favour infections of shoots and cones respectively (Tiberi – Battisti 1998, Masutti – Zangheri 2003).

It's possible to recognised infected trees by typical symptoms on the crown like: browning of the foliage, bark necrosis, resin flow around the infected area, dead of single branches. The disease develops quite slowly and thus most of the infected trees die after multiple infections or after root rot by opportunistic weak pathogenic fungi (Moriondo 1972, 1999).

2 MATERIALS AND METHODS

The goal of this study was to know the relationship between the occurrence of cypress canker disease and some environmental factors, following Tattar (1978), typical of Tuscany a region of central Italy, using GIS technology as tool to realize in the future a risk assessing map.
In the first step of this study it was necessary to realized i) a special collecting data form where all information about an infected plant were recorded and ii) a photo album, showing cypress trees having different intensity of infection to give a mark to every type of canker observed during the field work.

Surveys were conduced in the neighbourhood of Florence in Chianti's and Mugello's areas following national roads. The transect was about 60 km and South-North oriented and had a 100 m side buffer. A total of 6936 plants were collected and all data registered in a form composed by two parts: the first noted spatial data and the second canker disease data related to the photo album.
In the second step all data were put in a database software and analyzed by using software GIS (Geographical Information System) (Chirici et al. 2002, Chudamani et a. 2004, Godone et al. 2003, Maselli et al. 2003)

It was possible to realize some specific maps (TIN, slope, exposition, Bartorelli 1967) to study the relationship between cypress canker and some environmental factors that were supposed to have some influence about the occurrence and the spreading of the fungus (Van Staden 2003).

3 RESULTS AND DISCUSSION

Data analysed showed that quite often infections were more frequent in sites characterized by mild and wet periods during growing season and less frequent in sites having cold winters periods.

After the survey percent of infected tress were 3.2 in Chianti area (southern of Florence) and 2.3 in Mugello area (northern ward). Generally these plants were forming small groves. Most of cypress observed were adult trees showing old infections, only rarely (0.4%) it was possible to register new cankers recently developed. That would mean that the pandemic development of the disease as thought during the '90ties by Graniti, (1998) has been probably concluded.

Related to spatial data, cankers were most commonly found on sites where trees were supposed to have better growing conditions like: i) elevation about 400-500 m a.s.l. on South-East and South-West exposures but also 300 m on Northern sites, ii) annual precipitation higher than 700 mm and distributed all along the year.

4 CONCLUSION

As already reported by the literature under the Italian environmental circumstances Cypress trees have never dormant habit but on the contrary they growth as quiescent species related with photoperiodic conditions (Gellini – Grossoni, 2001). Therefore long and mild winter periods may enhance the susceptibility of trees to the disease (Moriondo 1972, 1999).

From this study we found that damages by *S. cardinale* occur mostly in those sites characterized by relatively high humidity and mild temperature during winter-spring periods corresponding to the stage of the most susceptible period for the host plants.

Considering that in previous studies the pathogen population resulted genetically uniform and no differences in virulence were found, we could conclude that the occurrence of the disease is strongly related with environmental and climatic conditions. Therefore GIS technology utilized in this study confirmed to be an important and useful tool for research in forest pathology.

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The Genetic Structure of Cypress Canker Fungus in Italy using RAPD and Minisatellite Markers

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Abstract – Over the past half century a destructive blight of *Cupressus* spp., caused by *Seiridium cardinale*, has spread worldwide from North America, devastating forests, plantations, and ornamental cypresses. The epidemic has been particularly severe in the Mediterranean region, on *C. sempervirens*. Seventy-seven isolates of *Seiridium cardinale* have been collected for the genetic characterization of the North-Italian populations of the fungus. Five *Seiridium* spp. isolates from different countries and different hosts growing in different parts of the world were used for comparison. The structure of the population has been analysed by means of Random Amplified Polymorphic DNAs (RAPDs) and Direct Amplification of Minisatellite-Region DNA (DAMD) PCR marker techniques by using the M13 core sequence. The results indicated a very high level of homogeneity in the North-Italian population of the fungus, whereas a certain variability was recognized in isolates from other hosts and other species. The isolates belonged to the North-Italian population appear to be very similar from the molecular comparison with both type of markers. The isolate from Greece was included in the same group of the Italian isolates. Only the *S. cardinale* from Chile was clustered at significant distance from the other *S. cardinale* isolates from Italy and Greece. The genetic homogeneity of the fungus in Italy suggests that this population has gone through a recent genetic bottleneck, perhaps from the introduction in Europe of few genotypes of the fungus. This supports the hypothesis that the pathogen was introduced to Europe during World War II on infected wood material from the United States. The results are discussed in relation to the introduction and spread of the fungus in Europe.

molecular markers / *Cupressus sempervirens* / *Seiridium cardinale* / population genetics / genetic variability / tree pathogen

Kivonat – A ciprusok kéregrákját okozó gomba genetikai struktúrája RAPD és miniszatellit markerekkel. A *Cupressus* fajokat pusztító *Seiridium cardinale* a múlt század második felében terjedt el Észak Amerikából világszerte, erdőkben, ültetvényekben és díszítő fajtákon. A járvány a mediterrán vidékeken különösen a *C. sempervirens* fajt pusztítja. A gomba észak-olaszországi populációjának jellemzésére hetvenhét *Seiridium cardinale* izolátumot gyűjtöttünk. Összehasonlításra öt, különböző országokból, különböző gazdanövényekről és világrészekről származó *Seiridium* spp. izolátumot használtunk. A populáció szerkezetét RAPD és DAMD PCR marker technikákkal elemeztük az M13 magszekvencia alkalmazásával. Az eredmények a gomba észak-olaszországi populációjában magas fokú homogenitást mutattak, míg más gazdanövények és más fajok esetében bizonyos változékonyság fordult elő. Az észak-olaszországi populációhoz tartozó izolátumok molekuláris összehasonlításban mindkét markertípus esetében nagyon hasonlítottak. A görögországi izolátum is az olasz izolátumok csoportjába tartozott. Egyedül a chilei *S. cardinale* izolátum különbözött szignifikánsan a többi,

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olaszországi és görögországi *S. cardinale* izolátumtól. Az olasz izolátumok genetikai homogenitása arra utal, hogy a gomba nemrég palacknyak-hatáson ment át (egyedlétszáma erősen lecsökkent), valószínűleg néhány genotípusának európai behurcolása óta. Ez alátámasztja azt a hipotézist, hogy a gomba a második világháború során, fertőzött faanyaggal került be Európába az USA-ból. Az eredményeket a gomba európai behurcolása és terjedése vonatkozásában vitatjuk meg.

Molekuláris markerek / *Cupressus sempervirens* / *Seiridium cardinale* / populációgenetika / genetikai változatosság / f a kórokozó

1 INTRODUCTION

The common cypress (*Cupressus sempervirens* L., Cupressaceae) has an important role in the characterization of Mediterranean landscape mainly for its aesthetic functions. This tree species grows under various Mediterranean climates (Emberger et al. 1963), from sea level up to 2000 m or more on a variety of soil types and in a variety of plant association (Zohary 1973).

The cypress is an hardy tree which grows in most environmental conditions although it dislikes severe winter temperatures. Two varieties can be found, var. pyramidalis, the most used as ornamental, and var. horizontalis with its broader more irregular crown.

Cypress has demonstrated to be an excellent pioneer species for reforestation of rocky, argillaceous, limestone, barren and superficial lands. It prevents the hydrogeological erosion and constitutes a source of yield for the good quality of its wood. It is also used like windbreak plant. The cypress., is native to Asia Minor and Persia and part of Greece, but nowadays it grows also along all Mediterranean basin: France, Spain, Portugal, former Yugoslavia and Italy as well as in North Africa (Teissier du Cros et al. 1999) where it was introduced presumably during the Roman era or even before, since the Phoenicians and Etruscans (Santini – Di Lonardo 2000, Giannelli – Bezzini 2002).

Over the past half century a destructive blight of *Cupressus* spp., caused by *Seiridium cardinale*, has spread worldwide from North America, devastating forests, plantations, and ornamental cypresses. The epidemic has been particularly severe in the Mediterranean region, on *C. sempervirens*. Three species of *Seiridium*, *S. cardinale*, *S. cupressi*, and *S. unicorne*, are associated with cypress canker (Swart 1973, Chou 1989), even though *S. cardinale* seems to be the most dangerous in Europe on *C. sempervirens*.

Great variation exists in the susceptibility of different species of *Cupressaceae* to *S. cardinale* infection (Andreoli 1979, Raddi 1979). Intraspecific variation in resistance has also been reported on *C. sempervirens* (Ponchet – Andreoli 1979, Xenopoulos 1990, 1991), with marked variations within provenances and families from controlled crosses (Graniti 1998).

The low temperatures, that the cypress has often to stand in the Northern area of the species distribution, act indirectly to increase the strength of penetration of *S. cardinale* spores by means of microlesions created by frost (Teissier du Cros et al. 1999). Several cypress improvement programs for resistance to canker and cold were set up with the attempt to cultivate resistant clones throughout wide-reaching territories and areas with highly diverse pedoclimatic conditions (Santini – Di Lonardo 2000, La Porta et al. 2004).

The possibility that, despite its cold susceptibility, future climate changes may render the North of Italy more favourable to cypress cultivation could aid the increasing spread of this arboreal plant on the amenities and encourages studies on the acclimatation of this conifer in the Italian northern regions (La Porta et al. 2004).

Effective exploitation of the cypress genetic resistance sources may enable the replacement of stands damaged by canker and by cold with more resistant selections.

As a matter of fact, so far several clones have patented by the breeding programs carried out in Italy and France in the last 15-20 years (Panconesi 1990, Teissier Du Cros et al. 1991, 1999, Danti et al. 2006) and a new breeding program started in North Italy (La Porta et al. 2004) intend to select clones resistant to canker and to cold. However, a strong effect of environment and of environment by genotype interaction on cypress clones has been noted (Santini et al. 1997, Giannini – Raddi 1992).

In this context it is very important to assess the real genetic structure and variability of the pathogen populations because an hypothetical high variability of the fungus may increase the possible outcome of hypervirulent strains that could compromise the stability of the acquired resistance of the patented clones (Santini et al. 1997). At the same time, an hopefully low variability of the pathogen would make easier the selection work of the strains used for the inoculation tests and more trustable and reliable the selection work.

The aim of this study was to compare the genetic variability among different provenance of North Italian *S. cardinale* isolates with the use of some close-related *Seiridium* spp outgroups. The final aim is clarify the supposed low genetic variability of *S. cardinale* in Northern Italy due to the missing of sexual reproduction and to the supposed introduction of few virulent genotypes of the fungus. These information are crucial for the maintenance of resistance stability in any breeding program before to obtain and to release the resistant patented cypress clones.

2 MATERIALS AND METHODS

2.1 Fungal cultures and DNA extraction

A total of 82 isolates found on various hosts of the family *Cupressaceae*, were used (*Table 1*). Seventy-seven isolates of *Seiridium cardinale* were used for the genetic characterization of the North-Italian population, and five *Seiridium* spp. isolates from different countries and different hosts were used for comparison.

This five isolates were included in this study to serve as out-group in the phylogenetic analysis. The North-Italian isolates were obtained from cones or infections of diseased and dead trees.

Cones and infected barks have been placed in humid room to the aim to favour the appearance of reproductive structures of the fungus, the conidia.

Single-spore isolates were cultured in Petri dishes on potato dextrose agar (PDA; Difco Laboratories, Detroit, USA) at 25°C in the dark and maintained in tubes on PDA at 4°C. For DNA extraction, cultures were grown on cellophane disc placed on potato dextrose agar (20 g/liter PDA, 20 g/liter agar; Difco Laboratories, Detroit, USA) in Petri dishes for 15-20 days at 25°C.

Once the mycelia had covered the disc, they were lifted from the cellophane membrane, frozen using liquid N2 and ground in a mortar with liquid N2.

Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) in agreement with the indications of the producer.

2.2 DNA amplification

Amplification of fungal DNA was obtained with eight Operon RAPD markers (Operon Kits B, E and P, Operon Technologies, Alameda, USA) with the optimized annealing temperatures: OPP8 at 50°C, OPP6 at 50°C, OPP12 at 49°C, OPP14 at 50°C, OPB11 at 42°C, OPB18 at 42°C, OPE14 at 42°C and OPB10 at 50°C.

Table 1. Seiridium cardinale (79) and other Seiridium spp. (3) isolates analysed in this study.

PROVENANCE	LOCALITY	CODE	LATITUDE	LONGITUDE	FUNGUS	HOST	COLLECTOR
TRENTINO - SOUTH TYROL	ALA	AL1	45°46'06"	11°00'05"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. FRIGIMELICA
	ALA	AL2	45°46'06"	11°00'05"			
	ALA	AL3	45°46'06"	11°00'05"			
	ALA	AL4	45°46'06"	11°00'05"			
	ALA	AL5	45°46'06"	11°00'05"			
	ALA	AL6	45°46'06"	11°00'05"			
	ALA	AL7	45°46'06"	11°00'05"			
	ALA	AL8	45°46'06"	11°00'05"			
	AVIO	AL9	45°44'15"	10°55'56"			
	AVIO	AL10	45°44'15"	10°55'56"			
	ARCO	AR1	44°55'03"	10°52'41"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. FRIGIMELICA
	ARCO	AR2	44°55'03"	10°52'41"			
	ARCO	AR3	44°55'03"	10°52'41"			
	ARCO	AR4	44°55'03"	10°52'41"			
	ARCO	AR5	44°55'03"	10°52'41"			
	MORI	RO1	45°51'09"	10°58'21"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. FRIGIMELICA
	MORI	RO2	45°51'09"	10°58'21"			
	ROVERETO	RO3	45°53'40"	11°01'58"			
	ROVERETO	RO4	45°53'40"	11°01'58"			
	BOLZANO	BZ1	46°29'22"	11°20'39"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. FRIGIMELICA
	BOLZANO	BZ2	46°29'22"	11°20'39"			
	PIANA ROTALIANA	PR1	46°13'17"	11°06'07"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. FRIGIMELICA
	PIANA ROTALIANA	PR2	46°13'17"	11°06'07"			
	PIANA ROTALIANA	PR3	46°13'17"	11°06'07"			
	PIANA ROTALIANA	PR4	46°13'17"	11°06'07"			
	PIANA ROTALIANA	PR5	46°13'17"	11°06'07"			
	PIANA ROTALIANA	PR6	46°13'17"	11°06'07"			
GARDA LAKE	NAGO	RG1	45°53'03"	10°52'55"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. PIVA
	NAGO	RG2	45°53'03"	10°52'55"			
	RIVA DEL GARDA	RG3	45°53'32"	10°50'37"			
	RIVA DEL GARDA	RG4	45°53'32"	10°50'37"			
	GARGNANO	LG1	45°41'48"	10°39'36"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. PIVA
	GARGNANO	LG2	45°41'48"	10°39'36"			
	LIMONE	LG3	45°49'16"	10°47'05"			
	LIMONE	LG4	45°49'16"	10°47'05"			
	PIEVE	LG5	45°46'07"	10°44'21"			
	PIEVE	LG6	45°46'07"	10°44'21"			
	PIEVE	LG7	45°46'07"	10°44'21"			
	PIEVE	LG8	45°46'07"	10°44'21"			
	SIRMIONE	LG9	45°29'56"	10°36'20"			
	SIRMIONE	LG10	45°29'56"	10°36'20"			
	SIRMIONE	LG11	45°29'56"	10°36'20"			
	SIRMIONE	LG12	45°29'56"	10°36'20"			
	SIRMIONE	LG13	45°29'56"	10°36'20"			
	SIRMIONE	LG14	45°29'56"	10°36'20"			
	VALEGGIO SUL MINCIO	LG15	45°21'04"	10°43'54"			
	VALEGGIO SUL MINCIO	LG16	45°21'04"	10°43'54"			
	VALEGGIO SUL MINCIO	LG17	45°21'04"	10°43'54"			
	VALEGGIO SUL MINCIO	LG18	45°21'04"	10°43'54"			
	GARDA	LG19	45°34'53"	10°42'14"			
	MALCESINE	LG20	45°46'23"	10°48'30"			
	SIRMIONE	LG21	45°21'04"	10°43'54"			
LAKE MAGGIORE	VERBANIA	LM1	45°56'10"	8°33'10"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. PIVA
	VERBANIA	LM2	45°56'10"	8°33'10"			
	VERBANIA	LM3	45°56'10"	8°33'10"			
	VERBANIA	LM4	45°56'10"	8°33'10"			
	VERBANIA	LM5	45°56'10"	8°33'10"			
	LAVENO	LM6	45°55'07"	8°36'59"			
	LAVENO	LM7	45°55'07"	8°36'59"			
	LAVENO	LM8	45°55'07"	8°36'59"			
	LAVENO	LM9	45°55'07"	8°36'59"			
	AZZATE	LM10	45°47'02"	8°47'28"			
COMO LAKE	BELLAGIO	LC1	45°59'08"	9°15'22"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. PIVA
	BELLAGIO	LC2	45°59'08"	9°15'22"			
	BELLAGIO	LC3	45°59'08"	9°15'22"			
	DERVIO	LC4	46°04'36"	9°18'08"			
	BELLANO	LC5	46°02'31"	9°17'46"			
	MOLTRASIO	LC6	45°51'59"	9°06'03"			
	MOLTRASIO	LC7	45°51'59"	9°06'03"			
ISEO LAKE	SARNICO	LI1	45°39'53"	9°57'11"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. PIVA
	LOVERE	LI2	45°48'51"	10°04'15"			
	CLUSANE	LI3	45°04'37"	9°59'58"			
	MARONE	LI4	45°44'03"	10°05'22"			
	ISEO	LI5	45°39'14"	10°02'46"			
FRIULI VENEZIA GIULIA	BASSOVIZZA	FR1	45°38'31"	13°51'04"	*S. CARDINALE*	*C. SEMPERVIRENS*	G. FRIGIMELICA
	BASSOVIZZA	FR2	45°38'31"	13°51'04"			
	SAN DORLIGO DELLA VALLE	FR3	45°36'37"	13°51'04"			
GREECE	KOS	GR			*S. CARDINALE*	*C. SEMPERVIRENS*	P. TSOPELAS
CHILE		CI			*S. CARDINALE*	*CUPRESSUS* SP.	A. WINGFIELD
NEW ZEALAND		NZ			*S. CUPRESSI*	*C. MACROCARPA*	S. CHOU
PORTUGAL		P			*S. UNICORNE*	*CUPRESSUS* SP.	A. GRANITI
AUSTRALIA		A			*S. EUCALYPTI*	*EUCALYPTUS DELEGATENSIS*	Z. Q. YUAN

Incubation was performed in a Mastercycler ep Gradient S Thermal Cycler (Eppendorf AG, Hamburg, Germany) using the following cycle parameters: 94°C for 60 s, from 42°C to 50°C in relation of the primer used for 60 s and 72°C for 60 s.

The total number of cycles was 35, with an initial denaturation step of 5 min at 94°C and a final extension step of 10 min at 72°C. A negative control with all reagents except DNA was included in all reactions.

The structure of the population was analyzed also by use of the core sequence of M13 minisatellite DNA (Innovagen, Lund, Sweden) (Stenlid et al. 1994). Amplification was carried in the above described Mastercycler. The cycling parameters with this primer were: 7 min pre-denaturation at 93°C; 45 cycles of denaturing: at 95°C for 30 s, annealing at 48°C for 30 s; extension 72°C for 30 s; and final extension 72°C for 10 min.

The amplification products were separated in 1,5% agarose gels (AquaPor HR GTAC, Atlanta, USA) in 0,5X TBE buffer (Tris-acetate 40 mM, EDTA 1 mM, pH 8), at a constant voltage of 100 V for 5 h at room temperature, and stained with 0,1% Ethidium bromide. The results were observed under UV light, and photographed with a digital camera.

2.3 Data analysis

A comparison of each profile was carried out on the basis of presence/absence (1/0) of amplification products of the same length. A binary matrix combined the complete data records for all the isolates from the eight RAPD primers and another from minisatellite M13.

Genetic distance was calculated with the GenAlEx 6 software using the formula of Nei's standard genetic distance (Nei 1972, 1978) between pairs of populations. A dendrogram for the RAPD primers was constructed by means of the UPGMA (Unweighted Pair Grouping by Mathematical Averaging) methods using the MEGA3 software (BETA version).

3 RESULTS

The total number of 82 isolates was used in the work. The sampling along Trentino-South Tyrol and the biggest lakes in North Italy provided a total of 77 isolates. Some extra 5 isolates were provided by several other colleagues (P. Capretti, P. Tsopelas, A. Wingfield; *Table 1*) including 3 isolates from *Seiridium* outgroup species: *S. cupressi*, *S. unicorne* and *S. eucalypti*.

The electrophoretic profiles of the isolates amplified by RAPDs exhibited amplified fragments ranging from 200 to 2300 bp. The total number of the fragments was 137 and only one of them was common to all the isolates, including the outgroups. Out of 137 fragments only 91 were found in the *Seiridium cardinale* and among them, 57 showed to be polymorphic. An example of the amplificated fragments are shown for RAPDs (*Figure 1*) and DAMDs (*Figure 2*).

The analyses of the RAPD amplifications with 8 primes revealed a substantial low genetic variability among the 11 Italian populations. As a matter of fact the UPGMA cluster analysis groups all the Italian populations very close each other, even though they are not totally identical (*Figure 3*). Only the *S. cardinale* from Chile was clustered at significant distance from the other *S. cardinale* isolates from Italy and Greece. About the other three species of *Seiridium* used as outgroups, all of three species were separated with RAPD primers from the *S. cardinale* strains. However, *S. cupressi* and *S. eucalypti* were closer each other than *S unicorne* with both molecular markers. The results obtained by DAMDs markers substantially confirm the same results observed by RAPDs.

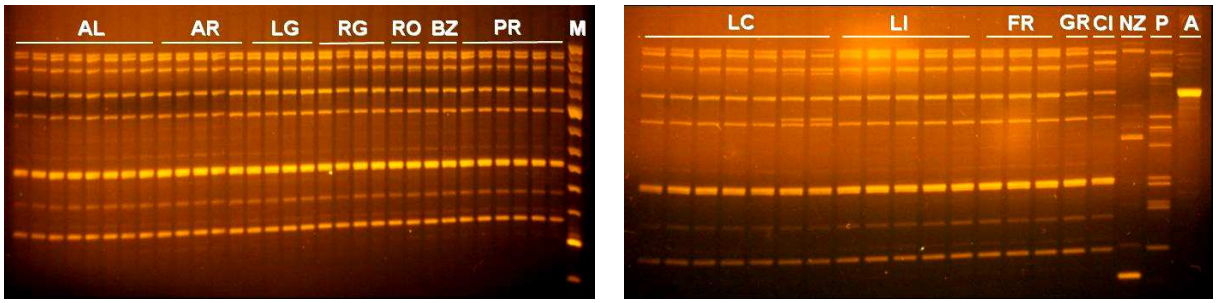


Figure 1. Example of RAPD profiles generated by primers OPP 8 for selected Seiridium spp. isolates. The acronyms of the populations are the following: AL (Ala), AR (Arco), LG (Garda Lake), RG (Riva del Garda), RO (Rovereto), BZ (Bolzano), PR (Piana Rotaliana), LC (Como Lake), LI (Iseo Lake), FR (Friuli Venezia Giulia), GR (Greece), CI (Chile), NZ (New Zealand), P (Portugal), A (Australia).

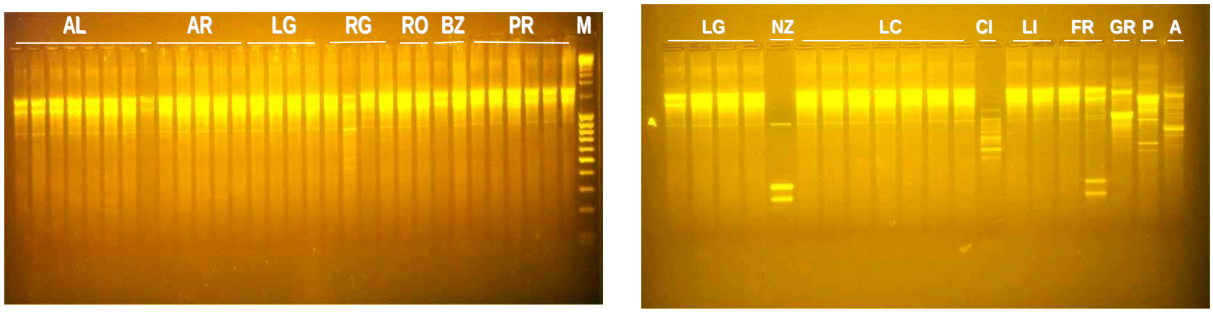


Figure 2. Example of minisatellite profiles generated by primers OPP 8 for selected Seiridium spp. isolates. The acronyms of the populations are the following: AL (Ala), AR (Arco), LG (Garda Lake), RG (Riva del Garda), RO (Rovereto), BZ (Bolzano), PR (Piana Rotaliana), LC (Como Lake), LI (Iseo Lake), FR (Friuli Venezia Giulia), GR (Greece), CI (Chile), NZ (New Zealand), P (Portugal), A (Australia).

4 DISCUSSION AND CONCLUSION

The results of the analysis of the Northen Italian populations of *S. cardinale* indicate a high level of homogeneity among them.

Viljoen et al. (1993) using sequences of ITS genes did not find any difference among and between 12 strains of *Seiridium*: *S. cardinale*, *S. cupressi* and *S. unicorne*, concluding that the three species are synonyms. Also Moricca et al. (2000), who compared by ITS2 of rDNA, didn't find any difference among five strains of Central and Southern Italian *S. cardinale* and with other European ones. Using ß-tubulin and histone sequences, Barnes et al. (2001) found very limited genetic variability among four strains of *S. cardinale*.

Later Krokene et al. (2004) was able to distinguish among the three *Seiridium* species, but no difference was detected among the three *S. cardinale* strains analysed. Regard *S. cardinale*, generally in all these previous works few strains were used in the analysis and even fewer of them were isolated from Italy. Sometimes, same strains were used in different works. A wide collection of strain was never analysed before to figure out definitely the variability among *S. cardinale*.

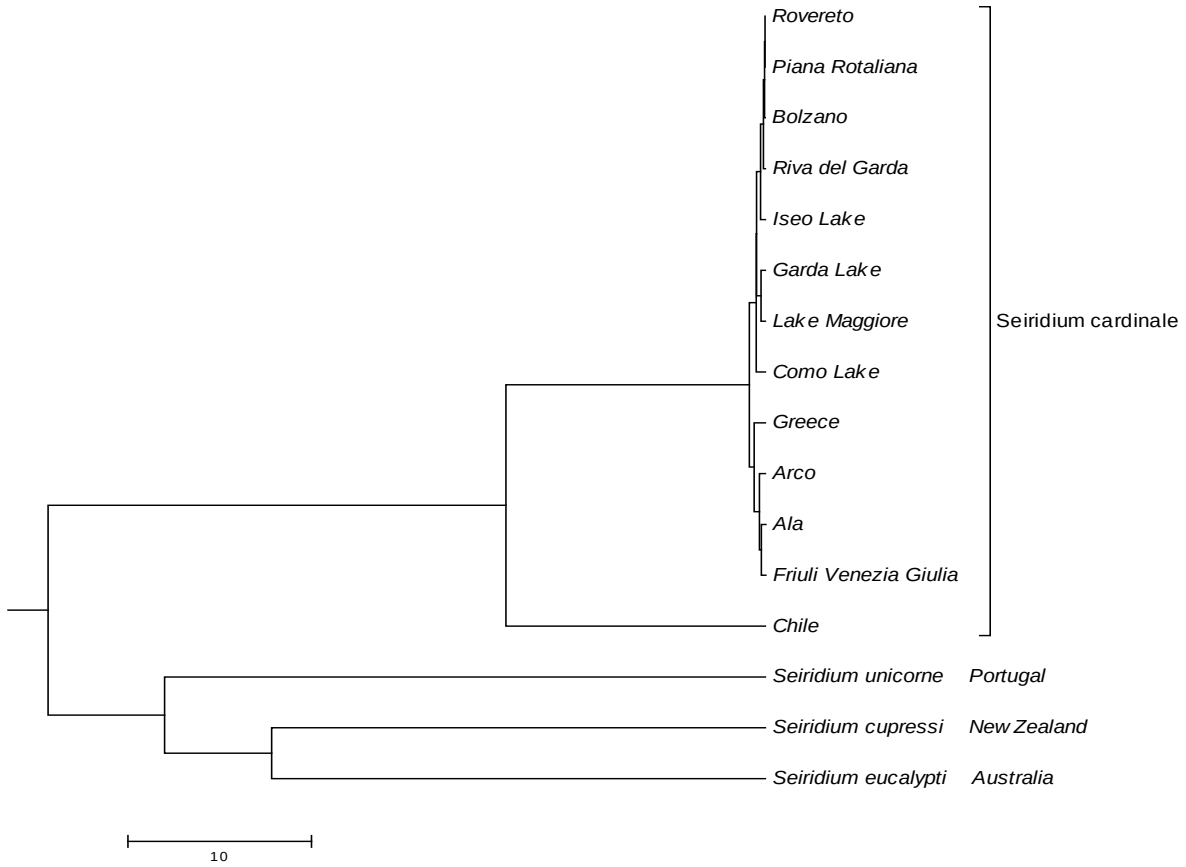


Figure 3. Dendrogram based on the similarity index matrix calculated from analysis of RAPD fragments obtained through the amplification of eight RAPD primers

In view of the extreme susceptibility of *C. sempervirens* to bark canker disease, however, it is also possible that only one or very few close relative strains of the fungus were introduced to Europe. Here they found extremely favorable conditions for their spread without facing an effective selection pressure from the host tree.

Based on our data, different populations of the fungus are morphologically indistinguishable from each other and are generally not separable on the basis of the host provenances they infect.

The use of RAPD technique is quite convenient especially when there is poor previous knowledge, about markers and sequences on the studied organism, to apply other techniques (Lynch – Milligan 1994, Diaz et al. 2001, Martinez et al. 2006, Monteleone et al. 2006, Valladares et al. 2006) including fungi (Hoegger et al. 1996, Santini – Capretti 2000, Dettman – Kamp 2001, Nagy et al. 2003). To improve the performance and the repeatability of this technique it is advisable to have higher stringency. It is possible to obtain this high performance selecting the RAPD primers that are able to amplify polymorphic fragments at higher annealing temperature (Wolf et al. 1999, Perez-Artes et al. 2000, HongYan et al. 2001, Sitthiphrom et al. 2005). In this study the 8 RAPD primers used were selected on a base of 80 different Operon primers and their annealing temperature were 49-50°C for 5 of them, while only for the remain three was 42°C. This kind of selection allowed a more stable results. The small differences osserved among the Italian *S. cardinale* strains were likely barely significant. Even though the preliminary data have to be confirmed by further analyses, the DAMD marker seem to show in this study a perfect similar pattern of amplified fragments

among the Italian strains even without the small minor differences showed by RAPDs. These results between the two markers types are similar with those obtained by other authors (Santini – Capretti 2000, Bhattacharya – Ranade 2001, Sabir 2006).

However, the high degree of similarity in *S. cardinale* is coherent with the common source of introduced isolates in Europe from the American continent about 60 years ago and suggest an occurring of a strong genetic bottleneck event.

Considering the number of samples analysed, this study suggests that *S. cardinale* is very homogeneous if not even para-clonal population, having the almost the same genotype in the whole of Italy and probably also throughout Europe. The pathogen, that for the first time was isolate in California in the 1928 (Wagener 1939), was probably introduced in Europe and in Italy from USA (Graniti 1998).

The finding that also the isolate from Greece didn't show any significant difference with the Italian populations is emblematic of the high level of genetical homogeneity of this fungus at least in Europe. This situation is even more evident in *S. cardinale*, because, at present, the sexual cycle is not known and in any case extremely rare (Graniti 1998).

The other species of *Seiridium* analysed in this work showed high significant differences each other and they where totally separated from the *S. cardinale* isolates as it was also found by Krokene et al. (2004). Similar results were found for several other virulent pathogens when they were introduced in a new environment or/and on the new host (Santini – Capretti 2000, Ristaino et al. 2001, Engelbrecht et al. 2004).

In conclusion, despite the fact that would be useful to confirm such results with other molecular markers, it seem quite clear from these preliminary data that *S. cardinale* has a narrow genetic homogeneity at least in the European continent. This fact, in absence of further introductions of higher virulent isolates of *S. cardinale* or other *Seiridium* spp., consents us to be relatively trustful regard the stability of the canker resistance acquired in the breeding programmes and the sustainability of the future issue on the market of resistant cypress clones.

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Identification of Gene Involved in Cypress Canker by PCR-Select Subtractive Hybridisation Approach

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Abstract – Cypress canker is the most serious biological threat faced by cypress in Europe and North America. Tree breeding strategies retain canker resistance the primary selection criterion.
Identification of genes activated or inhibited during the infection process is the basis to better understand the canker resistance. PCR-select (suppression subtraction hybridization) technique of isolation of genes specific for an infection process, was applied for analysis of host-pathogen interactions in the pathosystem *Cupressus sempervirens / Seiridium cardinale*. The subtraction, with RNA from the early stages of infection of *S. cardinale*, as a tester, and RNA from uninfected *C. sempervirens*, as a driver, enriched the pool of cDNA molecules with the ones specific for infection.
The first step, was to develop a critical protocol for RNA isolation from cypress bark to provide a good quality of RNA for the further analysis. In a second step, 5 years-old seedlings of *C. sempervirens* were artificially infected by virulent strain of *S. cardinale*. Particular attention was paid in the experimental design to avoid to select genes that were activated only by wounding. A third step, was the isolation of pathogen DNA, to monitor, by Real-time PCR, the pathogen spatial colonization in the bark along the stem. In the fourth step, a subtractive procedure to obtain an enriched library of cDNA, by PCR-Select, was carried out to select putative genes. To this purpose databank similarity searches were performed with the Blastx. program maintained at NCBI. In this study we succeeded in identifying about 100 cDNA clones significantly expressed in infected hosts but not in the uninfected control. The expression of several of these genes showing sequence similarity with resistance- or stress-related genes from other plant species were identified.

***Cupressus sempervirens* / *Seiridium cardinale* / gene expression / cDNA / fungal colonization profile**

Kivonat – A ciprusrákkal kapcsolatos gének azonosítása PCR alapú szubtraktív hibridizációs megközelítéssel. A ciprusrák Európában és Észak-Amerikában a ciprusok legjelentősebb biológiai veszélyeztető tényezője. A nemesítési stratégiák elsődleges szelekciós szempontja a rákkal szembeni ellenállóság. A rákkal szembeni ellenállóság jobb megértésének alapja a fertőzési folyamat során aktivált vagy gátolt gének azonosítása. A *Cupressus sempervirens / Seiridium cardinale* patoszisztéma gazda-kórokozó kölcsönhatásának elemzése céljából a fertőzési folyamatra specifikus géneket PCR alapú technikával izoláltuk. A szubtrakció, a *S. cardinale* fertőzés kezdeti szakaszából származó RNS teszterrel és a fertőzésmentes *C. sempervirens* RNS vezérlővel, megemelte a fertőzésre specifikus cDNS molekulák számát.

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Első lépésként kidolgoztuk az RNS kivonásának kritikus módszerét a ciprus kéregből, jó minőségű RNS nyerése céljából a későbbi vizsgálatokhoz. Második lépésben ötéves *C. sempervirens* csemetéket fertőztünk virulens *S. cardinale* törzzsel. Különös figyelmet fordítottunk a csak a sebzés által aktivált gének kiválasztásának elkerülésére. A harmadik lépés a kórokozó DNS-ének izolálása volt, Real-Time PCR módszerrel, a kéreg kórokozó általi térbeli kolonizációjának monitorozása céljából a törzs hosszában. Negyedik lépésben, a cDNS könyvtár bővítésére, PCR alapú szubtraktív eljárást folytattunk a szóba jöhető gének kiválasztására. E célból hasonlóság keresést végeztünk a Blastx nevű programmal a génbank (NCBI) adatbázisában. E munkával sikerült mintegy 100 olyan cDNS klónt azonosítani, amelyek szignifikánsan kifejeződtek a fertőzött gazdában, de a fertőzött kontrolban nem. Több olyan gén kifejeződését azonosítottuk, amelyek szekvencia azonosságot mutattak más növények rezisztenciához, illetve stresszhez kapcsolódó génjeivel.

***Cupressus sempervirens* / *Seiridium cardinale* / génkifejeződés / cDNA / gomba kolonizációs profil**

1 INTRODUCTION

Cupressus sempervirens L., (*Cupressaceae*) is native to the Iran, as well as Syria, Turkey, Cyprus and several Greek Islands (Crete, Samos, Rhodes ect.). It was introduced in most countries around Mediterranean and at present its natural geographic distribution is characterised by disjoint and often relic populations growing in Iran, Syria, Jordan, Lebanon, Libya, the Aegean Islands, Crete, Turkey, Cyprus (Zohary 1973). Probably during the Tertiary it occupied a larger areas (Axelrod 1958) that was reduced during millennia mainly by strong human pressure (Boscherini et al. 1994, Vendramin et al. 1995), intensive and unregulated forest utilisation, burning, grazing and cypress canker disease (Kayacik et al. 1979, Sumer 1987, Graniti 1998) leaving only small areas of forest. Since historical times, the cypress has been extensively cultivated far beyond its natural geographic range, in earlier times through its association with religious rites and later for aesthetic reasons. At present it grows also in Italy, France, Spain, Portugal and former Yugoslavia (Ducrey et al. 1999) where it was introduced presumably during the Roman era or even before, since the Phoenicians and Etruscans started to sail along the Mediterranean (Macina 2002). Such spread of cypress is still an ongoing process, not only in Mediterranean countries, but in every similar climatical area too, where the cypress is able to fit to the local environmental conditions (Santini – Di Lonardo 2000, La Porta et al. 2004).

Nowadays, the common cypress has an important role in the characterization of Mediterranean landscape mainly for its aesthetic function. Although there are likely no natural forests of *C. sempervirens* in Italy, this species is adapted very well to the Italian environment and in many sites shows a good natural regeneration to be considered a naturalized species (Ducrey et al. 1999). Cypress groves are present in coastal hills from Liguria to Calabria and in Sicily. In the central part of Italy, especially in Tuscany near Florence, Siena and Pisa, cypress woods are more present and productive. In the north Italy, cypress stands and groves can be mainly found around lakes where climatic conditions are favourable (Xenopoulos 1990, La Porta et al. 2004).

In the last fifty years the cypress has been attacked by a parasitic fungus, *Seiridium cardinale* (canker of cypress), which is seriously threatening the survival of this plant in Italy and in other Mediterranean countries (Panconesi 1990, Graniti 1998). Several cypress improvement programs for resistance were set up with the attempt to cultivate resistant clones throughout wide-reaching territories and areas with highly diverse pedoclimatic conditions. Several resistant clones were actually produced (Panconesi – Raddi 1990, 1991, Danti et al. 2006).

The cypress is a very plastic species: clones growing in completely different habitats take very different shapes in accordance with variations in environmental conditions, ecological

factors and soil characteristics. The strong effect of environment and of environment by genotype interaction on cypress clones has been noted (Santini et al. 1994). Similar conclusions are also being reached in works involving stability in the resistance to cypress canker disease. Clones to use should perhaps be tested locally before spread on a big area, instead of aiming the entire research effort at finding a universal clone adaptable to all environments (Giannini – Raddi 1992).

The low temperatures that the cypress has often to stand in the Italian northern regions act indirectly to increase the strength of penetration of *S. cardinale* spores by means of microlesions created by frost. In this context plants resistant to *S. cardinale* and adapted to cold northern regions guarantee a better protection against pathogen. Cypress clones resistant to canker show also fast recovering of physiological parameters (Muthuchelian et al. 2005a, Muthuchelian et al. 2005b). The presence of highly susceptible hosts and climatic conditions favourable for reproduction of the pathogen facilitated its establishment and spread in the Mediterranean area, where it has caused destructive and recurrent epidemics of canker that have decimated ornamental trees, windbreaks, natural stands and cypress plantations (Graniti 1986, Raddi et al. 1987).

Identification of genes activated or inhibited during the infection process is the basis to better understand the canker resistance. PCR-Select (suppression subtraction hybridization) technique of isolation of genes specific for an infection process, was applied for analysis of host-pathogen interactions in the pathosystem *S. cardinale*/*C. sempervirens*.

The aims of this study were to monitor spatial profiles of the pathogen colonization of susceptible and resistant trees and to identify important genes involved with resistance response to *S. cardinale* infection.

2 MATERIAL AND METHODS

2.1 Plant material and sampling

Two 5 year old clones of *C. sempervirens* growing at the plantation of IASMA Research Centre were used as host material. The two used *C. sempervirens* clones were the resistant patented clone called Bolgheri and a wild clone susceptible to pathogen.

Three resistant ramets and one susceptible ramet were located in greenhouse, thus minimizing the variation in microclimatic conditions. Two resistant ramets and one susceptible clone were inoculated with *S. cardinale*. One resistant ramet was used for control. Prior to inoculation, the fungus was grown on malt extract agar (1% malt extract, 1.5% agar, Difco Laboratories, Detroit, USA) in Petri dishes for 10-15 days at 25°C. Inoculations were made 50 cm above ground level on each stem. At each inoculation point, a plug of bark down to the sapwood surface was excised by using a 5-mm-diameter cork borer. A similarly sized agar plug containing the actively growing fungus was inserted into the hole, and the bark plug was replaced. A rectangular strip (2 x 10 cm) of bark containing phloem and cambium, with the inoculation site in the middle, was removed 7 and 30 days after inoculation. Immediately after excision, the samples were frozen in liquid N_2 and stored at –80°C.



Figure 1. Phases of sampling of the host stem tissue 30 days after inoculations.

2.2 DNA isolation and quantification of fungal colonization

The lesion length was recorded, and the upper half of one resistant and susceptible ramet at 30 dpi were sampled for DNA isolation and Real-Time PCR. Prior to sampling, the rhytidome and periderm were removed. The lesion was divided into sections (length, 1 cm; width, 5 mm; depth, approximately 3 mm), which were processed individually to obtain the fungal colonization profile upper the point inoculation. Fresh tissue was ground in liquid nitrogen with a mortar and a pestle. Genomic DNA was isolated from 50 mg of liquid nitrogen-ground powder by using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) in agreement with the indications of the producer. The DNA concentration was determined with a Dyna Quant Fluorometer 200 (Hoefer Scientific Instruments, San Fransisco, USA).

The primers wase designed with the Primer Express software, version 1.5, provided with the Applied Biosystems (Foster City, USA) Real-Time quantitative PCR system. A Phytocromo-P gene (GeneBank, AY380891) was used as the target for *C. sempervirens*. The designed forward primer, 5'-CCCGGTTTCTTTTCATGCA-3', and reverse primer, 5'-GGATCAGCACGGCAATCAG-3', amplify the region from base 95 to base 152. A ITS1 gene (GeneBank, AY687314) was used as the target for *S. cardinale*. The designed forward primer, 5'-CGGCGGATTTGTGGTATCC-3', and the reverse primer, 5'-CTGCAGCACCTGACAAAAGC-3', amplify the region from base 458 to base 521.

The Real-Time PCR detection of host and pathogen DNA was performed with SYBR Green PCR Master Mix with an ABI PRISM 7500 Real-Time PCR Instrument (Applied Biosystems).

For optimization of primers concentrations, in order to minimize the interference from competing reactions during multiplex PCR, was performed on samples with both host and fungal material present in known concentrations at a range of dilutions.

The primer concentrations selected were 300 nM of Phitochromo-P primer and 90 nM of ITS1 primer. Each 25 µl PCR was performed in SYBR Green PCR Mastermix (Applied Biosystems, Foster City, USA). As a template, 3 µl of the cDNA solution described above was used for each reaction. Each reaction was repeated twice. PCR cycling parameters were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The data acquisition and analysis were performed with the Sequence Detection System software package (version 1.7; Applied Biosystems, Foster City, USA). The amounts of pathogen and host DNA in the inoculated tissue samples were calculated by using the ΔCt based calculation procedure, with equations derived from the respective standard curve (Hietala et al. 2003).

2.3 RNA extraction and cDNA library

For construction of a forward-subtracted cDNA library, the resistant ramet used as control and a resistant ramet inoculated with *S. cardinale* and incubated for 7 days, were utilised.

Prior to RNA extraction, the rhytidome and the periderm were removed and the tissue subdivided into sections (length, 1 cm; width, 5 mm; depth, about 3 mm) that were processed separately. The sampled 1 cm long sections were frozen immediately in liquid nitrogen and ground with a mortar and a pestle. Approximately 300 mg of the powder was subjected to RNA isolation by following exactly the hot borate extraction method described by Moser et al. (2004). The precipitated RNA was dissolved in a 50 µl volume of RNase-free ddH_2O.

The RNA was purified by using the binding column of SpectrumTM Plant Total RNA Kit (Sigma Aldrich, St. Louis, USA) according to the manufacturer's instructions.

Total RNA was quantified with a VersaFluor fluorometer (Bio-Rad, Hercules, USA) and a RiboGreen RNA quantification kit (Molecular Probes, Eugene, USA) according to the manufacturer's instructions and was DNase-treated by using of Amplification Grade DNaseI (Sigma Aldrich, St. Louis, USA).

cDNA was synthesised from 1 µg of total RNA by using the BD SMART PCR cDNA Synthesis Kit (BD Biosciences, Clontech, East Meadow, USA) according to the manufacturer's instructions. The number of PCR cycles was optimised by LD PCR using 9700 ABI thermal cycler (Applied Biosystems, Foster City, USA) and BD Advantage 2 PCR Kit (BD Biosciences, Clontech, East Meadow, USA) according to the manufacturers' instructions. The optimal cycle number was defined as one cycle fewer than is required to reach the plateau phase. Ten PCR replicates were performed for the two experimental samples (RNA from control ramet and inoculated ramet) by using the obtained optimal cycle number 17. cDNA generated was directly used for construction of a forward-subtracted cDNA library by using Clontech PCR-SelectTM cDNA Subtraction Kit (BDBiosciences, Clontech, East Meadow, USA).

To make the subtracted cDNA library, PCR products from the secondary PCR were directly ligated into the pCR 2.1-TOPO cloning vector (TOPO TA Cloning, Invitrogen, Grand Island, USA) and transformed into One Shot Chemically Competent *E. coli* according to manufacturer's instructions.

For DNA sequencing, each reaction was performed with 4 µl Big dye terminator chemistry (Perkin Elmer, Waltham, USA) in a 20 µl reaction using an ABI Prism 3100 Genetic Analyzer 16 well capillary automated DNA sequencer. Nucleotide sequencing and data analysis were done after deleting the vector sequence. cDNA sequences were compared with GenBank database sequences using BlastX. Sequences for which no match was found were classified as unknown. A subset of interesting clones relevant in host defense and other cellular function was selected for differential screening.

3 RESULTS AND DISCUSSION

The spatial colonization profiles of *S. cardinale* show some difference between the two cypress clones. After 30 days of inoculation, the pathogen was detected until 24 mm high from inoculation point in the susceptible clone. In the resistant clone, it was found only 14 mm high from inoculation point (*Figure 2*). *S. cardinale* colonization levels were very similar in the two clones until the 6th mm from the inoculation point. In this interval, for both clones, there were the maximal amounts of pathogen with a slow decrease. Afterwards, in both clones, there was a rapid decrement in the amount of pathogen between the 6th and 12th mm. In this interval there was a relevant difference in the trend of colonization levels for two clones. At the 8th mm the amount of pathogen's DNA detected in the susceptible clone was about 20 times more present than in the resistant clone. Than, the pathogen was detected until the 14th mm in resistant clone, whereas in the susceptible clone with the regular and constant trend at down levels was found until the 24th mm.

The results of this spatial colonization profile of *S. cardinale* were used to detect the right distance of site where to make genes expression analysis. The sampled section was 6 mm away from the point of inoculation. We assumed that in this area it would be the greatest plant reaction to the pathogen, because we detected a progressive decrement of pathogen DNA.

Moreover, Real Time PCR technique on cypress to make spatial colonization profiles it will be an useful method for the development of early screening able to detect resistant cypress clones.

Expression profile of genes induced by *S. cardinale* in cypress was obtained by sequencing 120 subtractive PCR clones generated from mRNA of bark inoculated with the fungus after subtraction with that of non-inoculated bark. A subset of interesting genes representing important functional categories relevant in host defense and other cellular function from the cDNA library were selected. About 31% of the cDNA sequences had homology to known genes (*Table 1*).

In particular the sequences of chalcone synthase and thioredoxin-dependent peroxidase were obtained from three different PCR clones.

The peroxidases constitute an important group of enzymes associated with phenolic chemistry that are involved in defense-related processes such as lignification, cross-linking of cell wall proteins, auxin catabolism, production of oxygen radicals, as well as direct defense against pathogens (Campa 1991, Mohan et al. 1993, Otter – Polle 1997). Increased accumulation of peroxidases has been reported in several coniferous seedlings infected with pathogenic fungus (Asiegbu et al. 1999, Fossdal et al. 2001, Hietala et al. 2004).

Another interesting enzyme found is the chalcone synthase (CHS) catalyses, the first committed step in the biosynthesis of flavonoids. Flavonoids are implicated in several physiological functions and play a vital role in the interaction between plants and their environment. Stilbenes and flavonoids, together with the enzymes involved in their synthesis (e.g., PAL = phenylalanine ammonia lyase, CHS = chalcone synthase and STS = stilbene synthase; Franceschi et al. 1998, Chiron et al. 2000, Nagy et al. 2000, Viiri et al. 2001), are among the most intensively studied anti-fungal phenolic compounds in coniferous bark. Stilbenes and flavonoids, which are constitutively present as glycosides, represent a primary chemical barrier to invasion and are directly involved in defense against injury and fungal infection (Nicholson - Hammerschmidt 1992, Brignolas et al. 1995, Evensen et al. 2000).

This preliminary results could be useful to identify which genes could change its expression gene levels in responding to *Seridium* infection.

In a further step, a characterization of the most promising candidate genes, particularly chalcone synthase and peroxidase, will be performing by using of Real Time PCR technique on resistant and susceptible cypress clones at different inoculation times.

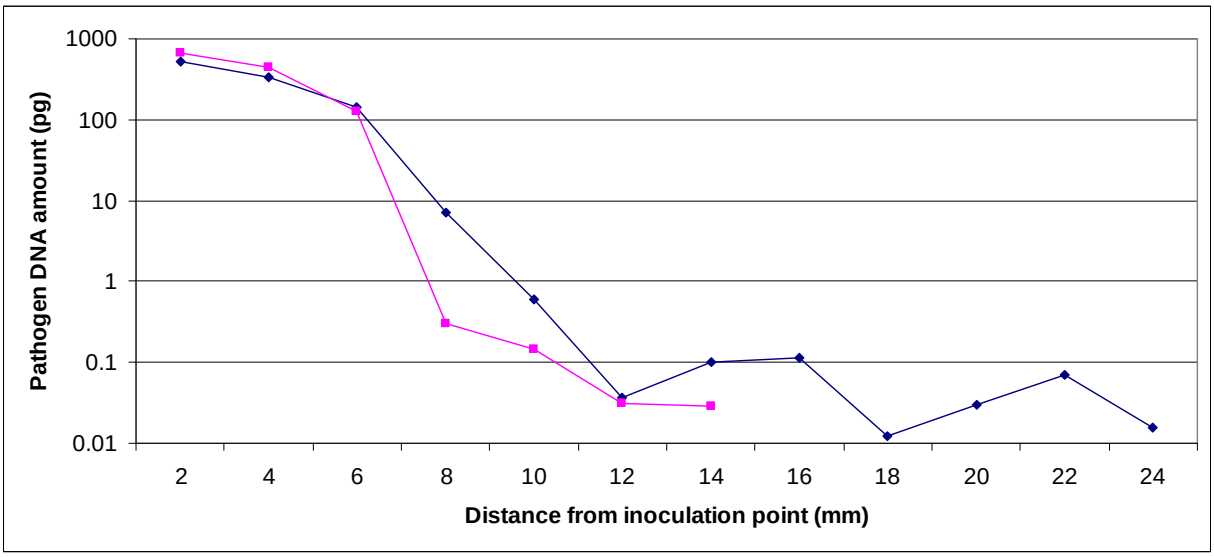


Figure 2. Total DNA yield from the sampled 1 cm-long slices covering the lesions. The lengths of the sampled lesions for resistant (pink) and susceptible (blue) clone.

Table 1. Features of some sequenced clones and results of BLAST search.

CODE	ACCESSION NUMBER	DNA HOMOLOGY	SPECIES	SIZE bp	SCORE	IDENTITIES	E-VALUE
L6	gi|21405653|gb|AY086929.1|	clone 29662 mRNA	*Arabidopsis thaliana*	1084	224 bits (113)	454/565 (80%)	6e-55
L12	gi|71000472|dbj|AB221012.1|	S-adenosyl-L-homocysteine hydrolase	*Beta vulgaris*	424	115 bits (58)	266/323 (82%)	1e-44
E13	gi|45477376|gb|AY559080.1|	LOS2 (los2) mRNA	*Capsella bursa-pastoris*	651	170 bits (86)	260/318 (81%)	5e-39
A15	gi|124358702|dbj|AB264257.1|	CC2188 gene for putative Adomet decarboxylase	*Chamaecyparis obtusa*	614	339 bits (171)	267/299 (89%)	8e-90
H10	gi|90704802|dbj|AB254820.1|	mRNA for putative peptidyl-prolyl cis-trans isomerase	*Cryptomeria japonica*	544	531 bits (268)	429/477 (89%)	9e-148
A17	gi|91075913|gb|DQ395330.2|	alkaline alpha galactosidase (AGA2)	*Cucumis sativus*	1062	81 bits (41)	86/101 (85%)	5e-12
F5	gi|20513302|dbj|AB075535.1|	Chs gene for chalcone syntase Chs	*Juniperus rigida*	405	268 bits (135)	213/239 (89%)	2e-68
F6	gi|20513302|dbj|AB075535.1|	Chs gene for chalcone syntase Chs	*Juniperus rigida*	449	426 bits (215)	323/359 (89%)	3e-116
H20	gi|20513302|dbj|AB075535.1|	Chs gene for chalcone syntase Chs	*Juniperus rigida*	333	280 bits (141)	219/245 (89%)	3e-72
I1	gi|109138844|gb|DQ629331.1|	Qiu 96270 large subunit ribosomal RNA gene	*Juniperus sp.*	432	775 bits (391)	399/402 (99%)	4e-23
E1	gi|924740|gb|U24586.1|	chloroplast 16S rRNA gene	*Juniperus virginiana*	410	670 bits (338)	365/370 (98%)	3e-13
K7	gi|924740|gb|U24586.1|	chloroplast 16S rRNA gene	*Juniperus virginiana*	402	712 bits (359)	373/375 (99%)	6e-18
I17	gi|56554971|gb|AY830127.1|	heat shock protein 70 (HSP70-1) mRNA	*Medicago sativa*	251	127 bits (64)	169/204 (82%)	2e-26
L19	gi|77993048|emb|CR955004.2|	chromosome 5 clone mte1-9e19	*Medicago truncatula*	878	83 bits (42)	93/110 (84%)	1e-12
F21	gi|92898848|gb|AC141323.9|	clone mth2-6a23	*Medicago truncatula*	1042	63 bits (32)	50/56 (89%)	1e-06
M23	gi|77993048|emb|CR955004.2|	chromosome 5 clone mte1-9e19	*Medicago truncatula*	875	83 bits (42)	93/110 (84%)	1e-12
B13	gi|2501849|gb|AF012823.1|	GDP dissociation inhibitor (GDI) mRNA	*Nicotiana tabacum*	546	186 bits (94)	387/479 (80%)	6e-44
M15	gi|51949799|gb|AY695052.1|	adenosine kinase isoform 1S mRNA	*Nicotiana tabacum*	625	167 bits (84)	228/276 (82%)	7e-38
M1	gi|115456202|ref|NM_001058237.1|	chromosome 1	*Oryza sativa*	512	73 bits (37)	58/65 (89%)	6e-10
A11	gi|115474008|ref|NM_001067138.1|	chromosome 7	*Oryza sativa*	733	54 bits (27)	30/31 (96%)	8e-04
E19	gi|115462424|ref|NM_001061347.1|	chromosome 5	*Oryza sativa*	1142	79 bits (40)	79/92 (85%)	2e-11
N21	gi|115474006|ref|NM_001067137.1|	chromosome 7	*Oryza sativa*	813	105 bits (53)	89/101 (88%)	3e-19
A23	gi|52788389|gb|AY705795.1|	clone 1R aldehyde dehydrogenase mRNA	*Pinus halepensis*	886	137 bits (69)	126/144 (87%)	9e-29
B22	gi|4138350|emb|AJ005119.1|	glutamine synhtetase	*Pinus sylvestris*	690	327 bits (165)	352/413 (85%)	3e-86
E7	gi|6752881|gb|AF220200.1|	nascent polypeptide associated complex alpha chain	*Pinus taeda*	756	115 bits (58)	160/194 (82%)	3e-22
D6	gi|6752881|gb|AF220200.1|	nascent polypeptide associated complex alpha chain mRNA	*Pinus taeda*	763	291 bits (147)	390/471 (82%)	2e-75
I11	gi|52851171|emb|AJ843119.1|	mRNA for thioredoxin-dependent peroxidase (tpx1 gene)	*Plantago major*	539	172 bits (87)	198/235 (84%)	9e-40
I15	gi|52851171|emb|AJ843119.1|	mRNA for thioredoxin-dependent peroxidase (tpx1 gene)	*Plantago major*	538	172 bits (87)	198/235 (84%)	9e-40
I7	gi|52851171|emb|AJ843119.1|	mRNA for thioredoxin-dependent peroxidase (tpx1 gene)	*Plantago major*	538	172 bits (87)	198/235 (84%)	9e-40
D11	gi|169704|gb|M64737.1|	ATP:pyruvate phosphotransferase (PK-p-beta) mRNA	*Ricinus communis*	925	127 bits (64)	358/456 (78%)	9e-26
L2	gi|14031062|gb|AY032884.1|	WD-40 repeat protein mRNA	*Solanum lycopersicum*	451	151 bits (76)	214/260 (82%)	3e-33
L3	gi|14031062|gb|AY032884.1|	WD-40 repeat protein mRNA	*Solanum lycopersicum*	445	151 bits (76)	214/260 (82%)	3e-33
G23	gi|410485|emb|Z21792.1|	DAHP synthase 1 precursor	*Solanum lycopersicum*	526	107 bits (54)	261/330 (79%)	5e-20
B1	gi|148538060|dbj|AK246826.1|	ribosomal protein	*Solanum lycopersicum*	923	97 bits (49)	106/125 (84%)	8e-17
L10	gi|13936692|gb|AF295670.1|	plastidic 6-phosphogluconate dehydrogenase (pgdP) mRNA	*Spinacia oleracea*	1188	107 bits (54)	99/114 (86%)	1e-19
D16	gi|147862488|emb|AM484542.2|	contig VV78X020249.4	*Vitis vinifera*	623	77 bits (39)	84/99 (84%)	5e-11

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Noteworthy Decline and Wood Decay on *Fagus sylvatica* L. by the Ascomycete *Annulohypoxylon cohaerens* (Fr.: Fr.) Y. M. Ju, J. D. Rogers & H.-M. Hsieh

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Abstract – Within the years 2005 to 2007 several declining and recently died beech trees (*Fagus sylvatica* L.) were investigated in a large city park in Dresden (Southern East Germany). The ascomycete *Annulohypoxylon cohaerens*, which was exclusively characterized as a saprophytic fungus by literature so far, had been identified as a conspicuous cause of the disease. The symptoms of the infection (changes of crown architecture and crown transparency, bark necroses), the morphological and physiological characteristics of the fungus *in situ* and *in vitro* (e. g. characteristics of fruit bodies, growth rate, colour and pattern of colonies, presence and structure of asexual reproductive states, potency and strategy of wood decomposition) as well as the factors of predisposition are presented in the article. Since *A. cohaerens* attains pathological importance and can be mistaken for some other ascomycetes, the distinguishing marks to related species (*Kretzschmaria deusta*, *Annulohypoxylon multiforme*, *Hypoxylon fragiforme*) are described. The significance of the fungus is evaluated for practice.

***Annulohypoxylon cohaerens* / *Fagus sylvatica* / vigour reduction / differential diagnosis / wood decay / pathogenicity test**

Kivonat – A *Fagus sylvatica* L. *Annulohypoxylon cohaerens* (Fr.: Fr.) Y. M. Ju, J. D. Rogers & H.-M. Hsieh tömlősgomba általi figyelemreméltó pusztulása és korhadása. A 2005 - 2007 években számos pusztuló, vagy frissen pusztult bükkfát (*Fagus sylvatica* L.) vizsgáltunk Drezda egyik városi parkjában (Délkelet-Németország). Az irodalomban mostanáig kizárólag szaprotrófként jellemzett *Annulohypoxylon cohaerens* tömlősgombát a betegség feltűnő okozójaként azonosítottuk. A cikkben bemutatjuk a fertőzés tüneteit (a koronaszerkezet változása, koronagyérülés, kéregnekrózis), a gomba morfológiai és fiziológiai sajátosságait *in situ* és *in vitro* (a termőtestek jellege, növekedési ütem, a telepek színe és mintázata, ivartalan szaporodási alakok megléte és felépítése, faanyagbontó képesség és stratégia), valamint a hajlamosító tényezőket. Mivel a patológiai jelentőségűvé váló *A. cohaerens* könnyen összetéveszthető több más tömlősgombával, ismertetjük a rokon fajoktól való megkülönböztető bélyegeket (*Kretzschmaria deusta*, *Annulohypoxylon multiforme*, *Hypoxylon fragiforme*). A gomba gyakorlati jelentőségét értékeljük.

***Annulohypoxylon cohaerens* / *Fagus sylvatica* / legyengülés / diagnózis / korhadás / patogenitási teszt**

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1 INTRODUCTION

In the year 2004 several 45 to 130 years old beech trees in a large city park in Dresden (Southern East Germany) showed a drastic lost of vigour. These trees manifested a noteworthy retarded sprout in spring, reduced smaller foliage as well as a strong blossom and fruit production (*Figure 1*). Some of the trees already had died in spring and had to be cut down for security reasons in autumn of 2005. Investigations on the fresh cut stumps and trunks showed an extended wood decay especially in the sapwood of the trees (*Figure 1*). On the bark of dead trees as well as on suffering but still living ones masses of fruit bodies of an ascomycete were found (*Figure 2*). Those were located on the lower part of the trunk, extending up to 4 meters height. Other fungi or causes for the disease could not be found.

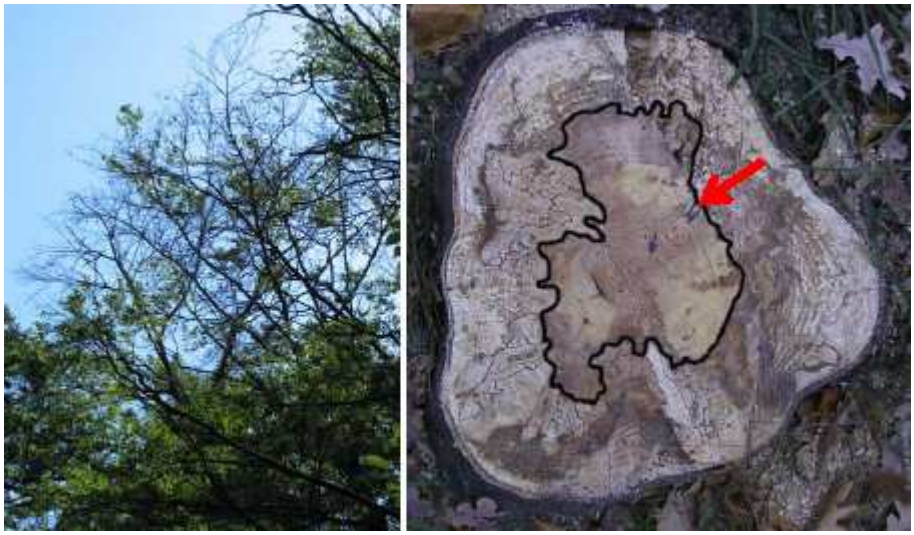


Figure 1. Crown symptoms of diseased beech trees (left) and fresh cut stump with extended decay in the sapwood (right)

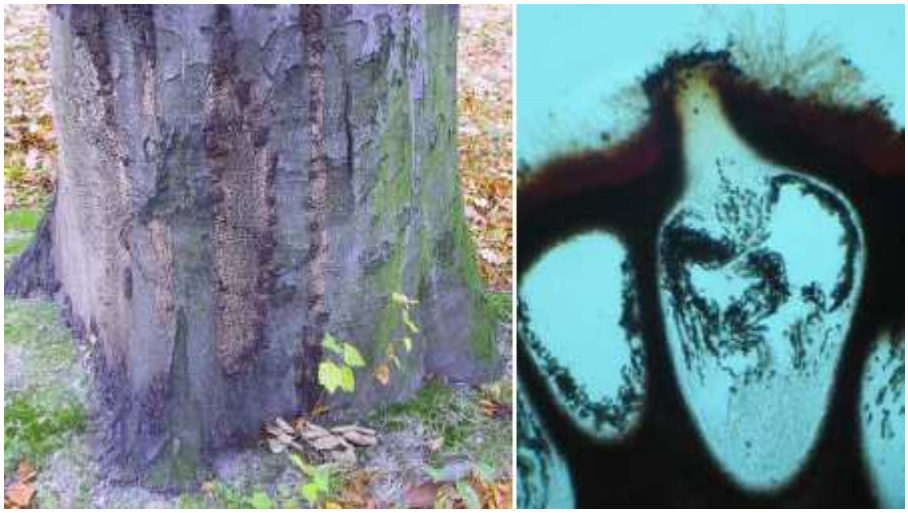


Figure 2. Butt base of a diseased beech tree with fruit bodies of Annulohypoxylon cohaerens (left) and microscopic details (cross-section) of the sexual reproductive state (perithecium)

2 MATERIALS AND METHODS

Field investigations were conducted in spring (April) and in autumn (November) of the year 2005. First of all the percentage of the damaged and already died trees were surveyed and the vitality of the still living trees evaluated. Fruit bodies of the fungus had been taken from all infected trees for further laboratory work.

The diagnosis of the collected fruit body samples and isolated single spore cultures were carried out with the aid of different keys (Munk 1957, Miller 1961, Jahn 1967, Greenhalgh - Chesters 1968, Jong 1970, Breitenbach – Kränzlin 1984, Petrini – Petrini 1985, Petrini – Müller 1986, Ju – Rogers 1996) using several chemicals (KOH, Melzer's reagent) and comparing cultures of the own institute. Macroscopic and microscopic investigations took especially place in order to distinguish the morphologically and physiologically similar ascomycetes *Annulohypoxylon cohaerens*, *A. multiforme*, *Hypoxylon fragiforme* and *Kretzschmaria deusta*. In this process the structure and colour of fruit bodies, texture and dimension of perithecia and ascospores, pattern and growth rate of cultures over a range of different temperatures (5, 10, 15, 20, 25 and 30°C) as well as the formation and morphology of anamorphs had been studied. Cultivation of isolates occurred on 2% Malt-Extract-Agar. In addition, physiological characteristics were obtained from the agent (enzymatic reaction in Guaic- and Tannin-Agar [Bavendamm 1929] and potency of wood decomposition). Cubes from healthy beech trees (sapwood and heartwood) of 20 x 20 x 45 mm^3 size were cut and successively dried (T = 105°C), weighted, moistened again and sterilized (T = 121°C). In each case one cube of sapwood and one of heartwood were placed onto Malt-Extract-Agar and incubated at 25°C for 50 and 100 days, respectively. At the end of the experiment cubes were superficially cleaned with 70% C_2H_5OH and dried again at 105°C. The differences of weights were noted. Wood decomposition was verified also by histological studies cutting naturally and artificially infested beech wood slices of 20 µm thickness in the radial-, tangential- and cross-section-area. Cuttings were mounted in 3% Safranin, 1% Auramin and 2% Methylene blue solution in order to accentuate the decomposition of cellulose or lignin.

3 RESULTS AND DISCUSSION

3.1 Differential diagnosis

All during the field investigations obtained fruit bodies could be assigned to the ascomycete *Annulohypoxylon cohaerens*. The fungus is not very frequent in Germany. In Saxony, where the investigations took place, *A. cohaerens* is even fixed in the "red list" as a missed species (Landesamt für Umwelt und Geologie 1990). The low knowledge about the fungus and its similarity to some other related ascomycetes can easily lead to wrong diagnoses. Under consideration of host species and macroscopic signs *A. cohaerens* could be especially mistaken for *Kretzschmaria deusta* or for *Annulohypoxylon multiforme*. *K. deusta* and *A. multiforme* create like *A. cohaerens* gregarious, confluent fruit bodies, which are discoid to pulvinate with a coarse or waved surface, dark brown to black. In addition, some confusion with *Hypoxylon fragiforme* may be possible because of the very similar young chestnut-brown gregarious fruit bodies (presence of anamorphs). All named ascomycetes are well able to grow on beech as host tree and their differentiation by signs is difficult. Therefore it could be useful to have additional distinguishing data. Beside the characteristics of ascospores and fruit bodies, the way of living (parasite or saprophyte), particular seasonal details or further characteristics (e.g. growth rate, production of imperfect reproductive states) can be helpful for determination of the species (*cf. Figure 3 - Table 1*).

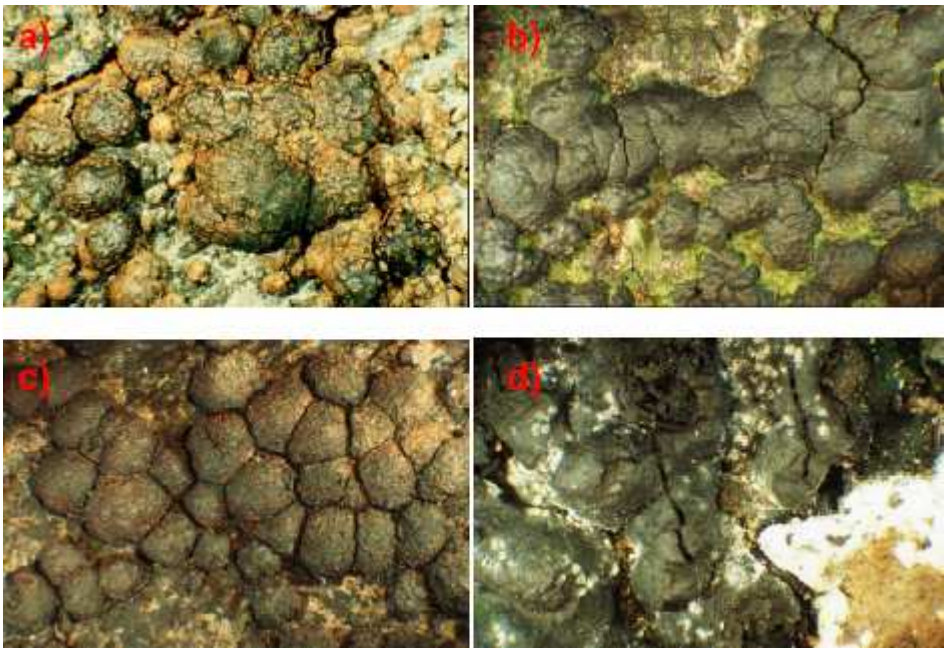


Figure 3. Gregarious fruit bodies of selected morphologically and physiologically similar ascomycetes on beech: a) Annulohypoxylon cohaerens, b) A. multiforme, c) Hypoxylon fragiforme, d) Kretzschmaria deusta

Table 1. Distinguishing marks of selected morphologically and physiologically similar ascomycetes on beech

Scientific Name	Way of living		Characteristic particularities		
	Sapro-phyte	Para-site	macroscopic	microscopic	seasonal
Annulohypoxylon cohaerens	+	(+)	gregarious fruit bodies, adnate to confluent, stroma inside brown to black	brown to dark brown spores of 9 to 12 µm length and 4 to 6 µm width and with *straight germ slit spore-length*	surface of young fruit bodies reddish to chestnut-brown, *discolours vinaceous in KOH*
Annulohypoxylon multiforme	+		gregarious fruit bodies, adnate to confluent, stroma inside brown to black	brown to dark brown spores of 9 to 12 µm length and 4 to 5 µm width and with *straight germ slit less than spore-length*	surface of young fruit bodies reddish to chestnut-brown, *does not discolour vinaceous in KOH*
Hypoxylon fragiforme	+		gregarious fruit bodies, but *not adnate to confluent*, stroma inside brown to black	brown, irregular ellipsoid spores of *10 to 15 µm length* and 5 to 7 µm width	surface of young fruit bodies *rust, bay, brick or orange-red*
Kretzschmaria deusta	+	+	gregarious fruit bodies, adnate to confluent, stroma inside *white to grey*	brown, ellipsoid spores of *25 to 35 µm length* and 7 to 8 µm width	young fruit bodies or incremental zone white to grey in spring, surface of older fruit bodies *carbonaceous and brittle*

3.2 Culture marks

In regard to the characteristics in pure culture (pattern and growth rate) most similarities can be ascertained for *A. cohaerens* and *H. fragiforme* (*Figure 4*). Cultures of both ascomycetes create a moderate aerial mycelium of initially white and later yellowish or reddish-brown, sometimes olive-coloured colouring. Growth rates of both species are high (optimum of *A. cohaerens* at 25°C: 5.7 mm/d and of *H. fragiforme* at 30°C: 5.5 mm/d), but growth is not considered uniformly concentric. *K. deusta* is distinguished from the previously compared species because of its more opulent and strictly white mycelium with the exception of creating sclerotic regions in advanced age. Between the studied fungi *K. deusta* has had the lowest growth rate (maximum: 3.7 mm/d at 25°C). Culture periphery grew equally to *A. cohaerens* and *H. fragiforme*. The aerial mycelium of *A. multiforme* is only slightly distinctive. Its grey-brown to grey-black colouration and its growth show a high uniformity. This fungus already achieves its optimal growth at 20°C (6.1 mm/d) and has therefore a more psychrophilic behaviour (growth rate at 30°C just 0.18 mm/d).

Differentiation of the species can also be effected by comparing asexual reproductive states. Especially *H. fragiforme* but also *A. cohaerens* formed opulently imperfect reproductive states within 14 days on Malt-Extract-Agar (2%). The formation of anamorphs of *A. multiforme* was very low and even absent for *K. deusta*. The asexual reproductive states of the related ascomycetes are assigned to different genera (Greenhalgh – Chesters 1968, Jong – Rogers 1972, Petrini – Petrini 1985, Petrini – Müller 1986). According to the actually available literature *A. cohaerens* and *A. multiforme* form an imperfect state which belongs to the *Virgariella*-type, the conidiogenous structure of *A. fragiforme* is *Nodulisporium*-like and that of *K. deusta* is *Hadrotrichum*-like.

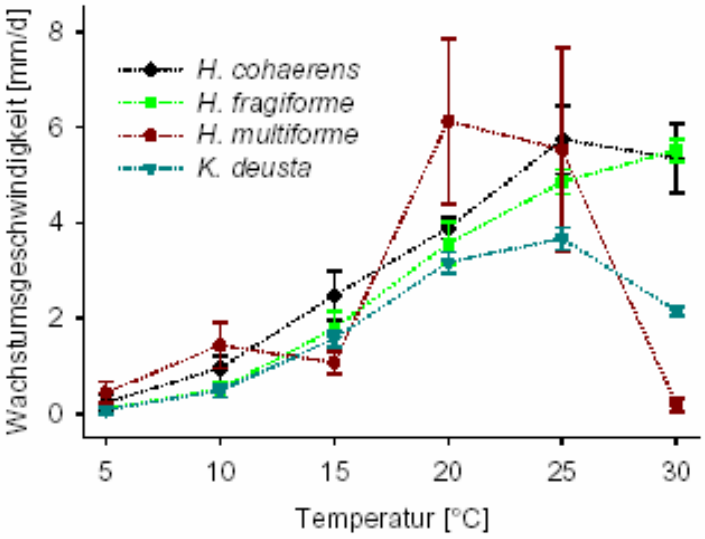


Figure 4. Growth rate of morpholigically and physiologically similar ascomycetes on beech

3.3 Wood decay

Tests according to Bavendamm (1928) resulted positive for all 10 tester strains cultivated on Tannin-Agar concerning the enzyme oxidase reaction. On the other hand on Guaic-Agar the evidence of extra cellular enzymatic activity was not provided.

The artificial inoculation of beech sapwood and heartwood samples demonstrated the potency of *A. cohaerens* to decompose wood. After 50 days the average loss of weight was 2.6% in the sapwood and 2.1% in the heartwood as well as 4.2% in the sapwood and 3.3% in

the heartwood after 100 days (*Figure 5*). Therefore *A. cohaerens* seems to be comparable with *K. deusta* in relation to its wood-decaying impact. Schwarze (1995) noticed a loss of weight by *K. deusta* of 4.6% after 85 days and Baum (2001 [a, b]) of about 10% after 100 days on artificially infected beech wood.

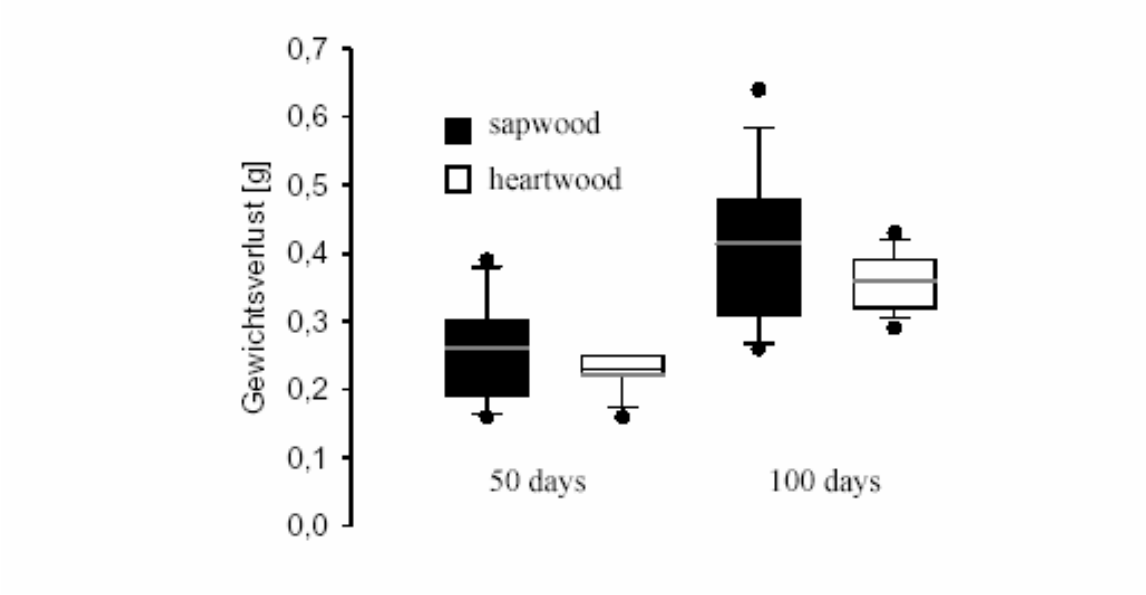


Figure 5. Wood decomposition by A. cohaerens on artificially infested beech wood

Microscopic analysis of the artificially and naturally infected material in reference to the strategy of spread and the establishment in woody tissue confirm the analogy between *A. cohaerens* and *K. deusta* (*Figure 6*).

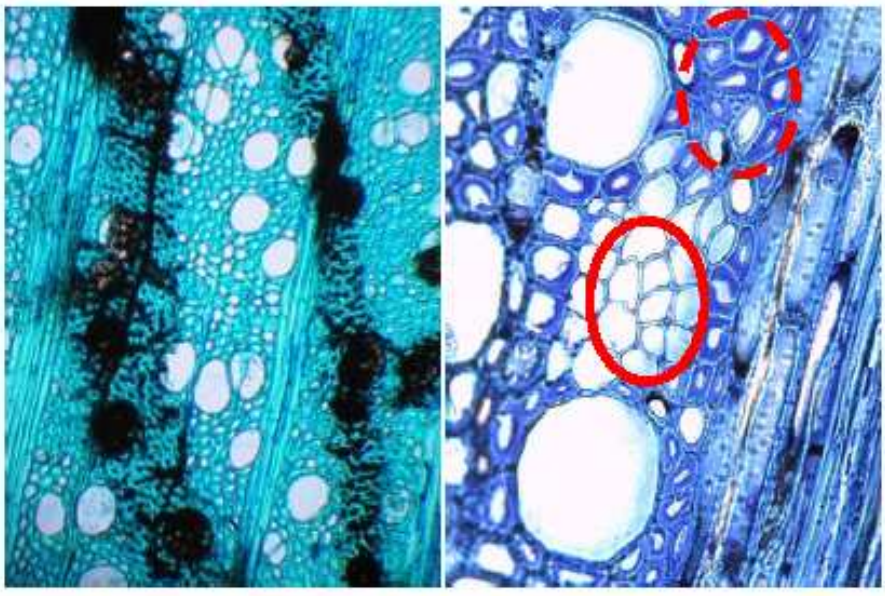


Figure 6. Establishment and decay by A. cohaerens in naturally infested beech wood. Creation of cavities and destruction of the secondary walls (left) and demarcations of pseudosclerotic plates (right)

Hyphes grow mainly in the secondary cell walls containing cellulose where the enzymatic wood corrosion leads to cavities (mouldering type). In consequence of the progressive decay (fusion of increasing holes) the whole secondary wall will finally be decomposed. To spread from cell to cell hyphes generally penetrate bordered pits. (Nilsson et al. 1989, Schwarze et al. 1995, Schwarze et al. 1999). Like *K. deusta* the fungus forms a distinct line of pseudosclerotic plates in decaying wood to be separated from healthy wood tissue or antagonistic fungi (Schwarze et al. 1993, Schwarze et al. 1999).

4 CONCLUSIONS

Up to now the rapid decline of older beech trees due to infection by the ascomycete *Annuloypoxylon cohaerens* can be seen as an exception. In Germany the fungus does not occur very frequent, it is more often found in European countries with stronger Atlantic influenced climate (Ireland, Great Britain and France). Usually *A. cohaerens* settles only saprophytic on beech wood, rarely it results as a week parasite on trees suffering from beech bark disease (Munk 1957, Ju – Rogers 1996, Sinclair – Lyon 2005). However, its aggressiveness and rate of spread in living beech trees recorded in a large city park in Dresden can be considered as parasitic. Infections effected without anthropogenic predisposition and caused mortality within a few years. Furthermore, *A. cohaerens* is able to cause an extended decay particularly in the sapwood, which belongs to the mouldering type.

Nevertheless by means of data is supposed that some abiotic influences led infested trees to a certain predisposition. While inundating by the river Elbe in 2002 some parts of the park were also flooded and had therefore increased groundwater levels for weeks. The following extreme hot and dry summer in 2003 aggravated the physiological stress of the trees. In connexion with the discussed climate change (e. g. increasing temperatures, decreasing of precipitation and concentration of climatic extremes) an impact of the existing host-pathogen interactions is probable. On the one hand stressed trees might be more susceptible against pathogens and on the other hand fungi might change strategies increasing their pathological importance.

In case of *A. cohaerens* can be presumed that diagnoses become more difficult and clear dissociation between *A. cohaerens* and *Kretzschmaria deusta* may be necessary if the fungus will be found more often in this aggressive, more parasitic manner.

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Cryphonectria parasitica in Sessile Oak in Hungary

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Abstract – Since 1999 occurrence of chestnut blight fungus (*Cryphonectria parasitica*) has been observed in sessile oak in Western and South-Western regions of Hungary in young and middle aged *Quercus petraea* stands mixed with *Castanea sativa*. Incidence and impact of the disease, vegetative compatibility type diversity of the pathogen, occurrence of the natural hypovirulence in oak, conversion capacity of the local hypovirulent strains and development of the symptoms in inoculated oak trees were studied in order to investigate the conditions of the biological control based on the hypovirulence in oak. In 2003 survey plots were designated in 12 infected oak stands. The rate of infection varied up to 23.02% and the mortality rate up to 5.76%. Isolations were performed in 2004 from survey plots and in 2006 from larger areas of two forest management units. In 2004 all isolates from oak were of virulent character, while 15.38% of the isolates showed hypovirulent features in 2006. Totally 15 VC types were delimited among the 174 isolates. The VC type diversity varied between 1 to 5 types/plot or forest subcompartment. Five local hypovirulent isolates showed full conversion capacity to the selected virulent strains of different VC types *in vitro*. Early appearing and progressive increasing of the symptoms were observed in the oak trees inoculated with virulent strains, however more than half of the trees healed during the second year after the inoculation. Some hypovirulent inoculations caused superficial altering of the suberisation, showing the establishment of the hypovirulent fungus in the bark. Our results show favourable conditions for successful application of the preventive control by disseminating the hypovirulent strains in young oak stands.

***Cryphonectria parasitica* / *Quercus petraea* / VC types / hypovirulence / conversion / symptom development / biological control**

Kivonat – ***Cryphonectria parasitica* kocsánytalan tölgyön Magyarországon.** A szelídgesztenye kéregrákját okozó *Cryphonectria parasitica* kocsánytalan tölgyön való tömeges előfordulását 1999 óta tapasztaljuk Nyugat- és Dél-Dunántúlon, fiatal és középkorú, gesztenyével elegyes kocsánytalan tölgy állományokban. A kórokozó elleni biológiai védekezés feltételeinek megismerése céljából vizsgáltuk a betegség előfordulását és jelentőségét, a kórokozó vegetativ kompatibilitási típusait, a hipovirulencia természetes előfordulását, a helyi hipovirulens törzsek konvertáló kapacitását és a tünetek kifejlődését mesterségesen fertőzött fákban. 2003-ban mintaterületeket jelöltünk ki 12 állományban, amelyeken a fertőzöttségi arány 23,02%-ig, a mortalitás pedig 5,76%-ig változott. 2004-ben a mintaterületek fertőzött fáiról, 2006-ban pedig két erdészet nagyobb területéről gyűjtöttünk izolátumokat. Hipovirulens törzseket a tölgyön 2004-ben nem találtunk, ezzel szemben 2006-ban a tölgyről származó izolátumok 15,38%-a hipovirulens jellegűnek bizonyult. A 174 izolátumból álló populációban összesen 15 VC típust különítettünk el. A VC típusok száma 1 és 5 között változott az egyes mintaterületekben, illetve erdőrészletekben. Öt helyi hipovirulens izolátum teljes konverziós kapacitást mutatott különböző VC típusú virulens törzsekkel szemben. Mesterséges fertőzési kísérlet első évében a tünetek gyors kifejlődését és fokozódását tapasztaltuk, de a fertőzés utáni második

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évben a fák több mint felénél a nekrózisok begyógyultak. A hiopvirulens oltások egyrészénél a parásodás felületi megváltozását észleltük, ami a hipovirulens gomba sikeres megtelepedésére utal. Eredményeink azt bizonyítják, hogy a hipovirulens törzsek terjesztése általi megelőző védekezés sikeres alkalmazásának feltételei kedvezőek a fiatal tölgy állományokban.

***Cryphonectria parasitica* / *Quercus petraea* / VC típusok / hypovirulencia / konverzió / tünetek kifejlődése / biológiai védekezés**

1 INTRODUCTION

Chestnut blight caused by *Cryphonectria parasitica* (Murrill) M.E. Barr was found first time in Hungary in 1969 in locality Nemeshetés county Zala (Körtvély 1970). The distribution and impact of the disease was investigated by the MÉM Laboratory for Chestnut Protection founded in order to find measures to reduce the spread of the epidemic (Eke - Gál 1975). Later investigations started since 1995 assessed the recent impact of chestnut blight in Hungary, occurrence of vegetative compatibility types and of the natural hypovirulence (Radócz et al. 1997). Successful application of the biological control based on the hypovirulence was performed in chestnut orchards and gardens in Sopron and surroundings (Vidóczi et al. 2005). Mass occurrence of the *C. parasitica* infection in oak stands (*Quercus petraea* (Mattuschka) Liebl.) was first observed in 1999 in Southwest Hungary (Surd 11D), where the assessment stated 14.9% infection and 5.7% mortality rate in 2001 (Gáncs 2002). Preliminary observations and data of the State Forest Service indicated that the disease is wide-spread in oak in South-western and Western regions of Hungary. Systematic investigations were started in 2003 in order to know the real impact of the disease and to find managing solution of this new forest protection problem.

Decreased virulence of *C. parasitica* strains containing cytoplasmatic dsRNS (*Cryphonectria hypovirus* 1, CHV1) constitutes the basis of the biological control of the pathogen. The isolates containing CHV1 are white or less pigmented in culture and do not form pycnidia or very sparsely only. These strains cause only superficial, non lethal symptoms in the bark of the hosts. The CHV1 can be transmitted from hypovirulent strains to the virulent ones by hyphal anastomosis, therefore the virulent strains become converted to hypovirulent. The transmission of the CHV1 happens only between the vegetative compatible partners belonging to the same VC type. The vegetative incompatibility system in *C. parasitica* is regulated by five to seven so-called *vic* gene. Compatible fungal strains belonging to the same VC type have identical alleles at all *vic* loci (Anagnostakis 1983, Bissegger et al 1997). The transmission of hypoviruses is negatively correlated to the number of *vic* genes that are different between VC types (Liu - Milgroom 1996). Increasing of the VC type diversity in the subpopulations of the fungus is possible by the recombination of *vic* genes during the sexual reproduction. The great diversity of the VC types influences negatively the success of the biological control. Natural hypovirulence occurs in many European countries and biological control programs were carried out in chestnut (Turchetti – Maresi 1991, Juhasova – Bernadovicova 2001, Heiniger – Rigling 1994, Vidóczi et al. 2005). Occurrence of *C. parasitica* in oak species also has been reported in some countries of Europe and USA (Torsello et al. 1994, Bissegger – Heiniger 1991, Luisi et al. 2002, Juhasova – Kulcsarova 2002, Radócz – Tarcali 2005), but the biological control in field has not been tried out in the field in connection with oaks.

2 MATERIALS AND METHODS

2.1 Incidence and impact of the disease in oak

Survey plots with numbered trees were established in 12 infected forest subcompartments distributed in 4 forestry districts. The rate of infection was assessed in the years 2003 and 2004 using an estimation scale: 1-trees free from symptoms, 2-trees with one perennial canker, 3-trees with more perennial cankers or diffuse canker, 4-dead trees. General healthy state and social position of the trees were also assessed as well as the position of the cankers: in trunk and/or in branches.

2.2 Isolation, native hypovirulence

Bark samples were taken from infected oak trees and some chestnut trees during the field evaluations. In 2004 samples were taken from all infected oak trees inside the survey plots. In 2006 regular field investigations and bark samplings were carried out in the area of two forest management units: Iharos (SEFAG Forestry and Timber Industry Co.) situated in South-Western Hungary and Hegyvidék (TAEG Co. Sopron) in the North-Western area of Hungary. The field investigations aiming to discover the infection of oak trees covered all oak stands presumed as threatened by the disease (oak stands of young and medium age mixed with chestnut). Bark samples were taken mostly from infected oak trees but also from some chestnut trees in order to investigate the VC type diversity of the subpopulations of the pathogen.

The isolation was performed on PDA after surface sterilization of the bark pieces using NaOCl solution (2 g/l active chlorine). The virulent or hypovirulent character of the isolates was estimated on the presence or lack of pigmentation and pycnidia in two week old cultures.

2.3 Vegetative compatibility

The vegetative compatibility was determined based on the formation or lack of merging barrage between the isolates in pairing test performed on PDA amended with pH indicator (Anagnostakis 1977, Powell 1995). The investigation of VC type diversity of the subpopulations was performed in several steps. First isolates from each forest subcompartiment were compared together, than one isolate was selected from each distinguished VC type and compared with the selected isolates from the other subcompartiments. Finally, the representative isolates of different VC types were paired with the tester strains EU 1-31 (Cortesi et al. 1998).

2.5 Conversion capacity of the hypovirulent strains

Native hypovirulent strains were selected for testing their conversion capacity against the local virulent isolates belonging to the most frequent and wide-spread VC types present in the forest subcompartments designated for the future field trials of the biological control. Six hypovirulent isolates were paired with 11 selected virulent strains in three repetitions. Conversion capacity of the hypovirulent strains was evaluated on the scale below:

-	no conversion
+	partial conversion: the growth reduction of the virulent strain is less than 50%
++	good conversion: the growth reduction of the virulent strain is more than 50%
+++	full conversion: the virulent strain is converted to hypovirulent in 100%

2.4 Inoculation tests

The inoculation experiment was performed in field in a *Q. petraea* stand of 11 years old near to Sopron, under permanent control. In May 2005 a number of 80 trees were inoculated with virulent isolates of *C. parasitica*, 10 trees with hypovirulent isolates and 10 trees were assigned as control. Four inoculation points were made in each tree. Holes of 5 mm diameter were bored in the bark and mycelial plugs from *C. parasitica* culture were placed in, than the wounds were covered with wax. Pieces of sterile medium were placed into the wounds of the control trees. The evaluation was effected at two weeks, 4 months, one year and two years after the inoculation. The symptoms cracking, deformation, sap flux, appearance of the stromata etc. were recorded and the length of phloem necrosis measured. The trees with developing stromata were regularly removed.

3 RESULTS AND DISCUSSION

3.1 Incidence and impact of the disease

The symptoms of *Cryphonectria parasitica* appeared mostly in form of perennial cankers in oak (*Figures 1 and 2*), however diffuse cankers also were observed in some weakened trees and dead trees killed by the fungus also occurred. Sexual stromata of the fungus were observed in most of the trees. Results of the field investigation are shown in the *Table 1*. In the year 2003 the rate of infection varied between 2.44 and 23.08% in the survey plots, mean 12.05%. The mortality was meanly 2.14% and varied up to 5.75%. In 2004 both the infection and mortality rates increased a bit in each plot, the mean values were 13.84 and 2.76% respectively. Trees with one perennial canker and with more perennial or diffuse cankers occurred approximately in the same rate, both a bit higher in 2004 than in the precedent year. Although the rate of mortality was not too high, the damages caused by the fungus are considerable, because of losses in quality timber due by deformed logs with large perennial cankers (*Figures 3 and 4*).

Table 1. Rate of C. parasitica infection in Q. petraea in the survey plots

Plot location (Forest subcompartment)	Nr. trees	2003				2004			
		Free from symptom	One canker	More/ diffuse cankers	Dead trees	Free from symptom	One canker	More/ diffuse cankers	Dead trees
		%	%	%	%	%	%	%	%
Csurgónagymarton 16A	155	86.45	8.39	3.23	1.94	81.94	11.61	3.23	3.23
Iharosberény 10H	155	81.94	7.10	5.81	5.16	81.94	6.45	6.45	5.16
Iharosberény 12C	149	90.60	3.34	2.68	3.34	90.60	4.03	2.01	3.34
Kőszeg 64D	123	97.56	2.44	0	0	95.93	3.25	0	0.81
Liszó 23F	150	88.67	5.33	3.33	2.67	86.00	6.00	5.33	2.67
Nagykanizsa 56E	148	81.76	8.11	10.14	0	79.73	6.76	11.49	2.03
Pogányszentpéter 5M	139	76.98	7.19	10.07	5.75	76.98	6.47	10.79	5.76
Simonfa 11J	134	93.28	2.99	2.24	1.49	93.28	2.99	2.24	1.49
Sopron 211C	131	95.42	3.05	1.53	0	93.13	3.05	3.05	0.76
Surd 9B	118	89.83	4.24	5.93	0	84.75	5.93	7.63	1.69
Total	**1402**	**87.95**	**5.35**	**4.56**	**2.14**	**86.16**	**5.78**	**5.28**	**2.76**





Figure 1. and Figure 2. Symptoms of Cryphonectria parasitica infection in Quercus petraea





Figure 3. and Figure 4. Large perennial cankers in Q. petraea caused by C. parasitica

3.2 Isolates, occurrence of native hipovirulence

In 2004 a number of 66 pure isolates were obtained from bark samples taken from infected oak trees inside the survey plots. All of them showed virulent features, intense pigmentation and abundant production of pycnidia *in vitro*. In 2006 totally 65 isolates were obtained from oak trees in forests of Iharos and Sopron. The 15.38% of these isolates were of hypovirulent character (*Figure 5*). In 2006 isolates were taken also from chestnut in order to investigate the population structure of the pathogen in the studied areas. The rate of hypovirulence was of 51.16% in the 43 isolates from chestnut bark (*Table 2*). The occurrence of hypovirulent strains in oak is an important result of this research. No former records of the natural

hypovirulence of *C. parasitica* in oak trees in Hungary. It was presumed, that only the virulent strains can infect the oak trees that are more resistant than chestnut.
The presence of hypovirulent strains in oak indicates the possibility of the biological control of the disease in oak. Since the hypovirulent fungus can stabilize in oak trees the conversion probability of virulent strains infecting them is more real. The great amount of natural hypovirulence among the isolates from chestnut indicates the reason of decreasing mortality in chestnut observed in the latest years.

Table 2. Occurrence of hypovirulence among the C. parasitica isolates from oak and chestnut

Year and location	*Quercus petraea*			*Castanea sativa*			TOTAL
	Total isolates No.	Hypo-virulent No.	Hypo-virulent %	Total isolates No.	Hypo-virulent No.	Hypo-virulent %	No.
2004							
Survey plots	66	0	0	0	0	0	66
2006							
Iharos	60	9	15.0	33	19	57.57	93
Sopron	5	1	20.0	10	3	30.0	15
Total 2006	65	10	15.38	43	22	51.16	108
TOTAL 2004 and 2006	131	10	7.63	43	22	51.16	174



Figure 5. Virulent and hypovirulent isolates of C. parasitica



Figure 6. Test of vegetative compatibility

3.3 Vegetative compatibility, population structure of the pathogen

Totally 174 isolates were involved in the VC compatibility test evaluated on the presence or lack of the mycelial barriers between the cultures (*Figure 6*). Mostly 1-3 VC types were distinguished inside of each forest subcompartment and survey plot, however 5 different VC types occurred among 12 isolates from the survey plot Liszó 23F (*Table 3*) .

Table 3. Vegetative compatibility of the isolates from survey plot Liszó 23F

	1	2	3	4	5	6	7	8	9	10	11	12
1		-	-	-	-	-	-	-	+	-	-	-
2	-		+	+	+	-	+	+	-	+	-	-
3	-	+		+	-	-	+	+	-	+	-	-
4	-	+	+		-	-	+	+	-	+	-	-
5	-	+	-	-		-	-	-	-	-	-	-
6	-	-	-	-	-		-	-	-	-	-	-
7	-	+	+	+	-	-		+	-	+	-	-
8	-	+	+	+	-	-	+		-	+	-	-
9	+	-	-	-	-	-	-	-		-	-	-
10	-	+	+	+	-	-	+	+	-		-	-
11	-	-	-	-	-	-	-	-	-	-		-
12	-	-	-	-	-	-	-	-	-	-	-	

Isolates No. 2, 3, 4, 5, 7, 8 and 10 represent the most frequent VC type in the site (identical with EU 12 tester strain). Isolates No. 1 and 9 belong to another VC type, while isolates No. 6, 11, and 12 are different from all and each represents a separated VC type.

Totally 15 VC types were distinguished in survey plots and two larger areas of the forest management units Iharos and Sopron. 12 VC types were compatible with one of the EU 1-31 tester strains, while 3 VC types (the representative isolates from Csurgónagymarton 4B, Iharosberény 21A and Iharosberény 10B) were different from all EU testers 1-31. (*Table 4*).

Table 4. Vegetative compatibility of C. parasitica isolates compared with EU 1-31 testers

Origin of the representative isolates	EU testers											
	1	2	3	9	10	11	12	13	14	15	20	28
Sopron 211C							+	+				
Nagykanizsa 56E					+				+			
Liszó 23F	+	+					+			+	+	
Surd 9B										+		
Csurgónagymarton 4B												
Csurgónagymarton 9C				+								+
Iharosberény 21A												
Iharosberény 12D											+	
Iharosberény 17I			+						+		+	
Iharosberény 18C								+				
Iharosberény 22H	+											
Iharosberény 8F								+			+	+
Iharosberény 10B												
Iharosberény 10C											+	
Iharosberény 10G									+			
Porrog 5H											+	

3.4 Conversion capacity of the local native hypovirulent strains

Different grades of reduction of virulent growth were observed at *in vitro* conversion test (*Figure 7 and Figure 8*). Five hypovirulent isolates (Hv) proved to possess a good and total conversion capacity to the most of the virulent strains (V) involved in the test (*Table 5*). Majority of the tested virulent strains representing different VC types were converted totally by at least one hypovirulent strain. However the hypovirulent isolate 104 Hv did not convert any of the tested virulent isolates and the virulent isolate 129 V was not converted by any of the hypovirulent strains. In this case we have to try further hypovirulent strains available in our collection of isolates.

Table 5. Conversion capacity of the selected hypovirulent isolates (Hv)

	30 V	31 V	32 V	45 V	49 V	52 V	56 V	57 V	105V	107 V	129 V
74 Hv	+++	++	+	++	+++	++	+	+++	++	+++	-
89 Hv	+	++	+++	+++	++	++	++	++	+	-	-
97 Hv	+	++	++	++	++	++	+++	++	+	-	-
104 Hv	-	+	-	-	+	-	-	-	-	-	+
112 Hv	+	++	+++	+++	++	+++	+	++	+	-	+
132 Hv	-	+++	++	+++	+++	+++	+	-	+	-	-



Figure 7. Different grades of conversion (white hypovirulent strains on left)

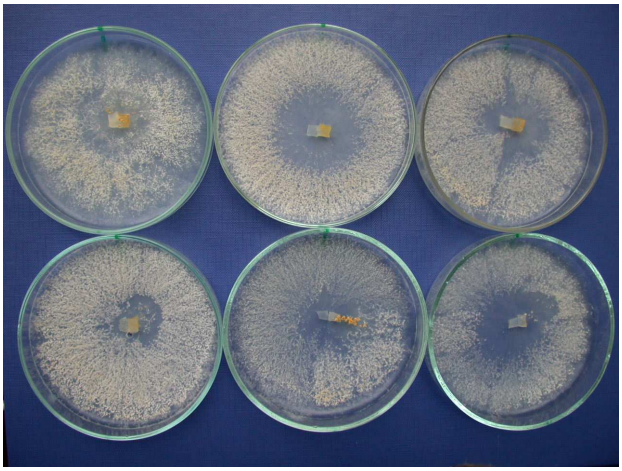


Figure 8. Examples of total conversion

3.5 Artificial inoculations, development of the symptoms

The field observations indicated that the incipient symptoms of *C. parasitica* infection are not easy to recognize in the oak trees, so a part of the infections may remain unobserved and unrecorded during the surveys. The inoculation tests aimed to observe the appearing and developing in time of the symptoms. The results are included in the *Table 6.*

At two weeks after the inoculation symptoms of cracking and deformation appeared in 28.75% of the trees inoculated with virulent strains and even the stromata of the fungus developed in 6.25% of the trees. The number of trees with symptoms and stromata increased progressively in the first year. Healing of the initial lesions was observed in more than half of

trees during the second year after inoculation (*Figure 9*). A rate of 56.25% of the inoculated trees apparently recovered in two years after the inoculation. The amount of 2.50 – 2.50% for the perennial cankers and dead tress is not relevant because of the regularly removing of the trees with stomata. The mean length of the phloem necrosis increased in the first year (21.48 mm to 33.94 mm), while in the second year decreasing of the average length was observed (15.24 mm) due by the callus building and healing of the lesions.

Table 6. Developing of the symptoms in Q. petraea inoculated with virulent C. parasitica

Date of inoculation:	Date of evaluation			
2005.05.05	Frequency of the symptoms (%)			
Symptoms	2005.05.20.	2005.09.08.	2006.06.20	2007.05.05
Cracking, deformation	28.75	51.25	20.00	-
Sap flux	8.75	1.25	-	-
Stromata (cumulative)	6.25	22.50	38.75	38.75
Healing (callus building)	-	52.50	5.00	-
Recovered (closed lesions)	-	-	35.00	56.25
Perennial canker	-	-	-	2.5
Dead	-	-	2.5	2.5
Mean necrosis length mm	21.48	33.94	15.24	-

The wounds recovered during the first year in the trees inoculated with hyporulent strains and control trees, no or minimum phloem necrosis occurred. Slight alteration of suberization was observed in some trees inoculated with hypovirulent strain indicating the presence of the hypovirulent fungus in the outer bark (*Figure 10*).



Figure 9. Healed virulent inoculations



Figure 10. Changing suberisation in the hypovirulent inoculations

4 DISCUSSION

Q. petraea is one of the most valorous forest tree species in Hungary covering an important amount of about 12% of the forested areas. At present infection of *C. parasitica* occurs in sessile oak in young and middle aged stands mixed with *Castanea sativa*. Regarding the excellent epidemic capacity of the pathogen, the threat of spreading of the disease is real to larger areas of oak forests towards the distribution of chestnut.

The natural hypovirulence occurs in 7.63% in oak and 51.16% in chestnut in the investigated areas. Decreasing of the disease severity in chestnut trees is a consequence of the natural spreading of the hypovirulent strains containing CHV. Since these strains are rare in oak trees, their dissemination may be a good way to decrease the incidence of the infection. The native hypovirulent strains possess a very good conversion capacity against the local virulent strains. The VC type diversity of the subpopulations of the pathogen is not too high in the most of the sites at this time. These circumstances offer favourable conditions for biological control of the disease in oak. The number of VC types is increasing in consequence of sexual reproduction, so continuous survey on population structure of the pathogen is needed for the success of the biological control.

Curative and preventive biological control technologies are known in the case of chestnut: inoculation with compatible hypovirulent material around the virulent bark lesions and dissemination of hypovirulent strains by inoculating sprouts, seedlings and healthy trees. Both procedures help the natural spread of the hypovirulent strains of the pathogen. Changes in population structure of the fungus favouring the rate of the hypovirulent strains occurs in natural way, too, but this process is slow and generally starts late after the establishment of the virulent strains. Introduction and dissemination of vegetative compatible hypovirulent strains capable to natural spread in oak trees seems to be the most practicable plan for the preventive control of the disease in oak stands.

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Chestnut Blight and its Biological Control in the Sopron Hills, Hungary

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Abstract – The distribution of chestnut blight caused by *Cryphonectria parasitica* was surveyed in the forests of Sopron Hills. The infection rate was different in the forest subcompartments ranging up to 35%. Six vegetative compatibility types were detected, two of them were wide spread and present all of the hills. Converted hypovirulent strains were tested for practical control of the disease. More than 90% of the treated trees healed in eight years after the inoculation in the experimental plot.

***Cryphonectria parasitica* / vc type / biological control**

Kivonat – A szelídgesztenye kéregrákja és az ellene való biológiai védekezés a Soproni-hegységben. Vizsgálatunk tárgya a *Cryphonectria parasitica* által előidézett szelídgesztenye-kéregrák a Soproni-hegység területén. Megállapítottuk, hogy a fertőzöttség mértéke változó, maximálisan 35%. A gyűjtött kéregmintákból a kórokozó hat különböző vegetatív kompatibilitási típusát különítettünk el. Közülük kettő széleskörűen elterjedt, jelen van a hegység egész területén. A betegség leküzdésére konvertált hipovirulens törzseket alkalmaztunk. A kísérleti területen a kezelt fák több mint 90%-a nyolc évvel az első kezeléseket követően meggyógyult.

***Cryphonectria parasitica* / vc típusok / biológiai védekezés**

1 INTRODUCTION

The causal agent of chestnut blight the ascomycete *Cryphonectria parasitica* was introduced into Europe from Asia, mediated in America. A few decades ago the disease reached Sopron Hills and caused epidemic infections and destruction of chestnut trees in plantations and semi-natural forest associations (Körtvély 1970). The control of the disease and the stop of the epidemic are possible only by one effective biological method based on dissemination of the hypovirulent strains of the pathogen. The cytoplasm of the hypovirulent strains contains double-stranded RNA (later named *Cryphonectria hypovirus 1*, CHV-1) that is related to the reduction of the virulence of these strains. The dsRNA can be transmitted to a virulent strain in laboratory and natural conditions, too. After this transmission the recipient virulent strain will become hypovirulent. The tree can overcome the attack of hypovirulent strains, the

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cambium and phloem remain living under the hypovirulent cankers. The transmission of CHV-1 is possible only between the vegetative compatible strains. By this reason the VC type diversity of the fungus populations is a cardinal condition of the success of the biocontrol. The biological control based on the hypovirulence was tried out successfully in practice in many European countries (Grente and Berthelay-Sauret 1978, Turchetti and Maresi 1991, Juhasova – Bernadovicova 2001, Robin – Heiniger 2001, Heiniger – Rigling 1994).

2 MATERIALS AND METHODS

2.1 Distribution of the disease

In autumn 1996 investigations were carried out on 4070 hectares of Sopron Hills (Sopron, Ágfalva and Harka localities). The determination of infection was performed by linear sampling in each forest subcompartment. Bark samples for further laboratory investigations were collected from each subcompartment where the disease occurred.

2.2 VC type diversity of the pathogen

The pathogen was isolated from the bark samples and a strain collection was established for later vegetative compatibility tests. A number of 51 isolates were analyzed by vegetative compatibility test using the pairing method developed by Anagnostakis (1977) (*Figure 1*). The delimited VC types were than identified by pairing with the EU tester strains 1-31 (Cortesi et al. 1998).

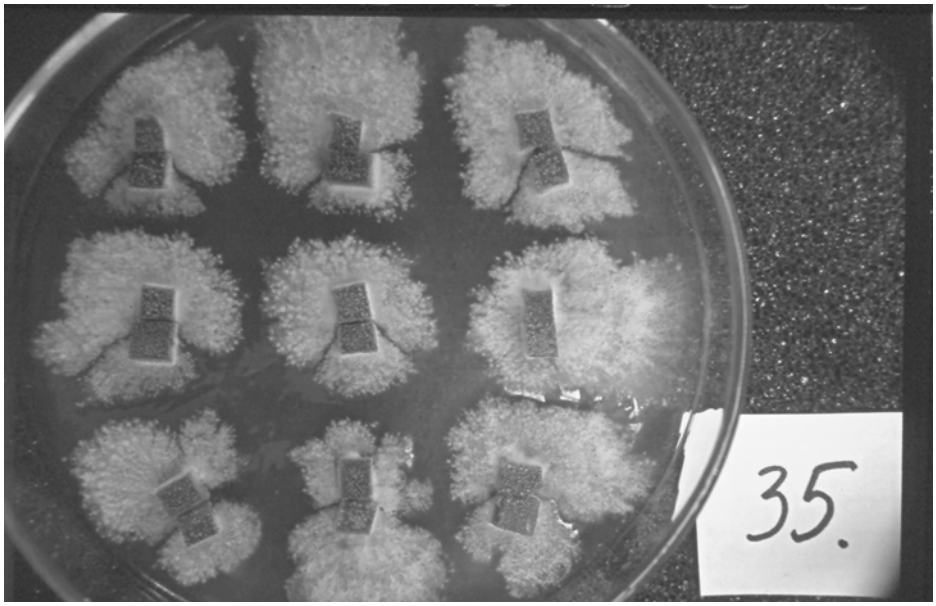


Figure 1. Compatibility test of the C. parasitica isolates

2.3 Hypovirulence, conversion of the local virulent isolates

The white color of the cultures indicates the presence of dsRNA. No native hypovirulent strains occurred among the isolates from Sopron Hills in 1996, so the identified vegetative compatibility types were converted in vitro with hypovirulent strains originated from other regions of West Hungary, where the natural hypovirulence was revealed a few years ago (Radócz et al. 1997). The character of the virulent strain turned into hypovirulent type after a short initial growth in case of positive conversion (*Figure 2*).

2.4 Prove of dsRNA content and genetic identity of the converted isolates

Molecular investigations were carried out in order to prove the presence of dsRNA in the converted strains and in some local isolates that seemed to be hypovirulent because of their intermediate morphological characteristics. The investigated strains were: S11, S18, S18xSa3, S21, S21xR5, S21xIhb2, An as hypovirulent control strain and S23 as virulent control. Isolation and analysis of dsRNA was performed by phenolic extraction method. Samples of extractions were separated by electrophoresis using 0,8% agarose gel in TBE buffer at 110 V, dsRNS molecules were stained with ethidium bromide (Wronski et al. 1997).

RAPD technique was used to determine the genotypes of the converted strains by using a version of Wronski et al. (1997). The DNA-containing supernatant was originated from the dsRNA extraction protocol. Polimerase chain reaction (PCR) was performed by six ten-base oligonucleotide primers. After PCR the DNA fragments were separated by electrophoresis using 1% agarose gel in TBE buffer at 110 V, after staining with ethidium bromide, and examined under UV light. Two virulent isolates, and three converted hypovirulent isolates were investigated. The molecular works were carried out in the laboratory of Österreichishes Forschungscentrum Seibersdorf.

2.5 Application of the biological control

Field trials with converted hypovirulent strains were carried out from autumn 1996 to 2000. In the experimental plot Ágfalva 37 diseased trees were treated. In addition, individual chestnut trees were inoculated in several gardens and orchards in Sopron. In the treated trees every canker was artificially inoculated roundly with mycelia of adequate (compatible) hypovirulent strain (*Figure 2*).



Figure 2. Inoculation holes in the bark

At the first step cork borer wounds were carried out in the living bark, in distances 4-5 cm from each other. These holes were filled with the hypovirulent inoculum (pieces of medium with mycelia), and finally the holes were closed with wax not containing fungicides. Parallel the inoculations the dried branches were removed. The inoculated trees were evaluated every year. In a few cases when the healing was uncertain the treatment was repeated using hypovirulent strains of two VC types. This was necessary because of occurrence of two VC types in the site, one dominant (SI) and one less frequent (SII). Strongly diseased, severe cankered trees were treated differently: not only cankers were treated roundly, but the stem also was inoculated vertically from the base to the branches up to the crown. Infected bark of heavy sporulated cankers was removed, in order to decrease the sources of inoculum.

3 RESULTS

3.1 Distribution of the disease

During the area surveying we recognized that in forests near the city chestnut blight is common, infection rate is more than 30%, and the blight occurrence in the farer areas is only sporadic. This result indicates that the pathogen was introduced first into the gardens and orchards inside the town and suburbs from where it was spread to the neighboring forest areas.

3.2 VC type diversity

Six vegetative compatibility types of the pathogen were delimited in the Sopron Hills. Three vegetative compatibility types are dominant (SI, SII, SIII), the other types (SIV, SV, SVII) are represented with only a few isolates. Near to the hills a seventh (SVI) type was detected. Six types were compared with EU tester strains 1-31.

The SI type was compatible with EU 13, this type is dominant in most of the studied areas, and 51.2% of the isolates belong to this type. SII representing 14.3% of the isolates was compatible with EU 6, 9 and 23. It is dominant in some forest areas closed to the chestnut cultures. SIII occurs locally in the West part of the Hills but there it causes massive infection of more than 30%. It is compatible with EU 1 and 17. The locally occurring SIV is compatible with EU 12, SV and SVII represented by a few isolates are compatible with a few EU types (*Table 1*).

Table 1. VC types of C. parasitica in Sopron Hills

VC Type	Compatible EU testers	Conversion dsRNA donor
SI	13	A3 x BF
SII	6, 9, 23	IHB2, R5
SIII	1, 17	S3
SIV	12	-
SV	1, 16, 22	-
SVII	1, 25, 28	-

3.3 Conversion of the local virulent strains

The isolates from Sopron Hills showed virulent character in culture, orange pigmentation and abundantly production of pycnidia. Local virulent strains were converted in vitro by using compatible hypovirulent strains as dsRNA donors available in our strain collection, originated from other regions of Hungary namely Zala and Somogy Counties (*Table 1*). The initial growth of virulent strain is changed into hypovirulent by the successful conversion (*Figure 3*).

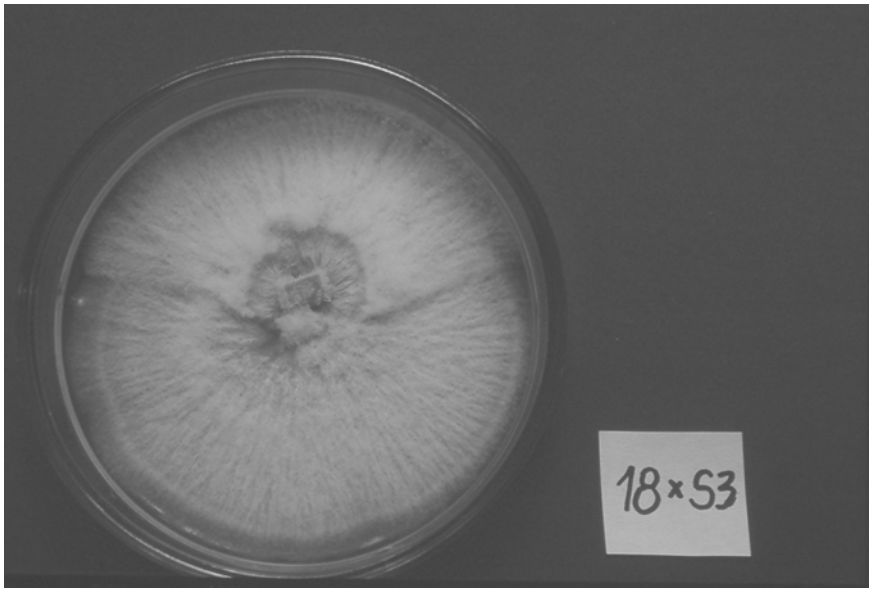


Figure 3. Successful conversion in vitro

3.4. Content of dsRNA and genetic identity of the converted strains

The conversions were verified by methods of molecular biology. The presence of dsRNA (molecular weight 12.7 kb) was detected in all of the converted strains. The dsRNA was not found in the local isolates, so the lack of natural hypovirulence was proved in the hills in 1996. The converted strains should be genetically identical with the original isolates, this fact is important for avoid the introduction of strange genotypes into the experimental plot. Genetic identity between the converted strains and original virulent strains was also demonstrated by RAPD technique with all the three converted strains.

3.5 Biological control

Efficiency of hypovirulent control against blight in field trials was investigated from 1996 to 2000. In 8 years 90% of treated trees recovered. The margins of the virulent lesions were healed and growth of the canker was stopped in the bark. (*Figure 4*). More than 90% of the treated trees healed in eight years after the inoculation in the experimental plot (*Figure 5*). The combined inoculations with two hypovirulent strains also were efficient. The strongly diseased, severe cankered trees treated by vertically inoculation in the stem also healed partially because of a protection zone created in the bark, which is not available for the virulent strains, so the water and nutrition transport is ensured and the trees survive the attack.



Figure 4. Healed cankers

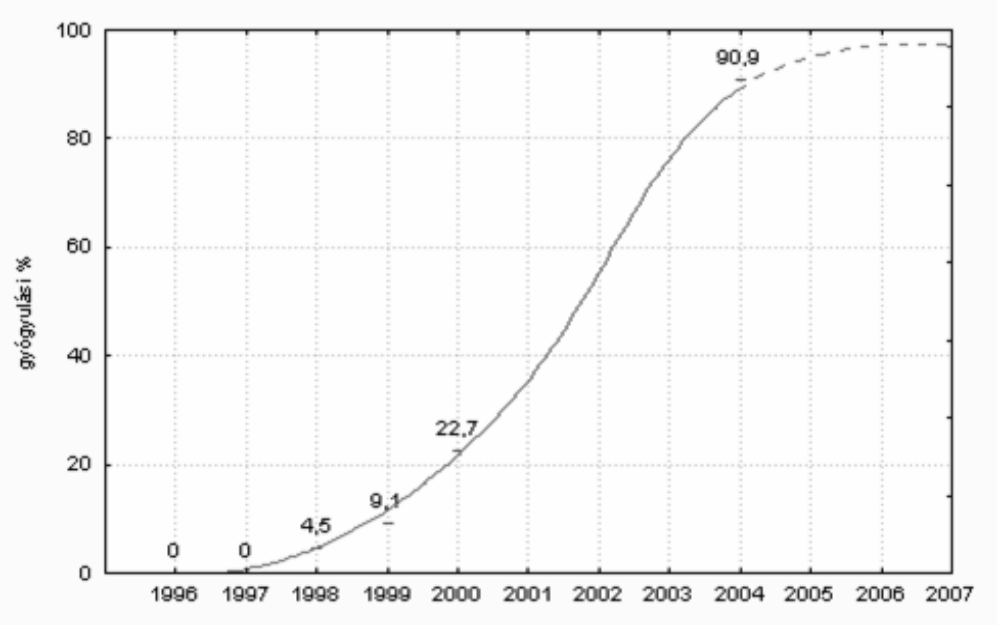


Figure 5. Healing rate of the treated trees in time

4. DISCUSSION

The aim of this study was to characterize the population of *C. parasitica* and to adapt the biological control onto the Sopron Hills. According to our results and the data regarding the distribution of VC types in Europe and Hungary (Robin – Heiniger 2001, Radócz 2001) we deducted to the spread of the epidemic. The first wave of the blight was entered from the Kőszeg Hills to the Sopron Hills. Later the second wave of epidemic has reached the hills from farer South-Western Transdanubia possible by anthropogenic effect. Then a vegetative compatibility type dominant in Austria occurred in the forests close to the border, this type has local area, but great infection capacity. The sexual propagation of the pathogen has begun.

During the treatments it was found that year for year new vegetative compatibility types appear, so the biological control needs continuous survey on population structure of the pathogen. The natural spread of hypovirulence started after the treatments by a meanly rate of approx. 2 m a year. In the knowledge of population structure of the fungus the first experiments in Ágfalva were extended and a practical biocontrol programme started in the chestnut stands in the surroundings of Sopron. Since *C. parasitica* causes necrotic cankers and destructions also on sessile oak, and in Sopron Hills the blight is common in oak forests mixed with chestnut, more investigations are needed on spreading of the pathogen and the hypovirulence on oak species.

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Other diseases

New Results of the Research on the Alder Phytophthora Disease in Hungarian Alder Stands

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Abstract – Department of Forest Protection of the Hungarian Forest Research Institute started a long term monitoring project in 2002 in order to study the Alder *Phytophthora* disease, particularly to determine the importance of the disease, the extent of the damage and the severity of the infection at different regions of the country. According to the results of four years investigations, *Phytophthora* and its typical symptoms are present all over Hungary. The rate of the infected trees, the intensity and spreading of *Phytophthora* infection, together with the rate of mortality shows, that this disease currently do not poses a direct danger to the existence of alder stands. *Phytophthora* symptoms were found in 75% of the stands but in 50.9% of the plots with *Phytophthora* present the infection rate is lower than 1%.

***Phytophthora alni* / Alder decline / *Phytophthora* disease of Alder**

Kivonat – Az éger fitoftórás betegsége kutatásának új eredményei a magyarországi éger állományokban. 2002-ben az ERTI erdővédelmi osztálya egy többéves kutatási programot indított a magyarországi égerpusztulással kapcsolatosan, melynek célja a fitoftórás megbetegedések országos elterjedésének felmérése, a károsodott területek nagyságának megállapítása, és a fellépő fertőzések mértékének meghatározása. Négy év kutatási eredményei azt mutatják, hogy a jellegzetes fitoftórás tünetek mindenütt jelen vannak a magyarországi égeresekben. A fertőzött fák aránya, a fitoftórás megbetegedések intenzitása és terjedése, a valamint a mortalitás mértéke azt mutatja, hogy ez a betegség nem veszélyezteti közvetlenül az állományok jövőjét. *Phytophthora* okozta tünetek az állományok 75%-ban fordultak elő, de csak a vizsgált mintaterületek 50,9%-ban volt magasabb a fertőzés mértéke 1%-nál.

***Phytophthora alni* / égerpusztulás / az éger fitoftórás betegsége**

1 INTRODUCTION

Common alder (*Alnus glutinosa*) covers 2.9% (47,000 hectares) of the forested area of Hungary. (ÁESZ 2002) The majority of alder forests can be found in Transdanubia (West of the Danube) and can be classified as "lowland fen type". Mountain riparian alder forests are less important from economical point of view (Danszky 1973; Bartha - Mátyás 1995) *(Figure1).*

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The alder decline caused by *Phytophthora* - which was already observed in several European countries - was first recorded in Hanság (NW Hungary), close to the Austrian border in 1999. (Brasier et al. 1995; Gibbs 1995; Erwin – Ribeiro 1996; Gibbs et al. 1999; Cech 1997, 1998; Varga 2000; Nagy et al 2000; Koltay 2001)

Department of Forest Protection of the Hungarian Forest Research Institute started a long term monitoring project in 2002 in order to study the decline, particularly to determine the importance of the disease, the extent of the damage and the severity of the infection at different regions of the country.

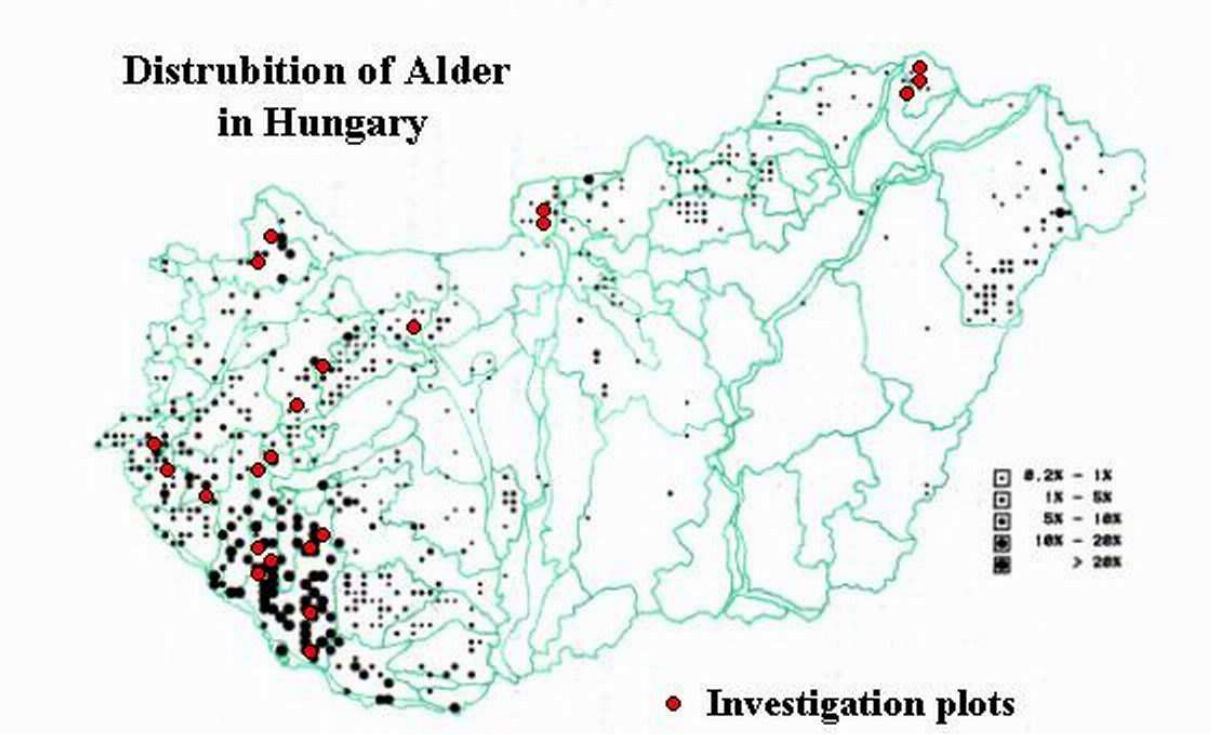


Figure 1.

2 MATERIAL AND METHODS

During 2002 and 2003 228 forest sub-compartments were investigated all over Hungary. In the investigated stands we counted the infected trees on randomly selected plots (0.25-0.50 ha each). The main symptom considered was the presence/absence of tarry spots on the stem and the root collar.

In addition to the country-wide survey, 22 long-term experimental plots were established in 2002 in different alder stands in order to carry out a more detailed study on the process and characteristics of the disease. *(Figure1)*

Stands with more than 3% of infected trees and belonging to different age classes and origin (seed/coppice) were selected as experimental plots. All plots contain 50 sample trees except two containing 100. The plots were examined once in 2002, and twice in 2003-2005, late spring and late autumn (Koltay et al. 2002).

3 RESULTS

Importance of the disease, the extent of the damage and severity of infection are different regions of the country. *Phytophthora* and its typical symptoms were found all over Hungary, both in riparian and fen type alder forests. *(Figure 2)*

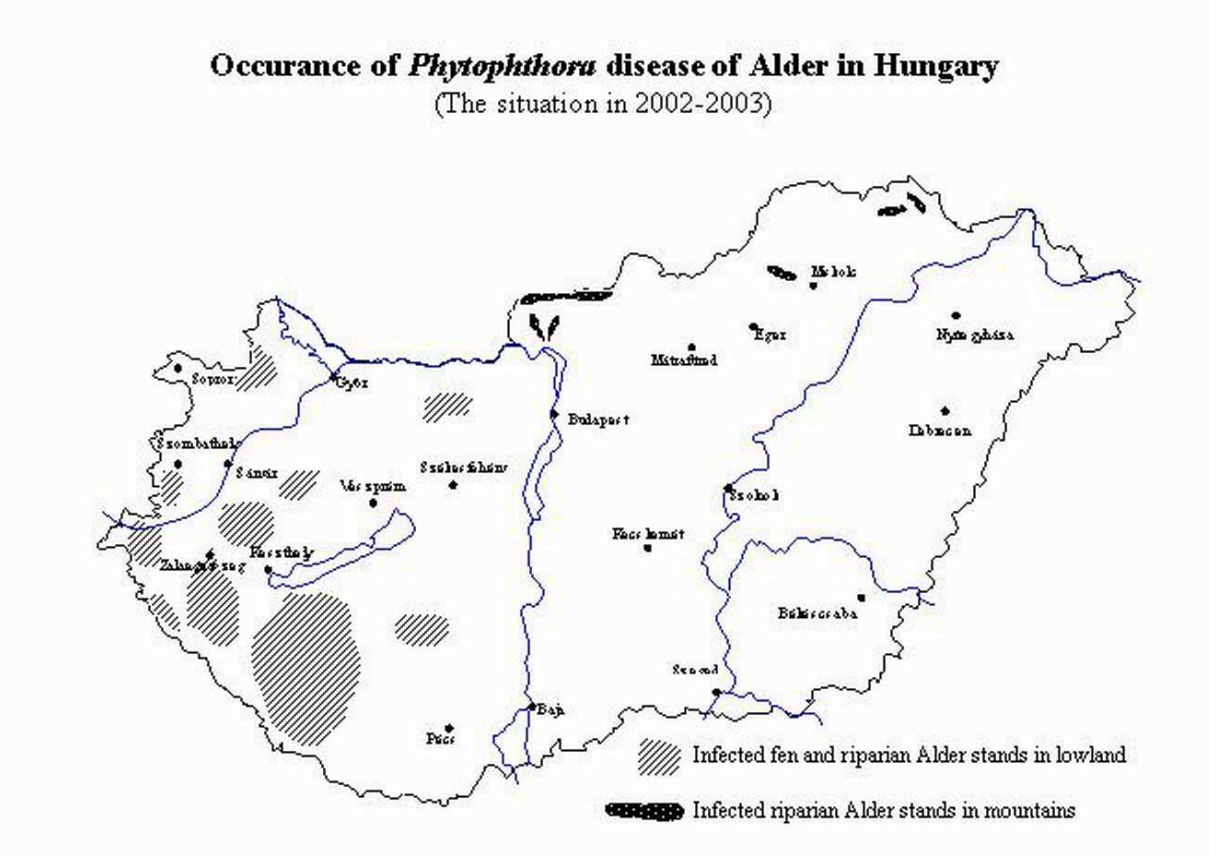


Figure 2.

Alder decline caused by *Phytophthora* can be observed in all age classes, independently of the height class of the trees. The measure of the infection and its distribution are very heterogeneous in Hungary. We found both heavily infected and healthy alder stands in each region. According to the data gained from the 228 plots investigated, symptoms of *Phytophthora* were found in 75% of the stands. *(Table 1)*

Table 1. Rates of Phytophthora infection on sample plots

Rate of Phytophthora Infection (%)	Investigated Alder Forests (2002-2003)	
	number	%
0	57	25,0
> 1	87	38,2
1 – 10	44	19,3
11 – 20	17	7,5
21 – 30	12	5,3
31 – 40	5	2,2
41 – 50	0	0
50 <	6	2,6
Sum	228	100,0

In 50.9% of the plots with *Phytophthora* present the infection rate is lower than 1%. In 25.7% of the plots the infection rate is between 1 and 10%, and in 23.4% of the plots the infection rate is higher than 10%. The heaviest infection - where tarry spots were found on 78% of the sample trees - was recorded in Hévíz (near the lake Balaton).

3.1 Characteristics of *Phytophthora* infection

The *Phytophthora* infection is indicated by the appearance of tarry spots on the bark. New tarry spots usually appear from autumn to spring. Most of the spots appear on the root swelling and lower part of the trunk. Sapwood always dies under the tarry spots.

Presence of tarry spots on trunk does not cause immediate crown decline. The crown usually looks healthy for 1 or 2 years after the tarry spots appeared. The rate of the crown decline depends on the progress rate of the sapwood necrosis.

After a longer or shorter time following *Phytophthora* infection, the crown becomes sparse and the leaves become abnormally small and yellow. More and more twigs and branches fall down and finally the tree dies.

3.2 Characteristics and progress of the disease

371 (30.9%) of the 1,200 sample trees at 22 experimental plots were infected by *Phytophthora* between 2002 and 2005. 75 (6.2%) trees were killed by *Phytophthora* infection during the same period.

9.3 % of the 75 dead trees died quickly (within two years), 18.6% died after three years and 72.1% died slowly, after four or more years following the infection. This means that trees usually die only longer time after the infection.

Health status of 5.3% of the infected did not change during the four years of investigation years and 2.1% of infected trees became healthy. These trees could fight the infection efficiently.

Incidence of *Phytophthora* infection is more frequent on intermediate and suppressed trees but there is no significant relation between infection frequency and the age of tree stands. *(Table 2)*

Table 2. Phytophthora infection frequency in different height class

	Height class							
	Prominent (1)		Dominant (2)		ntermediate (3)		Supressed (4)	
	db	%	db	%	db	%	db	%
Infected trees with tarry spots	41	25,6	226	28,6	73	39,9	30	45,5
Total trees	160	100	791	100	183	100	66	100

4 CONCLUSIONS

According to the results of four years investigations, *Phytophthora* and its typical symptoms are present all over Hungary. The rate of the infected trees, the intensity and spreading of *Phytophthora* infection, together with the rate of mortality shows, that this disease currently do not poses a direct danger to the existence of alder stands.

It seems, that by the start of the monitoring project in 2002 (or more likely even earlier) the epidemic already reached its peak. Since then both the number of new infections and diseased trees are decreasing slowly. *(Table 3)*

Table 3. Rates of new necrosis and *Phytophthora* infection on sample plots

	New mortality **%**	New *Phytophthora* infection **%**
2002	-	8,5
2003	3,3	6,2
2004	2,3	6,5
2005	1,3	5,3

For the forest-management – except for some extreme highly infested forest stands – the *Phytophtora* caused no remarkable economical damage, although the possibility of this is permanently present through rise of a new and more intense epidemic.

Besides, we found, that the rate of sudden decline is relatively low. In most cases the decline-process lasts more than four years. According to our monitoring, the trees sometimes are able to defeat the disease with their natural defensive mechanisms.

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Heterobasidion annosum Complex in Turkey

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Abstract – Presence of *Heterobasidion annosum* in Turkey has been known at least for seventy-five years. During this period information on host species and distribution has accumulated. The host range includes four *Abies* taxa, three species of pine, and *Picea orientalis*. The known distribution covers roughly the whole zone of forests surrounding the dry Central Anatolian plateau, i.e. the coastal regions of Black Sea, Aegean Sea, and Mediterranean Sea. However, there is very little information about the pathogenicity and damage caused by *Heterobasidion* species. The current knowledge about the known distribution of members of *H. annosum* complex, and pathogenicity on different hosts in Turkey is reviewed.

forestry / root and butt rot / conifer / host range / distribution

Kivonat – A *Heterobasidion annosum* komplexum Törökországban. A *Heterobasidion annosum* törökországi előfordulása legalább hatvanöt éve ismert. Ezen idő alatt felhalmozódtak a gazdanövény fajokra és a gomba elterjedésre vonatkozó információk. A gazdanövénykört négy *Abies* taxon, három *Pinus* faj és a *Picea orientalis* képezi. Az ismert előfordulási területek a száraz Közép-Anatóliai fennsíkot övező erdők, a Fekete-tenger, Égei-tenger és Földközi-tenger partvidékei. Nagyon kevés információnk van a *Heterobasidion* fajok patogenitásáról és az okozott károkról. A dolgozat a *H. annosum* komplex jelenleg ismert törökországi előfordulására és a különböző gazdákkal szembeni patogenitására vonatkozó ismeretek revízióját tartalmazza.

erdészet / gyökér- és tőkorhadás / fenyő / gazdanövénykör / elterjedés

1 INTRODUCTION

Species belonging to the *Heterobasidion annosum* -complex cause economically important root and butt rot of conifers in the northern boreal and temperate zones.

In Eurasia, three species of *Heterobasidion* have been identified: *Heterobasidion parviporum* Niemelä & Korhonen, *Heterobasidion annosum* (Fr.) Bref. sensu stricto, and *Heterobasidion abietinum* Niemelä & Korhonen (Niemelä – Korhonen 1998). *H. parviporum* occurs mainly in spruce and fir forests of northern and eastern Eurasia, whereas *H. annosum* s. s. grows mostly in pine forests and has mainly western distribution. The known distribution area of *H. abietinum* is smaller: it lives on several native species of *Abies* occurring in southern and central Europe, and Asia Minor (Korhonen – Dai 2005, Doğmuş-Lehtijärvi et al. 2006).

Presence of *H. annosum* sensu lato in Turkey (Asia Minor) has been known at least for seventy-five years. The first survey of Turkish macrofungi was done by Pilat (1932) who

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found *H. annosum* s.l. to occur on *Abies nordmanniana* ssp. *bornmülleriana* (Mattf.) Coode & Cullen in the Ilgaz Mountains north of Ankara. Since that date information on host species and distribution has accumulated. The aim of this paper is to briefly summarize the current knowledge about the host range and distribution of the members of *H. annosum* complex, as well as their pathogenicity on different hosts in Turkey.

2 DISTRIBUTION AND HOST RANGE IN TURKEY

2.1 Distribution

In Turkey, forests are located on the slopes of the mountain ranges that run parallel to the coast lines of the Black Sea and the Mediterranean Sea, while the dry inner part of the country is mainly grassland. The known distribution of *H. annosum* s.l. covers approximately the whole area of coniferous forests; records are missing mainly from pine forests near the Aegean coast. *(Figure 1)*. In southern Turkey *H. annosum* s.l. has been recorded from the provinces of Antalya, Isparta, Konya, Mersin, Adana, and Hatay (Selik 1973, Kotlaba 1976, Doğmuş-Lehtijärvi et al. 2006, Y. Balcı, Morgantown, 2007, personal communication, Lehtijärvi, unpublished data). In northern Turkey the distribution extends from Kazdağı Mountains in the Aegean part of Balıkesir province to Artvin near Georgian border (Pilat 1932, Lohwag 1957, Selik 1973, Balcı 1998, Doğmuş-Lehtijärvi et al. 2006, Doğmuş-Lehtijärvi et al. (in press), Y. Balcı, Morgantown, 2007, personal communication).

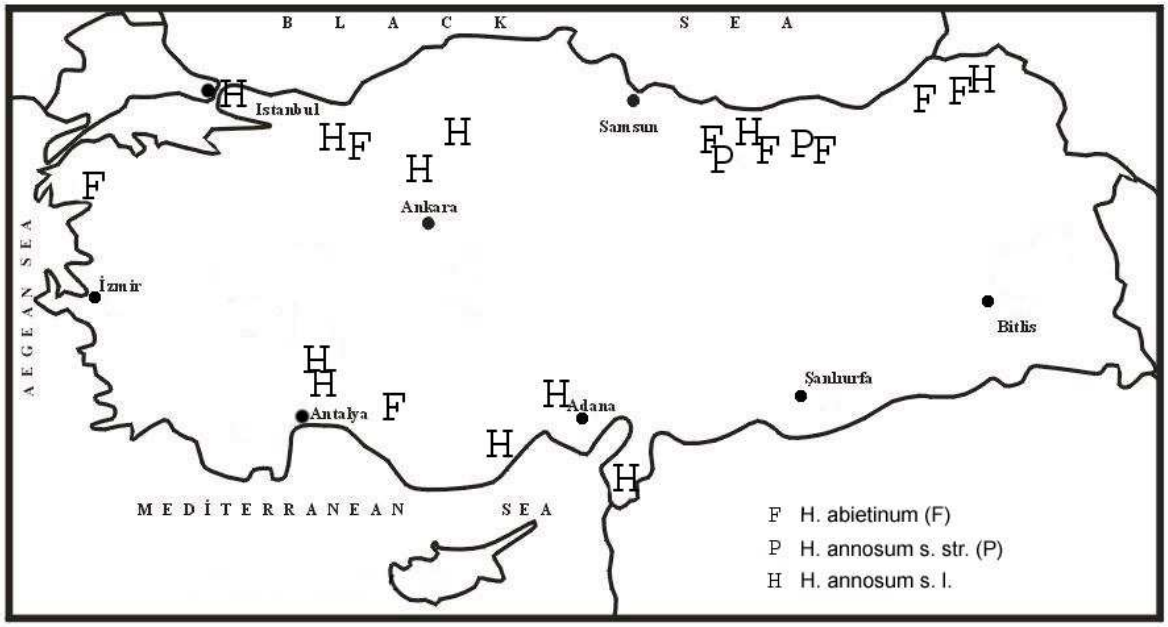


Figure 1. Distribution map of Heterobasidion in Turkey

2.2 Host range

The known host range of *H. annosum* s.l. in Turkey includes four *Abies* taxa, three species of *Pinus*, and *Picea orientalis* (L.) *(Table 1)*. These conifers are economically important and make up approximately 75% of the area of high forests in Turkey. So far *H. annosum* s.l. has not been reported from broadleaved trees.

Table 1. Records of Heterobasidion annosum s.l. on different hosts in Turkey

Host species	Province	References
Abies cilicica (Ant. & Kotschy) Carr.	Adana Antalya Isparta Mersin Konya	Selik 1973 Doğmuş-Lehtijärvi et al. 2006 Y.Balcı (2007 pers. comm.) Y.Balcı (2007 pers. comm.), Lehtijärvi (unpublished)
A. nordmanniana ssp. *equi-trojani* Syn: *A. equi-trojani* Aschers. et Sint.	Balıkesir	Balcı 1998, Doğmuş-Lehtijärvi et al. 2006
A. nordmanniana ssp. *bornmülleriana* Syn: *A. bornmülleriana* Mattf.	Çankırı / Kastamonu Düzce Bolu	Pilat 1932, Y.Balcı (2007 pers. comm.) Selik 1973 Selik 1973, Doğmuş-Lehtijärvi et al. 2006
A. nordmanniana (Steven) Spach ssp. *nordmanniana*	Artvin, Giresun, Gümüşhane, Ordu, Rize	Doğmuş-Lehtijärvi et al. (in press)
Picea orientalis (L.) Link	Giresun	Selik, 1973, Doğmuş-Lehtijärvi et al. (in press)
Pinus cf. *brutia* *P. brutia* Ten. Syn: *Pinus halepensis* var. *brutia* (Ten.) A. Henry	Hatay –	Kotlaba, 1976 Y.Balcı (2007 pers. comm.)
Pinus sylvestris L.	Ankara	Selik, 1973
Pinus nigra *Arn. ssp.* pallasiana *(Lamb.) Holmboe*	Antalya, Balıkesir	Lehtijärvi (unpublished)

Most of the records are based on morphological identification of basidiocarps of *H. annosum* s.l. Only recently mating tests have been used to identify the members of the *H. annosum* complex to species level. We found *H. abietinum* to be the dominating *Heterobasidion* species on *Abies cilicica* and all three subspecies of *A. nordmanniana* occurring on Asia Minor (Doğmuş-Lehtijärvi et al. 2006, Doğmuş-Lehtijärvi et al. in press). While *H. abietinum* has been found in several locations in western and north-eastern Turkey, *H. annosum* s.s. has been found so far only in two locations in north-eastern Turkey. We found basidiocarps of the latter species on stumps of *A. nordmanniana* ssp. *nordmanniana* in pure fir stands in Gümüşhane and Ordu provinces (Doğmuş-Lehtijärvi et al. in press). Most probably the distribution area of *H.annosum* s.s. in Asia Minor is fairly large as the species occurs not only in the north-eastern Turkey but also in neighbouring countries Bulgaria, Greece, and Cyprus (Tsopelas – Korhonen 1996, La Porta et al. 1998, Tsopelas – Nikolaou 2005). Moreover, some harvested pines in Turkey show typical symptoms of *H annosum* s.l. infections (Y. Balcı, Morgantown, 2007, personal communication).

3 PATHOGENICITY

There is very little information about the pathogenicity and damage caused by *Heterobasidion* in Turkish forests. Most of the studies on *Heterobasidion* in Asia Minor have been mycological surveys and the basidiocarps found on stumps or fallen trees (e.g. Selik 1973, Kotlaba 1976, Doğmuş-Lehtijärvi et al. 2006). Nevertheless, Selik (1973) states that decay can spread up to 6 m in infected stems of *A. nordmanniana* ssp. *bornmülleriana*.

Basidiocarps can be found on both standing and windthrown firs (Doğmuş-Lehtijärvi et al. 2006, Y. Balcı, Morgantown, 2007, personal communication). Basidiocarps were most common on *A. nordmanniana* ssp. *equi-trojani* in Kazdağı Mountains in Balıkesir, less common on *A. nordmanniana* ssp. *bornmülleriana* in Ilgaz Mountains in Kastamonu, and very rare on *A. silicica* in Mersin and Konya region (Y. Balcı, Morgantown, 2007, personal communication) found. Demirel (1999) found basidiocarps "on living conifers" in Artvin province. Observations on harvested pines have revealed typical signs of Heterobasidion infestation (Y. Balcı – Morgantown, 2007, personal communication). In short, field observations indicate that *H. annosum* s.l. is pathogenic on several hosts in Asia Minor although the matter has not been investigated thoroughly and there is no data about infection frequencies.

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Alien Species in Finnish Nurseries, *Phytophthora* spp.

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Abstract – International trade has increased the risk of pest spread. The climatic change will also improve the establishment of introduced species into new geographical areas. Since the beginning of 1990's *Phytophthora cactorum* has caused losses in agriculture on strawberries and caused stem lesions on silver birch seedlings in forest nurseries in Finland. *P. ramorum* was found in Finland for the first time in spring 2004 on marketed *Rhododendron* spp. plants originating from other EU member states. In August 2004 the pathogen was also found in one Finnish nursery on German *Rhododendron catawbiense* plants and on several other Finnish *Rhododendron* spp. cultivars. Most common microbes isolated from the lesions on the Finnish *Rhododendron* leaves collected in 2005 were *Pestalotiopsis* sp., *P. cactorum*, *P. inflata* and *P. ramorum*. In pathogenicity trials *P. inflata* was capable to infect most host plants used in tests including *Fragaria* x *ananassa*, *Betula pendula*, *Alnus glutinosa*, *A. incana*, *Picea abies* and *Vaccinium vitis-idea*. *P. ramorum* caused also stem lesions on birch and alder, but was less pathogenic than *P. inflata*. *Pinus sylvestris* was resistant to both *P. ramorum* and *P. inflata.*

***Phytophthora cactorum* / *P. ramorum* / *P. inflata* / hosts / pathogenicity**

Kivonat – Behurcolt fajok a finnországi csemetekertekben, *Phytophthora* spp. A nemzetközi kereskedelem növeli a károsítók terjedésének kockázatát. A klímaváltozás elősegíti a behurcolt fajok megtelepedését az új földrajzi területeken. A *Phytophthora cactorum* Finnországban az 1990-es évek elejétől a földiepren mezőgazdasági károkat, erdészeti csemetekertekben, a bibircses nyír magoncokon pedig szárnekrózist okoz. A *P. ramorum*-ot Finnországban először 2004 tavaszán találtuk meg, más EU tagállamokból származó kereskedelmi *Rhododendron* növényeken. 2004 augusztusában a kórokozót egy finn csemetekertben is megtaláltuk németországi származású *Rhododendron catawbiense* növényeken és több más, finnországi *Rhododendron* fajtán. A 2005-ben begyűjtött finnországi *Rhododendron* levelek nekrózisaiból leggyakrabban a *Pestalotiopsis* sp., *P. cactorum*, *P. inflata* és a *P. ramorum* mikroorganizmusokat izoláltuk. A patogenitási vizsgálatok során a *P. inflata* képes volt megfertőzni a legtöbb tesztnövényt, köztük a *Fragaria* x *ananassa*, *Betula pendula*, *Alnus glutinosa*, *A. incana*, *Picea abies* és *Vaccinium vitis-idea* fajokat. A *P. ramorum* is szárnekrózist okozott a nyíren és az égeren, de a *P. inflata*-nál kevéssbé bizonyult patogénnek. A *Pinus silvestris* mind a *P. ramorum* mind a *P. inflata* fajokkal szemben ellenálló volt.

***Phytophthora cactorum* / *P. ramorum* / *P. inflata* / gazdák / pathogenitás**

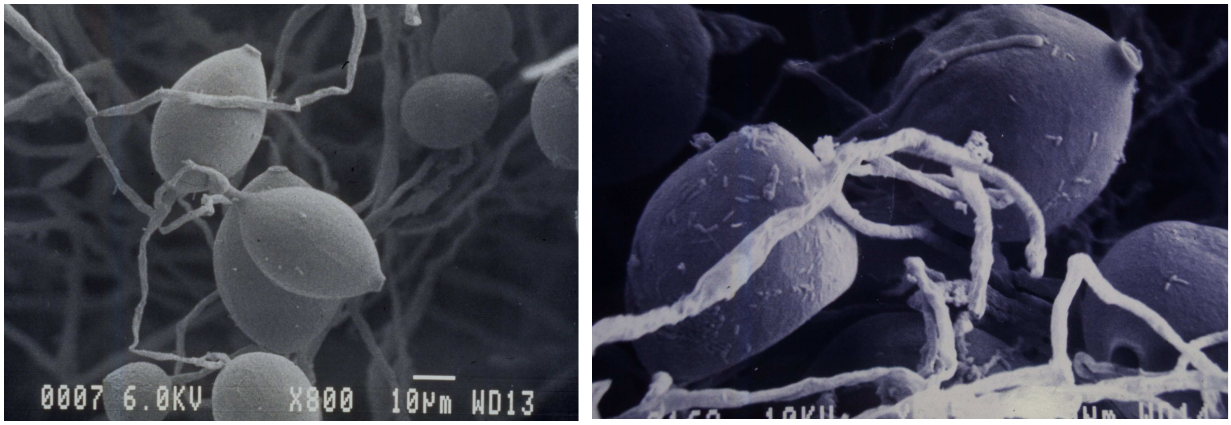
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Phytophthora cactorum

Phytophthora cactorum (Lebert and Cohn) J. Schröt is a pathogen which is transported with plant material as latent infections and can also survive in soil and plant debris. It is an economically important soil-borne pathogen of many herbaceous and woody species (Erwin and Ribeiro 1996). In forestry *P. cactorum* is known to cause root rot and stem cankers on many tree species (Erwin and Ribeiro 1996). In Sweden it was present in soil together with other *Phytophthora* species in stands where oaks (*Quercus robur* L.) showed tree crown defoliation (Jönsson et al. 2003). In inoculations it caused significant dieback of fine root and necrotic lesions on coarser root of oak seedlings (Jönsson, 2004). In Finland *P. cactorum* was isolated for the first time in 1990 from strawberry (*Fragaria* x *ananassa* Duch.) plants suffering from crown rot (Parikka 1991). A year later it was isolated from necrotic stem lesions on silver birch (*Betula pendula* Roth.) seedlings growing in forest nurseries (Lilja et al. 1996; Hantula et al. 1997, 2000). Since then this imported pathogen has caused crop losses in strawberry fields mainly as an agent of crown rot and increased culling of seedlings in forest nurseries.

Phythophthora cactorum and different host species

According to our observations the morphology of *P. cactorum* isolates from strawberry and birch differ from each other (*Figue 1a, b*).



(a) *(b)*

Figure 1. Sporangia of Phytophthora cactorum isolated from strawberry (a) and silver birch (b).

We carried out microscopic measurements and found several statistically significant differences in the sizes of the oogonia, oospores and sporangia (Hantula et al. 2000). However, the individual variation in morphological characteristics was evident, the microscopic examinations are insufficient for the identification of intraspecies genetic groups or host specificity among our isolates (Stamps et al. 1990). Both the random amplified microsatellite (RAMS) and Random Amplified Polymorphic DNA (RAPD) analysis showed substantial genetic variation among isolates of *P. cactorum*. However, Finnish isolates from birch and European crown rot isolates, including Finnish isolates, from strawberry formed own clusters in cluster analyses (Hantula et al. 1997, 2000, Lilja et al. 1998). Similarly in amplified fragment length polymorphism (AFLP) analysis 16 of 23 crown rot isolates from Europe, Japan, Australia and New Zealand were identical (Eikemo et al. 2004). However, the isolates from strawberry from different states or from different host in the USA or from strawberry from German or Canada, were polymorphic and formed 42 unique AFLP profiles (Huang et al. 2004). We also found high genetic variation within the North American

population of *P. cactorum* on strawberry fruits (Hantula et al. 2000). These results might suggest that the origin of *P. cactorum* is in the USA and that differences found in *P. cactorum* in Europe, Oceania, Asia and Africa are simply subsets of variation occurring in North America, which has been exported from there to other parts of the world (Hantula et al. 2000, Eikemo et al. 2004, Huang et al. 2004).

According to Seemüller and Schmidle (1979) leather rot of strawberry fruits is caused by different strain of *P. cactorum*, since in general leather rot pathogens are not able to cause crown rot, but crown rot pathogens can infect fruits. Most isolates from other hosts are also incapable to infect strawberry (Hantula et al. 1996, 2000, Eikemo et al. 2004). In our studies the isolates from strawberry infected birch only via wounds and the isolates from birch did not infect strawberry seedlings at all suggesting that there are differences in host specificities (Hantula et al. 1997, Lilja et al. 1998, Eikemo et al. 2004). This is in accordance with the observation of Seemüller and Schmidle (1979) who showed that *P. cactorum* isolates from strawberry and apple differ in their capability to cause symptoms on strawberry and apple. On *Alnus* the percentage of successful wound inoculations with a birch isolate was 40 and most lesions were small compared to those on the same size birch seedlings (Hantula et al. 1997). Based on the pathogenicity experiments done so far we conclude that *P. cactorum* strains cause more serious symptoms on the plants from which they are derived than on other plants, although variations exist between different host plants. Thus, strains tend to be more virulent on their hosts than on non-host plants.

We have also monitored the effect of *P. cactorum* infection on the development of container-grown, silver birch seedlings in nursery and after out-planting (Lilja et al. 1996, Lilja et al. unpublished). A PCR- based pathogen detection system, developed by us, was used to confirm the presence of *P. cactorum* in lesions after out-planting (Lilja et. al. 2006). In spring 1999 we collected diseased and healthy silver birch seedlings from a nursery field. Each seedling was assessed using a scale of 1 to 4 where: 1= no lesion , 2 = lesion < 5 mm^2, 3 = lesion > 5 mm^2, but not covering over half of the stem diameter, and 4 = lesion spread over half of the stem diameter, but not girdling the stem. On 4 May, after storage at + 4°C, the seedlings were out-planted. In the nursery stem lesions affected the height growth of birch seedlings, the shoot height of seedlings was related to the disease severity. Asymptomatic seedlings were taller than the diseased birches and the shortest were those with stem lesions covering over half of their stem diameter (*Figure 2*). After out-planting the stem lesions did not affect significantly on the mortality or the number of leader shoot changes. The height growth of seedlings in the reforestation increased with the disease rating. Growth of seedlings with stem lesions covering over half of their stem diameter grew more than healthy control seedlings or seedlings with smaller stem lesions (*Figure 3*). Thus the differences in shoot heights present in the nursery between diseased and apparently healthy seedlings were reduced, but not totally disappeared, after seven growing seasons in the field (*Figure 3*). However the mortality of seedlings increased with increased disease severity (*Figure 4*).

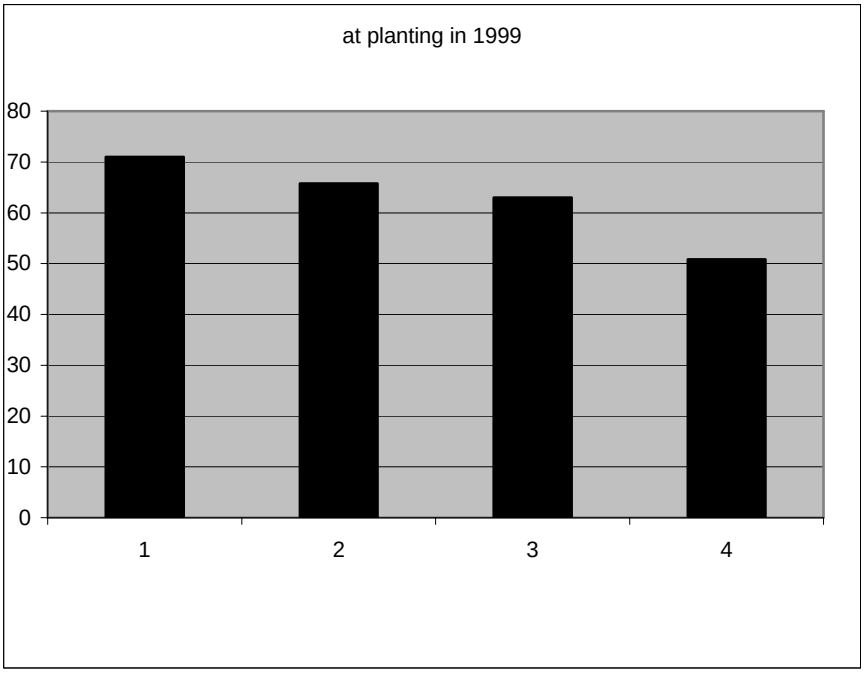


Figure 2. The height of silver birch seedlings before planting.
The health condition of seedlings was assessed using a scale of 1 to 4 where:
1= no lesion , 2 = lesion < 5 mm^2, 3 = lesion > 5 mm^2, but not covering over half of the stem diameter, and 4 = lesion spread over half of the stem diameter, but not girdling the stem.
Seedlings with lesions were infected with Phytophthora cactorum.
The number of seedlings in each disease severity category was 120

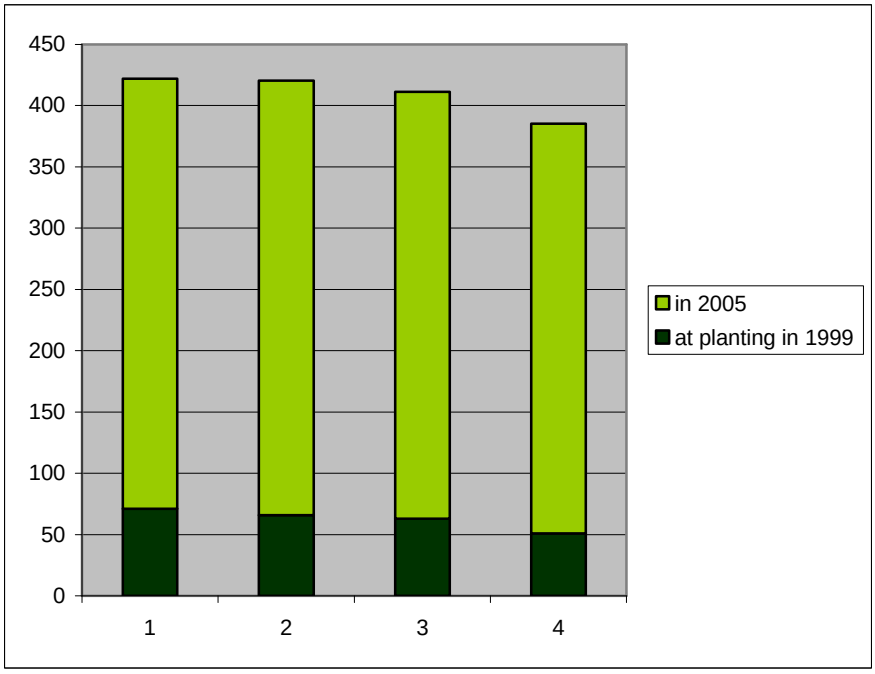


Figure 3. The height of silver birches seven years after out-planting.
The health condition of seedlings before planting was assessed using a scale of 1 to 4 where:
1= no lesion , 2 = lesion < 5 mm^2, 3 = lesion > 5 mm^2, but not covering over half of the stem diameter, and 4 = lesion spread over half of the stem diameter, but not girdling the stem.
Seedlings with lesions were infected with Phytophthora cactorum.
The number of seedlings in each disease severity category was 120

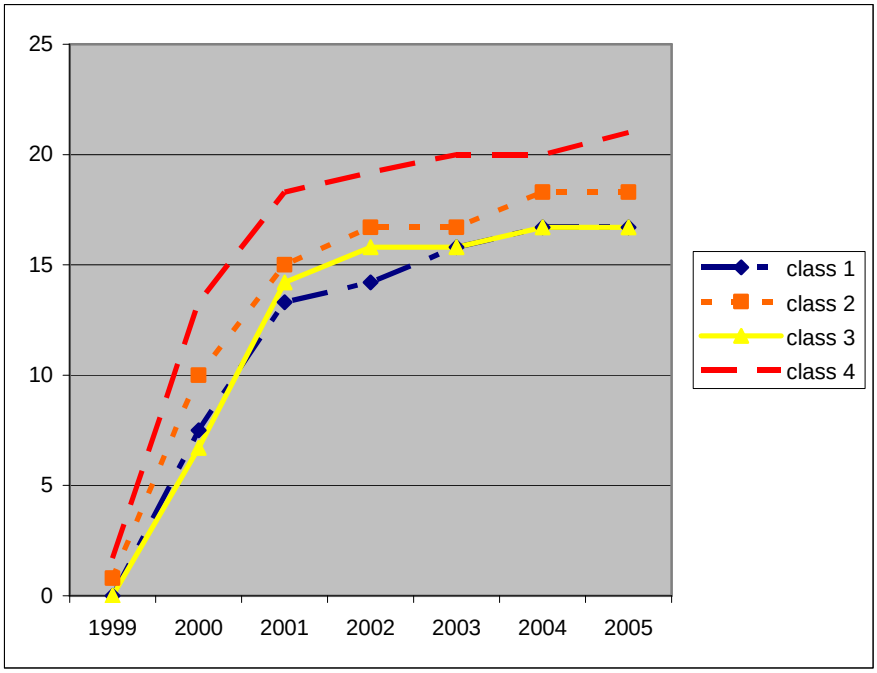


Figure 4. The mortality of seedlings after outplanting.
The health condition of seedlings before planting was assessed using a scale of 1 to 4 where: 1= no lesion , 2 = lesion < 5 mm^2, 3 = lesion > 5 mm^2, but not covering over half of the stem diameter, and 4 = lesion spread over half of the stem diameter, but not girdling the stem. Seedlings with lesions were infected with Phytophthora cactorum. The number of seedlings in each disease severity category was 120

Phytophthora ramorum

Phytophthora associated with a twig blight disease on rhododendron (*Rhododendron* sp.) and viburnum (*Viburnum* sp.) in Germany and the Netherlands was described as a new species, *Phytophthora ramorum* (Werres, de Cock & Man in't Veld) in 2001 (Werres et al. 2001). Later the same species was found to be responsible for the Sudden Oak Death disease (SOD) of oaks (*Quercus* spp.) and tanoaks (*Lithocarpus densiflorus* Hook. & Arn., Rehd) in California, USA (Rizzo et al. 2002). The spread of *P. ramorum* in North America has been very rapid. The disease was first discovered on tanoaks near Mill Valley in 1995. Since then it has spread throughout coastal counties around the San Franscisco Bay area where many tanoaks, coast live oak (*Q. agrifolia* Née), and California black oaks (*Q. kelloggii* Newb.) have been killed (Rizzo et al. 2002, Davidson et al. 2002, 2005). It has now spread to Washington and Oregon (Davidson et al. 2005, Hansen et al. 2003, Rizzo et al. 2005). In Europe *P. ramorum* was first found and identified in Germany and the Netherlands (Werrs et al. 2001). Later it was proved to be present in many other countries, but in Europe there has not been such epidemic as the SOD in the western North America. *P. ramorum* has mainly occurred as a cause of leaf, twig and shoot blight on different ornamental hosts in nurseries and gardens in Belgium, Finland, Denmark, France, Italy, Ireland, Norway, Poland, the UK, Slovenia, Spain, Sweden and Switzerland (Delatour et al. 2002, Moralejo and Werres 2002, Orlikowski and Szkuta 2002, De Merlier et al. 2003, Beales et al. 2004a, b, Heiniger et al. 2004, Zerjav et al. 2004, Lilja et al.2007). More recent isolations have been done in Estonia (Hanso, personal communication). In general the native, European oak species such as common oak (*Q. robur*) or sessile oak [*Q. petraea* (Matt.) Liebl] are more resistant than American oaks, although in inoculations individual trees have shown different susceptibility

(Denman et. al. 2005). In some countries finds locate also in mature trees as American southern red oak (*Q. falcata* Michx.) (Brasier et al. 2004). Other infected trees in the UK and the Netherlands have grown in large gardens and the source of infection has been rhododendrons.

It is not known how *P. ramorum* originally entered Europe or North America, but the mating type, morphology, growth characters and population distribution suggests that separate introductions into Europe and into North America may have occurred from a third unknown location (Brasier 2003, Ivors et al. 2004, Rizzo et al. 2005, Werres et al. 2005). It is, however, probable that imported, infected ornamentals have been the main source of the pathogen.

In Finland *P. ramorum* was found for the first time in spring 2004 on marketed rhododendron plants originating from other EU member states. In August 2004 the pathogen was also found in one Finnish nursery on rhododendrons (*Rhododendron catawbiense* Michx) and on several other cultivars produced in Finland by micropropagation. Most common microbes isolated from the lesions on the leaves of a Finnish cultivar 'Elvira' were in 2005 *Pestalotiopsis*, *P. cactorum*, *P. inflata* and *P. ramorum* (Lilja et al. 2007).

Phythophthora ramorum and different host species

P. ramorum spreads mostly aerially and it has not been shown to infect roots. It has many hosts in different plant families: Aceraceae, Anacardiaceae, Betulaceae, Caprifoliaceae, Ericaceae, Fagaceae, Hippocastanaceae, Lauraceae, Oleaceae, Pinaceae, Pittosporaceae, Primulaceae, Rhamnaceae, Rosaceae, Taxaceae, Taxodiaceae and Theaceae and thus the disease expressions differ depending on hosts (Knight 2002, Hong 2003, Henricot - Prior 2004, Hüberli et al. 2004, 2005, Lane et al. 2004, Denman et al. 2005). On woody shurbs and other understory hosts, which can serve as a source of inoculum for trees, *P. ramorum* mainly causes leaf lesions or/and twig blight. In larger trees, symptoms vary between tree species. Leaf lesions occur always first at the tip or edges of the leaves. Bark infections cause cankers with tarry or rusty colored exudations. The leaves of infected oak trees may turn brown over a short period, but death may take one or more years (Garbelotto et al. 2001). On tanoak the pathogen affects both bark and leaves and death can be rapid. On the stem base the infections cause wilting and death eg. on viburnum (Werres et al. 2001). On conifers, *P. ramorum* causes needle blight and dieback of young shoots. Myrtlewood tree/ Bay laurel (*Umbellaria californica* Matt.) harbors the pathogen without suffering serious damage itself, while supporting abundant production of spores and thus caused the epidemic in California (Davidson et al. 2005, Maloney et al. 2005).

In pathogenicity tests run by us *P. ramorum* caused stem lesions on silver birch and common alder (*Alnus glutinosa* (L.) Gaertner), but was less pathogenic than *P. inflata.* Scots pine (*Pinus sylvestris* L.) was resistant to *P. ramorum.*

Phytophthora inflata

Phytophthora inflata (Caroselli & Tucker) was described by Caroselli and Tucker (1949) as a pathogen causing cankers on elms (*Ulmus american* L. and *U. fulva* Michaux) in the USA. Later it has been reported from rotten roots of nursery plants of elder (*Sambucus tenuifolium* L.) and common lilac (*Syringa vulgaris* L.) in the UK (Hall et al. 1992). Within surveys for *P. ramorum*, *P. inflata* was isolated from rhododendrons (*R. ponticum* L.) with wilting foliage and blackened shoot tips in a nursery in Scotland (Schlenzig 2005). Later the same pathogen was recovered from single plants of lingonberry (*Vaccinium vitis-idaea* L.) and salal (*Gaultheria shalon* L.), both with leaf lesions and dieback symptoms, from another nursery in

Scotland (Schlenzig 2005). The results by Schlenzig (2005) and Testa et al. (2005), that *P. inflata* was found in surveys for *P. ramorum,* were in accordance with our experience. We also isolated *P. inflata* from the cultivars known to be infected with *P. ramorum* (Lilja et al. 2007).

In pathogenicity trials *P. inflata* was capable to infect most host plants used in tests including strawberry, silver birch, common alder, grey alder [*Alnus incana* (L.) Moench], Norway spruce (*Picea abies* L. Karst.) and lingonberry. Scots pine was resistant to *P. inflata*.

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Biological Sprout Control with *Chondrostereum purpureum* – Preliminary Results from Field Trials in Finland

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Abstract − The aim of our ongoing project is to test the efficiency of the indigenous decay fungus, *C. purpureum*, as a biocontrol agent against stump sprouting in Finland. *Chondrostereum purpureum* was applied to freshly cut birch (*Betula pendula, B. pubescens*) stumps at 12 different time points during the growing season. The most effective treatment time seemed to be the early summer, at least on birch. Efficacy of *C. purpureum* on aspen (*Populus tremula*), grey alder (*Alnus incana*) and willows (*Salix spp*.) was also tested. Treatment was done in July. One year after the treatments *C. purpureum* seemed to have a slight reductive effect on sprouting on all these tested tree species. According to the preliminary results high enzymatic activity of the fungus and good growth ability on wood chips in laboratory did not necessarily guarantee the good ability to prevent sprouting in the field. However, there were differences in the ability of different isolates of *C. purpureum* to prevent sprouting and it is worth to try to find more aggressive isolates in the future for biocontrol purposes. Preliminary results showed that the use of *C. purpureum* is a promising method for biological sprout control in Finland.

Basidiomycetes/ Vegetation management/ Mycoherbicide

Kivonat – Biológiai sarjadzásgátlás *Chondrostereum purpureum*-mal – a szabadföldi kísérletek előzetes eredményei Finnországban. Projektünk célja a *Chondrostereum purpureum* őshonos farontó gomba hatásának kimutatása a tuskósarjak visszaszorításában, Finnországban. A *C. purpureum*-ot friss nyírfa tuskókon alkalmaztuk a vegetációs időszak 12 különböző időpontjában. A leghatásosabb kezelési időpont a kora nyár volt, legalábbis a nyír esetében. A *C. purpureum* hatásosságát rezgőnyáron (*Populus tremula*), hamvas égeren (*Alnus incana*) és füzeken (*Salix* spp.) is kipróbáltuk. A kezeléseket júliusban végeztük. Egy év elteltével a *C. purpureum* gyenge visszaszorító hatását tapasztaltuk e fafajok sarjképzésére. Előzetes eredményeink szerint a gomba magas enzim-aktivitása és laboratóriumi jó növekedési képessége nem garantálja szükségszerűen a szabadföldi jó sarj-visszaszorító képességet. Különbségeket észleltünk az egyes izolátumok sarjadzásgátló képessége között, ezért érdemes megpróbálni agresszívebb izolátumokat találni a jövőbeni biokontrol céljaira. Előzetes eredményeink azt mutatták, hogy a *C. purpureum* felhasználása ígéretes módszer a sarjadzás biológiai gátlására, Finnországban.

Bazídiumos gombák / vegetáció kezelés / mikoherbicid

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1 INTRODUCTION

Sprouting of fast growing broadleaved trees cause problems in newly planted and young coniferous stands, where undesirable vegetation contend for space, light, water and nutrients with valuable conifer trees. Sprouting is a problem also under power transmission lines, at roadsides and railways. Use of chemicals to prevent sprouting has nowadays awakened environmental concern. Hence, the most common method to prevent sprouting is mechanical cutting, but it is ineffective for many species as they can resprout vigorously without further treatment.

Researchers in the Netherlands and in Canada have demonstrated the potential of white rot fungus, *Chondrostereum purpureum* (Pers. ex. Fr.) Pouzar, to successfully control stump sprouting of many hardwood species (Scheepens – Hoogerbrugge 1989, De Jong 2000, Wall 1990, Wall 1994, Dumas et al. 1997, Jobidon 1998, Becker et al. 1999, Harper et al. 1999, Pitt et al. 1999). *C. purpureum* is a basidiomycete commonly found throughout the temperate regions of the world. It is an early colonizer of fresh wounds on many broadleaved trees, logging slash and stored logs (Brooks – Moore 1926, Rayner 1977, Spiers – Hopcroft 1988). *C. purpureum* is also known as a pathogen responsible for silver-leaf disease of many fruit trees (Brooks – Moore 1926).

We report here the preliminary results of biological sprout control with *C. purpureum* in Finland. We tested if the application time has an effect on the efficacy of the *Chondrosteum purpureum* treatment on birch and investigated the usefulness of *C. purpureum* as an inhibitor of stumps sprouting on aspen (*Populus tremulae*), grey alder (*Alnus incana*) and willows (*Salix* spp.). In addition, we studied if the activity of several secreted hydrolytic and oxidative enzymes and growth ability on wood chips *in vitro* correlates with the ability of the fungal isolates to prevent growth of new sprouts in the field.

2 MATERIALS AND METHODS

2.1 Effect of the application time of the stump treatment on birch

The experimental site located in Orivesi, Central Finland, about 200 km north of Helsinki. The site was a 15-year-old spruce stand with birches (*Betula pendula, B. pubescens*) as a mixed forest. Plots were established in 12 different time points, with intervals of two weeks, from the 2th of May to 12th of October in year 2005. Birches were manually cut with brush saw and the entire surface of the cut stems was treated with inoculum. The inoculum contained active mycelium of *C. purpureum* pre-grown on potato dextrose liquid medium containing 24 g/l potato dextrose broth and 20 g/l silica powder in distilled water. Control stumps were left untreated (control 1) or they were treated with a blank inoculum (control 2). Plots were circular and variable in diameter. Each plot contained at least twenty cut stems and four replicate plots were established for each treatment. At least three meters untreated buffer zones were left between plots. Plots were examined for the first time in late summer 2006, about one year after experiments were established. Twenty identified stumps per plot were observed. The amount of sphorosphores of *C. purpureum* on stumps was defined, the number of living sprouts per stump was counted and the height of the tallest living sprout was measured.

2.2 Efficacy of *C. purpureum* on aspen (*Populus tremula*), grey alder (*Alnus incana*) and willows (*Salix spp.*)

The experimental sites located in Parikkala, eastern Finland, about 350 km northeast of Helsinki. Field trials were established in July 2005. Trees were cut by brush saw and sprayed immediately with the mycelium of *C. purpureum* growing in liquid culture as described previously. Control stumps were treated with the blank substrate or left untreated after cutting. Plots were circular and variable in diameter. One plot contained at least twenty cut stems and four replicate plots were established for each treatment. Plots were examined first time in late summer 2006, one year after the experiment was established. The amount of sphorosphores of *C. purpureum* on each stump was defined and the number of living sprouts per stump was counted. In addition the height of the tallest living sprout was measured.

2.3 Production of wood decaying enzymes and fungal biomass production *in vitro*

We tested 21 isolates of *C. purpureum* in the laboratory to find out if the activity of several secreted hydrolytic and oxidative enzymes and growth ability on birch wood chips correlated with the ability of the fungal isolate to prevent sprouting. Activity of pectinases, proteases, cellulases, laccases and peroxidases was tested. Agar plates for the detection of pectinases, proteases, and cellulases were modified from Hagerman et al. 1985. ABTS and Poly R-plates used to test the ligninolytic enzyme activities (laccases, peroxidases) were modified from Steffen et al. 2000. Each screening plate was inoculated with a 6-mm diameter agar plug of a precultured mycelium. Two replicate plates of each strain/substrate combination were used. Cultures were incubated at 25 ºC and visually observed. Intensity of color reactions on plates was classified by minus and plus marks. If there was not any observable color reaction, minus mark was given. Three plus marks indicated a strong color reaction. General enzymatic activity value for each isolate was defined as the sum of plus marks given.

Fungal growth in wood chips was investigated using fluorescein diacetate (FDA) method (Boyle – Kropp, 1992). FDA is a fluorogenic substrate that becomes fluorescent upon enzymatic cleavage by a number of nonspecific enzymes of living cells and it has been used previously as a test of fungal viability (Söderström 1977, Schnürer – Rosswall 1984, Barak – Chet 1986). The product of this enzymatic conversion is fluorescein, which can be quantified by a spectrophotometer. The fresh birch (*Betula pendula*) wood chips (particle size 1 mm) used in this experiment were sawed and chipped in Ruotsinkylä, Finland. Wood chips in 20-ml glass vials were inoculated with the mycelium of *C. purpureum* growing in liquid culture and cultivated at 25 ºC. The amount of growing mycelium on wood chips was measured at four different time points: 3, 7, 10 and 14 days after inoculation.

Strain selection for the field was done based on tests made *in vitro*. Different isolates were put in order based on their general enzymatic activity value and FDA absorbance value. Totally 8 isolates of *C. purpureum* were chosen for the field trial. Four isolates with good ability to grow in woodchips and high general enzyme activity and four isolates with lower ability to grow in woodchips and with lower general enzyme activity were selected.

Field trial was established in Juupajoki, Central Finland, about 200 km north of Helsinki. Experimental site was a 13-year-old spruce stand with birches as a mixed forest. The plots were established in the middle of June 2006. A total of 40 treatment plots were established, consisting of four replicates of each treatment. Plots included control stumps, which were cut but treated with the blank inoculum or left untreated. Plots were circular and variable in diameter. Diameter was determined by the area required to locate at least 30 birch stems in each plot for each treatment. A minimum of a 2-m-wide buffer zone separated the plots from each other. Birches were manually cut using a brush saw and the entire surface of the cut stems was immediately treated with one of the eight selected isolates. Plots were examined

first time in late summer 2006, 14 weeks after the experiment was established. The amount of sphorosphores of *C. purpureum* on each stump was defined, the number of living sprouts per stump was counted and the height of the tallest living sprout was measured.

3 RESULTS

3.1 Effect of the application time for the birch stump treatment

One year after the treatments, the treated birch stumps had far more fruiting bodies than control stumps, irrespective of treatment time. The amount of fruiting bodies on treated stumps varied according to treatment time. The highest number of fruiting bodies was found from stumps, which were treated between early May and late June; 60-85% of treated stumps had fruiting bodies. Also control stumps from that time had the highest number of fruiting bodies; 0-14,5% of control stumps had fruiting bodies.

The percentage of sprouting stumps was decreased by the application of the fungus in each treatment time. The biggest difference between treated and control stumps was observed in the middle of July; only 20 % of treated stumps were resprouting whereas 90 % of control stumps put out new sprouts.

Number of living sprouts per stump decreased due to the application of the fungus. The reduction of the living sprouts per stump varied according to the time of cutting. Reduction was 60-80% when the fungus was applied to the stumps between the 4th of May and the 14th of July. When fungus was applied to the stumps between 29th of July and 15th of September the reduction of the number of living sprouts per stump was only 30-50% compared to control stumps. Significant reduction of living sprouts per stump could not be found in treatments done in late September and October. Although the number of living sprouts per stump decreased because of *C. purpureum* treatment, it had no effect on the maximum height of new sprouts in any treatment times.

3.2 Efficacy of *C. purpureum* on aspen (*Populus tremula*), grey alder (*Alnus incana*) and willows (*Salix spp.*)

Fruiting bodies of *C. purpureum* were found from every observed tree species one year after treatments. Control stumps had far less fruiting bodies than treated stumps on every tree species. Fructification was most abundant in willows; about 90% of treated stumps had fruiting bodies. About 60% and 30% of treated alder and aspen stumps had fruiting bodies, respectively. Only 0-2,5% of control stumps had naturally occurring fruiting bodies depending on tree species.

One year after the treatments, the percentage of non-sprouting stumps was increased and the number of living sprouts per stump was reduced by the application of *C. purpureum* on all tested tree species. However, the difference between treated and control stumps was not very clear and statistical significance between treated and control stumps could not be found in any observed tree species one year after the treatments. The maximum height of the living sprouts did not differ between treatments in any observed tree species.

3.3 Correlation of *in vitro* characteristics to inhibition of sprouting of birch in the field

Laccase activity was present in all isolates of *C. purpureum*, but there were clear differences between the isolates. Only four isolates bleached Poly-R, indicating manganese peroxidase (MnP) activity. Pectinase activity was scarce in *C. purpureum*; only two isolates produced detectable amounts of pectinases. Activity of proteases, lipases and cellulases was present in all isolates, although there was a lot of variation in the production levels of these enzymes.

There were clear differences in biomass production of mycelium in wood chips between different *C. purpureum* isolates. The amount of growing mycelium on wood chips was measured in four different time points, 3, 7, 10 and 14 days after inoculation, and differences were seen clearly between different isolates just until 10 days after inoculations.

Assessments conducted in the field 14 weeks after the treatments revealed that there were differences between different *C. purpureum* isolates in their ability to prevent sprouting on birch. However, differences seemed not to correlate with high or low enzymatic activity on agar plates or growth rate in the laboratory tests.

4 DISCUSSION

Preliminary results showed that *C. purpureum* is a promising method in biological sprout control in Finland. On birch it seems to reduce sprouting quite well and the most effective treatment time seems to be in the early summer.

C. purpureum treatment seemed also to have a slight effect on sprouting also on aspen, alder and willows. On these tree species the effect of the treatment seemed not to be as effective as on birch. However, one must remember that these are just preliminary results one year after treatments and the situation can still change in the following years.

According to the first year's results high enzymatic activity and good growth ability on wood chips in the laboratory do not seem to correlate with the good ability to prevent sprouting in the field. The hydrolytic enzymes tested here appeared not to be crucial for the biocontrol efficiency. Virulence of *C. purpureum* is most likely dependent on several factors, including phytotoxins, which were not analyzed in our study. However, there are differences in the ability of different isolates of *C. purpureum* to prevent sprouting and it is worth to try to find more aggressive isolates in the future for biocontrol purposes. Other characteristics, which could affect to the ability to prevent sprouting, could also exist.

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EXTENDED ABSTRACTS

New Reports of Dothistroma Needle Blight in Eurasian Countries

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Extended abstract – One of the most serious needle diseases that affect pines (*Pinus* spp.) is Dothistroma needle blight (DNB). Two species of fungi are responsible for causing this disease (Barnes et al. 2004). These are *Dothistroma septosporum* (teleomorph: *Mycosphaerella pini*) that has a worldwide distribution and infects a wide range of *Pinus* spp. and *D. pini* (teleomorph unknown), which has thus far been reported only from the North-Central U.S.A. on the non-native *Pinus nigra* (Barnes et al. 2004). In recent years, there have been increasing numbers of reports of DNB from new hosts and new geographic regions of the Northern Hemisphere (Bradshaw 2004, Bednářová et al. 2006). Moreover, there has been an increase in the intensity of this disease in some parts of Europe and North America (Koltay 2001, Aumonier 2002, Brown et al. 2003, Jankovský et al. 2004, Woods et al. 2005).

Since 2004, we have conducted surveys and inspections of trees in Austria, Bhutan, Hungary, Ukraine and South-Western Russia. These have helped to document DNB on several native and non-native pine species, and to unmask its presence in situations where disease symptoms and signs were not obvious or not typical (Barnes et al. 2007). In 2004, non-native *Pinus peuce* trees in an arboretum in Vienna (Austria) were found to suffer from DNB. In 2005, a non-native *Pinus radiata* tree in Western Bhutan was found with typical DNB symptoms. Further east in Central Bhutan, native *Pinus wallichiana* trees in conifer forests at high elevations, had needle blight symptoms atypical of DNB. These, and other pine needle collections from Hungary, Ukraine and South-Western Russia, with typical DNB symptoms formed the basis of this study.

Isolates from the various hosts and countries were examined morphologically and compared using DNA sequence data. Conidia for all collections were elongated, straight to slightly curved, hyaline and they had one to five septa. Conidial dimensions varied considerably when measured from conidiomata produced on needles and in culture. Detailed measurements indicated that *D. pini* has slightly wider conidia than *D. septosporum*, as has

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previously been shown by Barnes et al. (2004). However, these differences are so small that distinguishing *D. septosporum* from *D. pini* based on morphology is virtually impossible.

Comparisons of DNA sequence data for the ITS region of the rDNA and parts of the β-tubulin gene region, were used to unambiguously identify the *Dothistroma* pathogens collected from symptomatic needles. *Pinus peuce* from Austria, *P. radiata* and *P. wallichiana* from Bhutan as well as *Pinus mugo* from Hungary were all found to be infected by *D. septosporum*. In contrast, *Pinus pallasiana* from the Kherson region in Ukraine and the nearby Rostov region in South-Western Russia were infected by *D. pini*. These results represent a new host record for *D. septosporum* on *P. peuce* and a new country report for *D. septosporum* from Bhutan. They also confirm that *D. septosporum* occurs in Hungary. Results also provide a new host report for *D. pini* on *P. pallasiana* and new country reports for *D. pini* in Ukraine and South-Western Russia. The latter records represent the only reports of *D. pini* from outside the North-Central U.S.A. The new host and country records provided here are consistent with the continuing trend of reports of the DNB pathogens from new hosts and new geographic areas during the last two decades, particularly in the Northern Hemisphere.

***Dothistroma septosporum* / *Dothistroma pini* / *Mycosphaerella pini* / Bhutan / Russia / Ukraine**

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Exposing the Enigma of Dothistroma Needle Blight Using Molecular Markers – a Progress Report

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Extended abstract – *Dothistroma septosporum* is a fungal pathogen causing a disease known as either red band needle blight or Dothistroma needle blight (DNB) on *Pinus* species worldwide. The three morphological varieties of this pathogen originally recognized based on differences in conidial length (Thyr – Shaw 1964, Ivory 1967) have not been supported by DNA sequence analyses (Barnes et al. 2004). However, phylogenetic relationships of *Dothistroma* isolates from various countries, based on DNA sequences for portions of the rDNA ITS, β-tubulin and TEF 1-α gene regions, revealed that DNB is caused by two distinct fungal species (Barnes et al. 2004). One species, *Dothistroma septosporum,* has a world-wide distribution and a very wide host range (Barnes et al. 2004, Bradshaw 2004, Bednářová et al. 2006). It is the pathogen responsible for the devastating losses to pine plantations in many Southern Hemisphere countries (Gibson 1974). The teleomorph state of this fungus is *Mycosphaerella pini* (Funk – Parker 1966). In contrast, *Dothistroma pini* is known only from the non-native *Pinus nigra* in the North-Central U.S.A and from *P. pallasiana* plantations in Ukraine and South-Western Russia, outside the natural range of this pine species (Barnes et al. 2004, Barnes et al. 2007).

Although morphologically very similar, conidial widths for *D. pini* are on average, slightly wider than those of *D. septosporum*. Despite this, unambiguous identification of these two fungi based solely on morphology is virtually impossible. Specific mating type primers were developed for both *Dothistroma* species (Groenewald et al. 2007). The primers have a dual function in that they can be used to discriminate between *D. septosporum* and *D. pini* and also to identify the mating type of individual isolates. Screening populations from various countries using the primers has shown that both mating types of *D. septosporum* are present in Europe, America and Africa, but that only one mating type is present in some of the countries in the Southern Hemisphere, with monoculture plantings of *Pinus radiata* and where the sexual state has not been found. The fact that both mating types of *D. pini* are present in the U.S.A. provides a clue to where a teleomorph for this species might be found.

Microsatellite markers that have also been designed specifically for *D. septosporum*, support the mating type results. These indicate that clonal populations exist in some of the Southern Hemisphere countries, while populations in Europe, on native tree species, have

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high levels of diversity. Ongoing research using these markers will provide an opportunity to study the population structure and world-wide movement of these pathogens. The emerging results should also be valuable in facilitating the development of management strategies to reduce the impact of Dothistroma needle blight, by either containing the pathogens within areas or minimizing their introduction into other countries.

Dothistroma pini / Dothistroma septosporum / Mycosphaerella pini / *mating type genes / microsatellite markers / phylogeny*

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Common Needle, Shoot, Branch and Stem Diseases of Conifer Trees in Bhutan

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Extended abstract – Bhutan is a small, landlocked, densely forested country in the South-Eastern Himalayas (FAO 1999, 2001). Forests are of immense importance for the ecology, economy and social well-being of this country and for the livelihood of its people. In mountainous areas at elevations between about 2100 and 4200 m asl., temperate conifer forests form the natural vegetation in this part of the Himalayas. These forests occupy about 24% of the total area of Bhutan and they consist mainly of Eastern Himalayan fir (*Abies densa*), Eastern Himalayan spruce (*Picea spinulosa*), Himalayan hemlock (*Tsuga dumosa*) and Himalayan Blue pine (*Pinus wallichiana*) (Grierson – Long 1983, Rosset 1999). Other conifers and various broadleaved tree species (*Rhododendron* spp., *Betula* spp., *Populus* spp., *Acer* spp., *Sorbus* spp. and *Salix* spp.) are often admixed to the aforementioned major conifer species or sometimes dominate forest stands on specific sites (Grierson – Long 1983, Rosset 1999). Another important conifer in Bhutan is Chir pine (*Pinus roxburghii*), which occurs mainly in sub-tropical and warm temperate forests (Grierson – Long 1983). This pine does, however, not form part of cold temperate conifer forests.

In the 1980's conifer forests in Bhutan were affected by two serious, large-scale forest health problems, namely decline of fir (*Abies densa*) (Donaubauer 1986, 1987, 1993, Ciesla – Donaubauer 1994) and outbreaks of the bark beetles *Ips schmutzenhoferi* on *P. spinulosa* and *P. wallichiana* and *Ips longifolia* on *P. roxburghii* (Schmutzenhofer 1987a, 1987b, 1988, Holzschuh 1988, Tshering – Chhetri 2000, Kirisits et al. 2002). Fir decline and bark beetle outbreaks have for the first time shown that diseases, insect pests and abiotic damaging factors can pose a great threat to the forests of this Himalayan country and can greatly upset the aims of forest management and conservation. These two forest health problems were also the starting point for research in forest entomology, forest pathology and forest protection in Bhutan and mark the begin of the collaboration between Bhutan and Austria in these fields. Following research and training activities in the 1980's, collaboration in forest pathology and

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forest protection between Austria and Bhutan has been continuing since 2001 as part of the Conifer Research and Training Partnership (CORET, http://woek.boku.ac.at/coret/) between the University of Natural Resources and Applied Life Sciences, Vienna (BOKU), Austria and Renewable Natural Resources (RNR) Forest Research of Bhutan, in which scientists from the Forestry and Agricultural Biotechnology Institute (FABI) of the University of Pretoria, South Africa also participate.

Surveys and studies, starting in the 1980's greatly increased knowledge about diseases and insect pests of conifer trees in Bhutan (e. g. Donaubauer 1986, 1987, 1993, Schmutzenhofer 1987a, 1987b, 1988, Chhetri 1990, 1995, Schieler 1992, Ciesla – Donaubauer 1994, Nedomlel 1995, Rosset 1999, Tshering – Chhetri 2000, Kirisits et al. 2002, 2007, Van Wyk et al. 2004, Coetzee et al. 2005, Konrad 2006, Dorji 2007, Barnes et al. in press). These studies helped to define potential threats of conifers and resulted in suggestions for integrated disease and pest management. Here, we provide an overview on common needle, shoot, branch and stem diseases of conifer trees in Bhutan, based on the work conducted during the past 25 years.

The most important pathogen of *Pinus wallichiana* is Himalayan dwarf mistletoe (*Arceuthobium minutissimum*) (Hawksworth – Wiens 1996). This minute parasitic plant is widespread and very damaging in dry Blue pine forests in Western Bhutan (districts Paro, Ha and Thimphu) (Donaubauer 1986, Chhetri 1990, 1995, Tshering – Chhetri 2000, Kirisits et al. 2002, Dorji 2007). *Taxillus kaempferi*, a leafy mistletoe, also commonly infects Blue pine in Western and Central Bhutan (Donaubauer 1986, Chhetri 1990, Kirisits et al. 2002, Dorji 2007). This mistletoe also occurs on *Tsuga dumosa* and *Picea spinulosa* (Grierson – Long 1983, Donaubauer 1986). Blister rust on branches and stems of Blue pine, caused by *Cronartium ribicola* or a related species occurs occasionally on young trees (Donaubauer 1987, Chhetri 1990, Kirisits et al. 2002). Needle diseases of *P. wallichiana* include Dothistroma needle blight caused by *Dothistroma septosporum* (Barnes et al. in press), needle rust caused by a *Coleosporium* sp. (Donaubauer 1987), needle cast caused by a *Rhizosphaera* sp. and infestation by sooty moulds. Hysterothecia of *Lophodermium* spp. are common on Blue pine needles (Kirisits et al. 2002), but the species have not yet been determined. *Lophodermium* spp. may be endophytes or saprophytes becoming apparent on needles affected by other needle pathogens. There are also records of a needle cast caused by cf. *Meloderma desmazierii* on *P. wallichiana* (Donaubauer 1986, Chhetri 1990). Needle rust, caused by a *Coleosporium* sp. (Chhetri 1990) and *Lophodermium* spp. have also been documented on *P. roxburghii*.

Picea spinulosa is affected by Sichuan dwarf mistletoe (*Arceuthobium sichuanense*), which has been recorded only from the districts Ha and Paro in Western Bhutan (Donaubauer 1987, Hawksworth – Wiens 1996, Thering – Chhetri 2000, Dorji 2007). This dwarf mistletoe is much less prevalent than *A. minutissimum* on Blue pine and has thus-far not caused economic damage (Donaubauer 1987, Dorji 2007). Sirococcus shoot blight, caused by the P type of *Sirococcus conigenus* was found for the first time on *P. spinulosa* in 2001 and this record also represented the first report of the disease and the associated pathogen from anywhere in Asia (Kirisits et al. 2002, 2007, Konrad 2006). At higher elevations, current-year spruce shoots frequently suffer from infection by a rust fungus resembling *Chrysomyxa woroninii*, which causes hypertrophy, intense yellowing and finally death of shoots (Donaubauer 1987, Kirisits et al. 2002). A second *Chrysomyxa* sp. causes needle rust, with symptoms and signs resembling those of needle rust diseases of other spruce species in the Northern hemisphere (Kirisits et al. 2002).

The most important forest health problem of *Abies densa* is a syndrome known as fir decline (Donaubauer 1986, 1987, 1993, Cielsa – Donaubauer 1994). In the 1980's numerous stands over an extensive area in Western Bhutan were affected and at many sites a large

portion, if not virtually all trees were killed. This dramatic fir decline was explained as a complex / decline disease (Ciesla – Donaubauer 1994), with prolonged drought and probably also frost as the main inciting factors and various biotic agents (stem and root rot fungi) as predisposing and/or contributing factors (Donaubauer 1986, 1987, 1993, Cielsa – Donaubauer 1994). Little is known about needle, shoot, branch and stem diseases of *Abies densa*. Needle blight caused by a fungus resembling *Lirula nervisequia* was prevalent in the 1980's (Donaubauer 1987). Trees of all age classes and especially also old trees were affected by this needle blight. Needle rust, caused by an undetermined rust fungus was observed once during the disease survey in 2001 (Kirisits et al. 2002).

Few, if any diseases have thus-far been documented on other temperate conifer trees in Bhutan. A needle cast caused by *Rhizosphaera* sp. occurs on *Tsuga dumosa* (Donaubauer 1987), and anecdotal reports suggest the occurrence of juniper rust (caused by *Gymnosporanium* sp.) on Black juniper (*Juniperus pseudosabina*) and Weeping blue juniper (*Juniperus recurva*). The latter is supported by the occurrence of *Gymnosporangium* spermogonia and aecia on wild apple (*Malus* sp.) trees. No diseases have been recorded on Eastern Himalayan larch (*Larix griffithiana*), Sargent spruce (*Picea brachytyla*), Bhutan pine (*Pinus bhutanica*) and Yew (*Taxus baccata*).

Results of the disease surveys since the 1980's form the basis for future surveys and studies on diseases of conifer trees in Bhutan. Our ultimate goal will be to publish a guide to important and/or common diseases affecting conifers in this Himalayan country. This guide would be a useful tool in facilitating the diagnosis, prevention and management of tree disease problems. It would also be helpful for the training of students and forestry staff in Bhutan to increase their knowledge and understanding in forest pathology. As the main objective of CORET is the education of Bhutanese scholars, researchers and practitioners and thus human capacity building in various disciplines of forest science, this guide would also immensely contribe to the success of this partnership program between Austria and Bhutan.

forest pathology / forest protection / disease survey / *Pinus wallichiana* / *Picea spinulosa* / *Abies densa*

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Black Stain Root Disease Studies on Ponderosa Pine – Parameters and Disturbance Treatments Affecting Infection and Mortality

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INTRODUCTION

Black stain root disease of ponderosa pine (*Pinus ponderosa* Doug. Ex Laws.), caused by *Leptographium wageneri* var. *ponderosum* (Harrington & Cobb) Harrington & Cobb, is increasing on many eastside Sierra Nevada pine stands in northeastern California. The disease is spread from tree to tree via root contacts and grafts but overland spread of the disease is most likely due to woody root feeding bark beetle (*Coleoptera:Scolytidae*) vectors. Soil and site relations along with disturbance are factors in the etiology of the disease (Harrington and Cobb 1988). Thinning and prescribed burning are important silvicultural tools in maintaining forest health in eastside pine stands. Because soil compaction is a concern in many sites, skid trails are treated by subsoiling equipment to alleviate compaction where this might be an issue. However, little is known of the effects of these silvicultural treatments on incidence of black stain root disease on sites with high disease risk. Because the woody root feeding insects that vector the disease respond to disturbance (Otrosina – Ferrell 1995), understanding consequences of different disturbances resulting from silvicultural treatments is essential for devising management plans to mitigate disease impact This paper summarizes preliminary results from two long-term studies initiated in 1996 and 2000 to address these issues.

MATERIALS AND METHODS

In 1996 and 2000, ponderosa pine sites were selected in the Modoc National Forest and near Poison Lake on the Lassen National Forest, respectively, in northeastern California. The first study (Modoc National Forest) objective was to determine effects of high impact and low impact thinning conducted in spring or fall seasons. Fifteen 2.5 ha plots, including unthinned controls, were located and marked for randomly assigned thinning treatments. Codominant tree age in the plots was approximately 100 years. Thinning treatments were conducted during spring and fall of 1995 and consisted of 1) low impact thinning involving rubber tired

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skidders on designated skid trails and chainsaw falling only, and 2) High impact thinning involving use of tracked shears and skidding at operator discretion. Each treatment was replicated 3 times in a random design.

Plots were visited every year post treatment until 2002. Thereafter, a final measurement to date was conducted on all plots during 2006. Data on symptoms and mortality were recorded for all years. Only dead or moribund trees with confirmed black stain root disease as determined by chopping into the root collar and observing characteristic black streaking were documented as mortality due to blackstain root disease.

In the second study conducted in the Lassen National Forest, we addressed effects of prescribed burning and subsoiling on black stain root disease development. The study design was a randomized complete block with four replications. Each treatment plot was 2.5 ha, with four treatments per block. Treatments were 1) underburning only, 2) subsoiling skid trails only, 3) underburning and subsoiling, and 4) untreated control. The entire study site was 80 ha and had been thinned one year prior to plot and treatment establishment. Prior to thinning, the average stand basal area was 263 ft^2 / acre (60 m^2 / ha) and the average QMD (quadratic mean diameter) was 7.9 inches (20 cm). Post thinning stand density was 121 ft^2 / acre (28 m^2 / hectare) with an average QMD of 14.8 inches (38 cm).

In addition to conducting 100 percent yearly surveys on each plot to record symptom development and mortality, experiments involving large woody root inoculations with *L. wageneri* var *ponderosum* were also conducted. These experiments were designed to provide information on the minimum amount of spores carried by insects necessary to start root infection. Spore suspensions containing 50, 500, 5,000, 50,000, and 3,050,000 spores were injected in artificial wounds created by coring to 2 cm depth in the xylem with a 4 mm diameter increment hammer. The spore suspensions were placed into roots of randomly selected trees in the burn only and control plots. Lesions, including sterile control wounds were measured after 9 weeks. During 2002, woody roots of trees with fire scorch damage and those without were inoculated with *L. wageneri*-infested 4 mm diameter cores. Roots were re-excavated after 9 weeks and lesions measured. Data on stem cambial sucrose synthase activity, a surrogate for determining stress via carbohydrate status of the trees, was also obtained during the 2002 and 2003 season as in Otrosina et al. (1999).

We also carried out insect trapping using Lindgren flight traps during the 2002-2003 seasons to determine treatment effects and potential relationships with subsequent disease occurrence. A cluster of four traps per plot was used and baited alpha pinene and ethanol. Traps were checked every 2-4 days during the flight season and trapped insects were counted and sorted by species.

RESULTS AND DISCUSSION

Modoc National Forest

Cumulative mortality for the 10 years since treatment initiation is presented in *Figure 1*. Excluding the control plots, the high impact thinning treatment conducted in the spring resulted in the most mortality. The low impact spring thinning did not have mortality due to blackstain root disease. This must be interpreted with caution because by chance, the assigned treatment on these plots happened to be on soils not favorable for black stain root disease development. There may be a correlation between certain soil series, vegetation types, and occurrence of the disease (Kliejunas – Otrosina 1998).

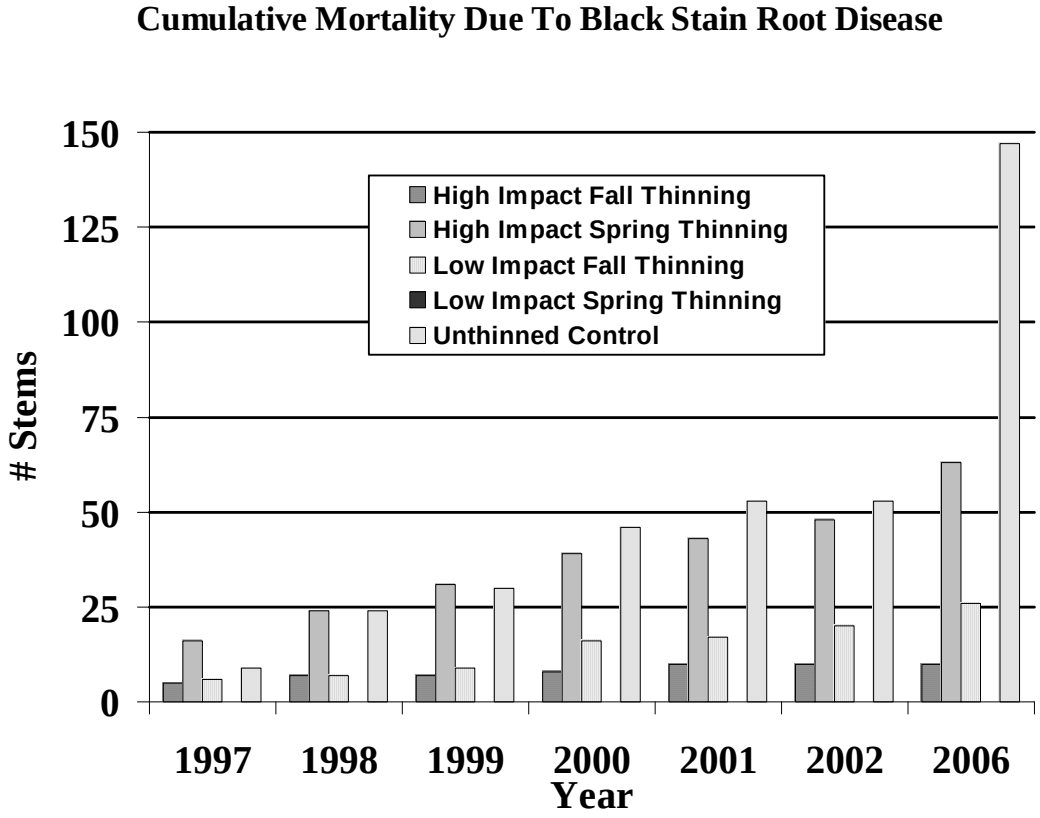


Figure 1. Mortality resulting from thinning treatments over 10 years following four thinning treatments. Note that low impact spring thinning plots never had blackstain root disease due to soil factors.

The control plots had dramatically more mortality than any of the thinning treatments. This is significant because it illustrates the benefit of lowering stand density and therefore stress in mitigating disease impact. Excessive stand density coupled with high mortality rates from black stain root disease can greatly increase risk of catastrophic wildfire in unthinned stands.

Lassen National Forest

While the study is long-term, intermediate results are interesting. Several root feeding insect species of interest, suspected to be potential vectors of *L. wageneri*, were caught during the two seasons. Among the more common species were *Hylastes macer*, *Hylurgops subcostulatus*, and *Hylurgops porosus*. Treatment differences in total insect trap catches are not obvious, although the underburn only plots tended to have slightly higher catches during the latter half of the flight season. In 2002, this trend appeared to be more marked, with greater catch numbers later in the season (*Figure 2*). Recently, DNA evidence indicated the insect species mentioned above, among others trapped on the study plots are carrying *L. wageneri*, presumably as spores (Schweigkofler et al., 2005). Such insect species have been suspected but heretofore have not been confirmed to be carrying *L. wageneri* in ponderosa pine stands. This confirms the long held notion that root feeding Scolytids serve as potential vectors of the fungus, critical for spread of the disease over longer distances.

Between 2001 and 2005, the burn only treatment had the highest mortality (*Table 1*). Scorching was evident on most of the mortality trees, which succumbed within two years following treatments. It has been approximately a century since fire last occurred in these stands. The subsoiling and burn treatment had considerably less mortality than the burn only

treatment. The subsoiled skid trails may have served to mitigate at least partially fire severity or intensity in these plots (*Table 1*). Consequently, caution should be exercised when reintroducing fire to stands that have not been burned for a considerable time.

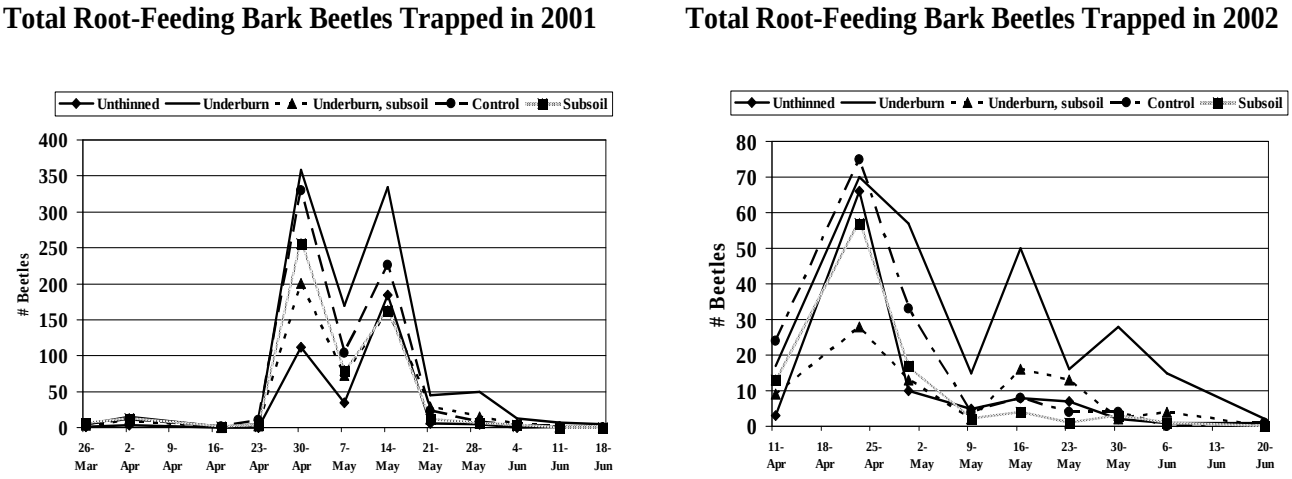


Figure 2. Summary of trap catches of root feeding Scolytids during the 2001 and 2002 trapping season. Note differences in scale between years.

Table 1. Poison Lake Mortality/Symptomatic Tree Data Summary

Treatment	Total # Dead Trees (2001-2005)		Symptomatic Trees		Confirmed Blackstain	
Underburn	322	(40.00)[1]	18	(3.87)	11	(2.99)
Underburn, Subsoil	174	(40.00)	12	(2.16)	2	(1.00)
Subsoil	35	(10.24)	33	(8.54)	15	(6.18)
Control	37	(10.31)	25	(5.25)	8	(4.00)

[1] Standard deviation (n=4)

Findings in 2004 inoculation experiments using the three dosages of a local isolate of *L. wageneri* spores (Otrosina et al 2004) are presented in *Figure 3*.

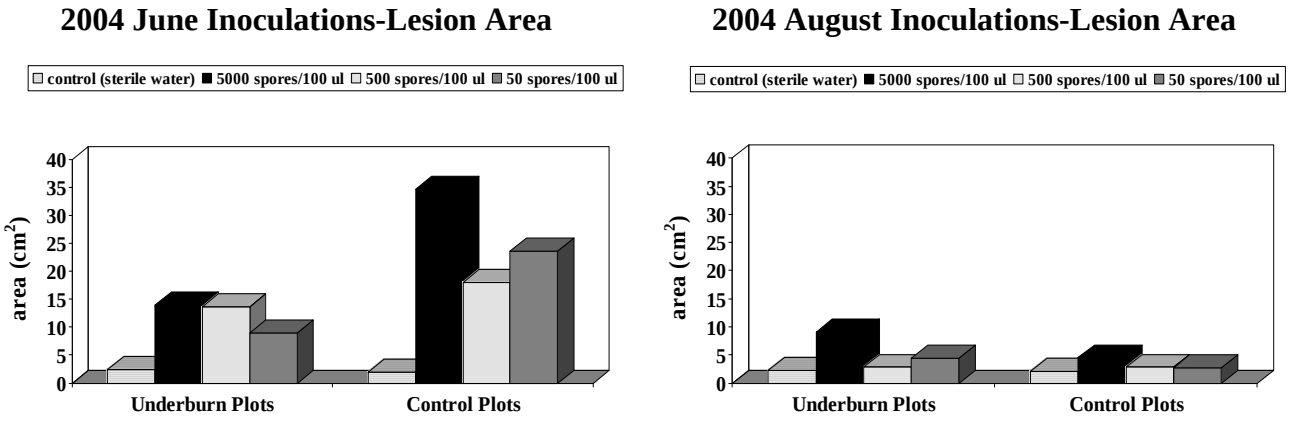


Figure 3. Lesion areas resulting from different L. wageneri spore dosages between underburned and control plots for June and August inoculations.

1) The June inoculations produced larger lesions in roots after 9 weeks than the August inoculation.
2) The lowest spore dose, 50 spores, produced lesions that were significantly larger than controls (June inoculations). This is noteworthy because it is consistent with the lower range of spore numbers found on potential insect vectors as determined by DNA analyses (Schweigkofler et al 2005).
3) Lesions in underburned plots trended smaller than control plots,
4) Lesions from August inoculations were significantly smaller than June inoculations
5) We recovered *L. wageneri* from lesions approximately one year (June 2005) after inoculation (June and August 2004) with the 5,000 spore inoculum dosages or mycelial inoculum.

Sucrose synthase activity, a measure of tree physiological status, shows a seasonal trend between the sampled months in 2003, and 2004. Peak activity is attained during July and August and drops rapidly during September. This is consistent with other data reported for ponderosa pine (Otrosina et al, 1996). These data seem to be negatively correlated with the lesion sizes in the June and August inoculations. The meaning of these relationships is unclear at this time but the physiological status of the tree and interactions with insect feeding, infection, and subsequent disease expression are important research topics that must be addressed.

In 2005, 100% surveys of each plot showed symptomatic trees, based upon crown characteristics, were distributed evenly among treatments, and few confirmed black stain root diseased trees were found. This is to be expected due to the longer time interval we anticipate from treatment initiation to infection, colonization, and symptom expression in the trees. Thus, further long-term monitoring of these study plots is necessary and planned.

Acknowledgements: The authors wish to thank Al Vazquez, Silviculturist, Lassen National Forest, and Jeff Withroe, Forest Ecosystems Manager, Lassen National Forest, for their continued interest and generosity in providing very significant assistance during various phases of this project. We also thank James Cunningham, Jeffrey Magniez, Chris Crowe, and Michael Thompson for their essential contributions in the lab and field phases of this project.

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Mycotoxin Producing *Fusarium* Species – the Cause of Primary Stem Canker of Deciduous Forest Plants

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OBJECTIVE

Fusarium species are spread worldwide and causing damages at different stages of tree development as well as are involved in many complex diseases of forest ecosystems. Most impact was assessed in forest nurseries with seedling diseases like root and hypocotyls rot and wilt. Moreover, afforestations and natural stands of young forest broadleaved trees were affected by a range of species with symptoms of foliage withering and dieback of branches as well as bark necrosis and canker. Because those alarming symptoms apeared more widespread in the last decade, *Fusarium* strains were isolated from affected young trees of black locust, birch, alder, and aspen from nurseries, afforestations and from natural stands in Germany. The isolates were classified in *F. avenaceum*, *F. tricinctum*, *F. sporotrichioides*, and *F. sambucinum* by conidia morphology and ITS sequencing of rDNA. Fifteen isolates were examined regarding their pathogenicity on six broadleaved tree species with artificial inoculation under glasshouse conditions. Furthermore, the mycotoxic properties of these strains were investigated from cell extracts produced in six different culture media by means of on-line couplings LC-PDA-Q-TOF-ESI-MS as well as LC-UV-NMR, and MALDI-TOF-MS.

MATERIAL AND METHODS

Fusarium strains were isolated from bark necroses of black locust, birch, alder, and aspen selected in nurseries and plantations. The isolates were determined by conidia formation and by partial 16S rDNA analysis. Inoculation experiments were carried out with containerized two year-old plants of *Sorbus aucuparia*, *Acer platanoides*, *Tilia cordata*, *Prunus avium* (all tested in 2005 and 2006), *Fraxinus excelsior* (2005), and *Quercus robur* (2006) by artificial inoculation with conidia suspension (~10^4 conidia / ml) with six replications. Control plants were grown without conidia treatment. *Fusarium* strains tested in 2005 and 2006 resp. were *F. avenaceum* (9 and 7 strains, resp.), *F. sporotrichioides* (2 and 4, resp.), *F. sambucinum* (2 and 4, resp.), and *F. tricinctum* (1 strain). Plants were estimated regarding formation of necroses and canker symptoms by a gradual score after 9 months: Without

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symptoms =1, spot infection =2, small necrosis not broader than the half of shoot =3; extended necrosis with canker =4, canker with withering and dieback =5.

Mycotoxin analysis of fungal strains: Rapid identification of secondary metabolites from pure culture (PDA) was carried out by combination of MALDI-TOF- and -TOF/ TOF-MS, as well as by on-line couplings LC-PDA-ESI-Q-TOF-MS and LC-UV-NMR. The mutual completion of structure information delivered by the spectroscopic methods UV/VIS, MS and NMR is of special importance for rapid identification of secondary metabolites directly in crude extracts.

RESULTS

Inoculation experiments had shown that all isolates caused shoot necrosis, canker, and dieback symptoms of tree species tested (*Figure 1*, *2*). Differences between *Fusarium* species were visible resulting in different intensity of symptoms. These can be traced back to the secondary metabolite profiles of strains showing the appearance of a range of metabolites known for their phytotoxic properties and of novel metabolites. Strains of *F. sambucinum* and *F. sporotrichioides*, which have caused severe damage, produced mainly mycotoxins from the trichothecene group. *F. tricinctum* inoculation induced only light damage. This species accumulated mainly cyclodepsipeptides such as enniatins. Between the different isolates of *F. avenaceum* a high variation of virulence was determined. The mycotoxin profiles of those strains had shown a large spectrum of compounds ranging from formation of cyclodepsipeptides alone up to cyclodepsipeptides and trichothecene mycotoxins.

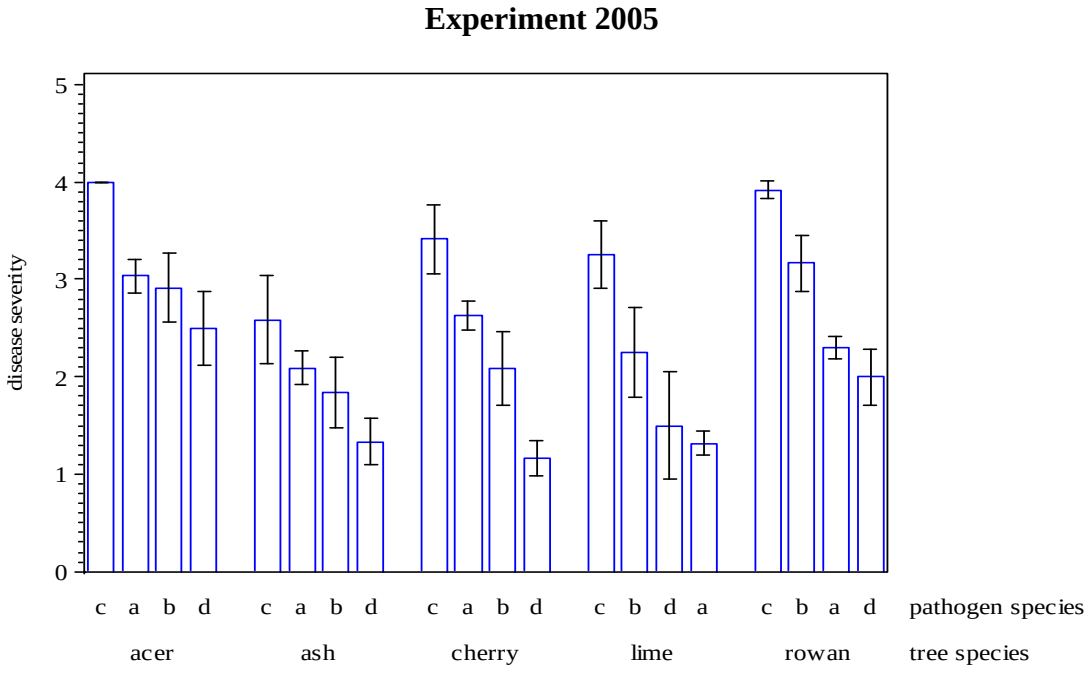


Figure 1. Mean of disease severity of deciduous tree species caused by four different Fusarium species 9 months after artificial inoculation from experiment 2005; the letters are indicating the fungal species: a F. avenaceum (n=54), b F. sporotrichioides (n=12), c F. sambucinum (n=12), d F. tricinctum (n=6).

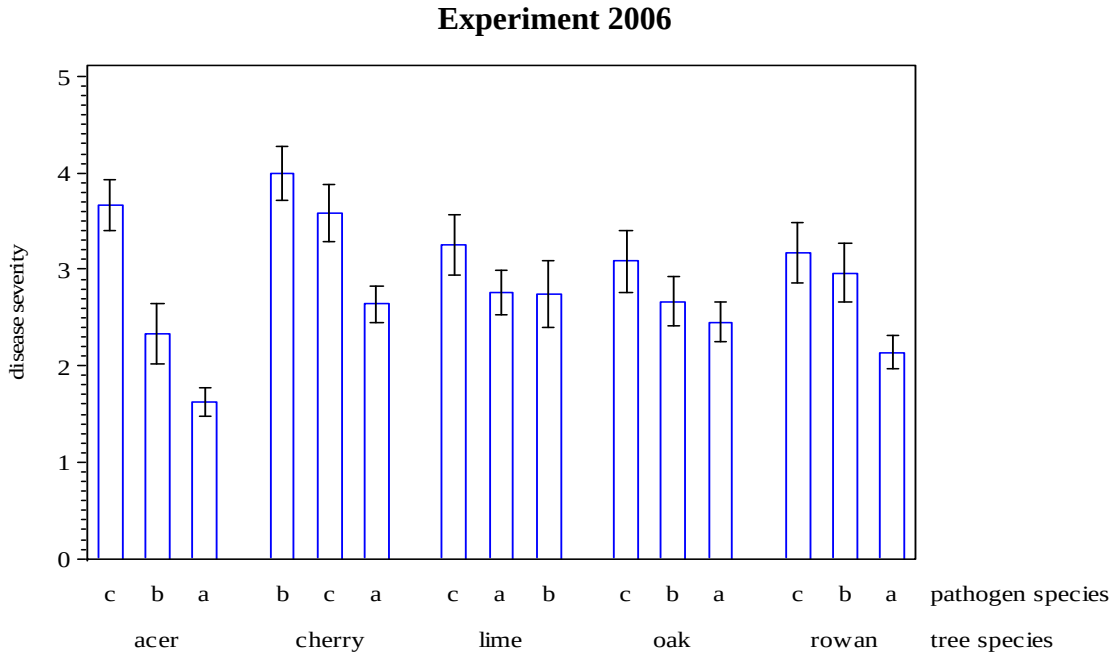


Figure 2. Mean of disease severity of deciduous tree species caused by three different Fusarium species 9 months after artificial inoculation from experiment 2006; the letters are indicating the following fungal species: a F. avenaceum (n=42), b F. sporotrichioides (n=24), c F. sambucinum (n=24).

DISCUSSION

Our results had shown the phytotoxic effect of a range of mycotoxin producing *Fusarium* strains on six different forest tree species was not specific. *F. sambucinum* had proven as a species with the highest pathogenicity to the young broadleaves tested followed by *F. sporotrichioides* and *F. avenaceum*.

Reports about damage in terms of bark necrosis, canker, wilt and dieback caused by *Fusarium* species are more common in the last years. Particularly the neophytic tree species *Robinia pseudoacacia* was investigated regarding these pathogens because it plays an increasing role in woody biomass production (Szabó 2000, Halász 2002).

Pathogenic *Fusarium* species are characterized by the formation of a large variety of toxic metabolites. More than 100 toxigenic secondary metabolites have been described (DeNijs et al.1996). Enniatins were long known as phytotoxins from *Fusarium* species and associated with plant diseases characterized by wilt and necrosis formation. Furthermore, beauvericin, moniliformin, as well as toxins from the trichothecene group are produced by members of the genus (Logrieco et al. 2002). The interaction between the trait of mycotoxin-production of a strain and their virulence could be proven at *F. graminearum* and *F. avenaceum* (Desjardins et al. 1996), where trichothecene-nonproducing and enniatin-nonproducing mutants resp. showed a reduced virulence at their hosts.

The recent study showed the possible role of members of this genus to evolve into serious pathogens for different broadleaved tree species in forested landscapes. This may become important under the aspect of transformation of arable land to areas for production of woody biomass.

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ABSTRACTS

A Comparison of *Dothistroma septosporum* Isolates from Two Forest Districts in Britain

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Abstract – Red band needle blight is a potentially lethal disease of pine trees, caused by the fungus *Dothistroma septosporum*. Until the late 1990's this disease was not considered to be a major problem in Britain, however it is now found throughout the country causing widespread damage, primarily on Corsican pine (*P. nigra* ssp. *Larico*).

This study sought to establish whether there is any variation *in vitro* between isolates from two Forest Districts (East Anglia and New Forest) in Britain. To do this linear growth rates on artificial media, biomass production, culture morphology and spore size was examined. Although there were differences in linear growth, biomass and culture morphology between isolates, there was no distinct grouping between isolates from the two geographical locations. In contrast, there was pronounced geographical variation in conidial length and width. Further studies are underway to investigate the genetic variability of these isolates.

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The Relationship Between Climate and the Incidence of Red Band Needle Blight in the East Anglia Forest District, Britain

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Abstract – Since the late 1990's there has been a dramatic increase in the extent and severity of red band needle blight (*Dothistroma septosporum*) in Britain, particularly in the East Anglia Forest District. In Britain and the main infection period for *Dothistroma septosporum* is thought to be between May and September. Meteorological data from East Anglia suggests that since the late 1990's the climatic conditions appear to have been favourable to the disease, with mean annual maximum temperature and rainfall having increased since 1998 by 0.9˚C and 0.3 mm respectively. In addition, as was found by Woods (2005) in British Columbia, the frequency of prolonged periods of precipitation and temperatures of 18 – 20˚C during summer months has increased during this period

These factors are likely to have influenced the rate of colonisation of *D. septosporum*. The increase in temperature over the past eight years supports the prediction of Broadmeadow and Ray (2005) that mean annual temperature will increase by between 3 - 6˚C by 2080. The increase in temperature observed in this study, if it continues as experts predict, is likely to benefit the spread and severity of RBNB.

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Bionomy and Symptoms of Dothistroma Needle Blight in the Czech Republic

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Abstract – The first symptoms on the needles infected in the current year appear in late summer (end of August, beginning of September) in the form of unspecific yellow spots on needles. Finally the needles get dry from tips and dead tissues are at first of straw – brown colour. In the course of September, at first dark brown and later narrow black strips are formed on dead parts of needles. Acervuli are formed from October and characteristic red strips appear. In acervuli, conidia can be formed till the end of November (depending on climatic conditions).

Under strong infection pressure, needles die already during the year of infection, namely rather early, from August till September. In the same year, acervuli can be formed even with accompanying symptoms as the occurrence of red strips.

Typical manifestations related to needle dying of the lower part of a crown and the abundant occurrence of red strips on dead tissues of needles were noticed in the CR primarily on Austrian pine *Pinus nigra*.

Dothistroma needle blight / symptoms

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Quambalaria Species: Leaf and Shoot Pathogens of Increasing Concern to Eucalypt Plantation Forestry

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Abstract – With the rapid expansion of eucalypt plantation forestry globally, the past decade has seen a sharp rise in the number of reports of *Quambalaria* (previously *Ramularia* and *Sporothrix*) species causing foliage, shoot and stem diseases on eucalypts. The severity of disease, the variety of symptoms and host species infected, as well as the geographical range in which *Quambalaria* species occur, have also increased. DNA sequencing has facilitated accurate diagnosis of the four species (*Q. pitereka, Q. eucalypti*, *Q. cyanescens*, and *Q. coyrecup*) associated with diseases. In this review, we attempt to summarize all published data on these species, and to compare them in terms of phylogeny, symptoms caused, host range, and current distribution. Phylogenetically the four species form a monophyletic lineage within the Microstromatales, a basidiomycete order closely related to several other orders of smut fungi (Ustilaginomycetes). *Quambalaria pitereka* is known as a shoot and leaf pathogen of a number of *Corymbia* species and has been reported from New South Wales, Queensland and Western Australia. The first discovery of this pathogen outside of Australia was made in 2006 when young *Corymbia citriodora* were found infected in Guangdong, China. *Quambalaria eucalypti* is the only *Eucalyptus* pathogen in the group that has not been found in Australia. It causes serious leaf and shoot blight of seedlings and hedges on several *Eucalyptus* spp. in South Africa, Brazil and Uruguay. The pathogen causing cankers and death of adult *C. calophylla* trees in Western Australia, has recently been described as *Q. coyrecup* (= *'S. destructor'*). The remaining species, *Q. cyanescens*, has been reported from New South Wales and Western Australia, occurring most often in combination with *Q. pitereka* or *Q. coyrecup* on both *Eucalyptus* and *Corymbia*. A currently inexplicable phenomenon is that *Q. cyanescens* had also frequently been isolated from humans (not as pathogens), soil, air, and as associates of various hardwood-infesting bark beetles, from several Eurasian countries from as far West as the Netherlands to as far east as Syria and Iran. The increasing severity and economic impact of disease outbreaks and the expanding geographic and host ranges of *Quambalaria* species, clearly shows that these pathogens should be seriously considered in eucalypt breeding programmes. For effective control measures to be developed, an appropriate understanding of the general biology, life cycles and infection strategies of these fungi will be required, since virtually nothing is known about these aspects of *Quambalaria* species.

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Red Band Needle Blight in Britain

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Abstract – Red Band Needle Blight is caused by the pathogens *Dothistroma septosporum* and *Dothistroma pini.* Until recently the disease was primarily of concern in the Southern Hemisphere, particularly on radiata pine (*Pinus radiata*). However, since the 1990's there has been an increase in disease incidence in Europe, mainly on sub-species of black pine (*Pinus nigra*) and also on lodgepole pine (*Pinus contorta* ssp. *latifolia*) in Canada.

In Britain the disease was first recorded in 1954 on pine nursery stock in Dorset. Disease outbreaks reoccurred sporadically at the same location until 1966 and there were two occurrences in Wales on plantation trees in 1958 and 1989. However, apart from these records there were no reports of the disease the late 1990s. Since then, the incidence of the disease in Britain has increased markedly, particularly on Corsican pine (*Pinus nigra* spp. *laricio*), with 20 reports of the disease between 1997 and 2002.

As a result of the increase in disease reports, in 2006 all Corsican pine stands under the age of 30 years on the Forestry Commission estate were assessed for disease incidence. The survey revealed that the disease was present in all Forest Districts surveyed in England and Wales and half of those assessed in Scotland. Overall, the disease was found to be present in 71% of the stands assessed covering a total of 7,051 hectares. The disease was also reported on a further 13 pine species in Great Britain during this survey, with lodgepole pine the second most frequently infected species. The increased incidence of the disease has resulted in a five year moratorium on the planting of Corsican pine, one of the most important softwood timber species grown in southern Britain. The threat this disease poses to lodgepole pine (*Pinus contorta latifolia*) which is grown mainly in Scotland, is also of concern.

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Actual and New Needle Diseases of Christmas Trees in Austria

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Abstract – The main conifer species cultivated as Christmas tree in Austria are Nordman's fir (*Abies nordmanniana*), Grand fir (*A. grandis*) and Colorado spruce (*Picea pungens*). Since there is no Austrian seed-production, seeds or young plants are imported. Therefore the risk of introduction of invasive pathogens is high. Furthermore, the production of Christmas trees in monocultures creates favourable conditions for the spread of pathogens.

This contribution gives examples for actual and new fungal pathogens on Christmas trees in Austria as well as for probable future invasive species.

The most common fungal species in Christmas tree plantations, especially on Nordman's fir, is Grey mould (*Botrytis cinerea* Pers.), which spreads in cool and rainy spring periods leading to irregular branching by killing young shoots. Another needle cast-species of Nordman's fir and Grand fir is *Kabatina abietis* Butin & Pehl. This species has become of increasing importance during the last decade. The characteristic symptom of *Kabatina abietis*, the reddening of the distal needle half, can probably also be produced by other fungal species, which are under investigation at present.

Three species of rust fungi cause loss of needles in Christmas tree plantations:

- *Pucciniastrum epilobii* (Pers.)Otth on firs growing in vicinity of *Epilobium* – species.
- *Chrysomyxa abietis* (Wallr.)Unger on spruces has only local importance.

The alpine rust *Chrysomyxa rhododendri* (DC.)De Bary infects only *Picea abies* growing close to Rhododendron-species.

Local epidemics by species of *Rhizosphaera* are a rare phenomenon in Austria.

Rhizosphaera kalkhoffii Bubák sometimes produces needle cast of spruces, on firs it is also *Rhizosphaera oudemansii* Maubl.

Colorado spruces are often infected by Lirula-needle cast (*Lirula macrospora* (R.Hartig)Rehm)). The infected needles of the previous year show a violet colour.

Since 2004, local epidemics of the hyphomycete *Thysanophora penicillioides* (Roum.) Kendrick are known from *Abies nordmanniana*. Thysanophora penicillioides, commonly known as a non host specific rather saprophytic fungal species, sporulates on green needles, which are shed subsequently. *Phaeocryptopus nudus* (Peck)Petr., known from Scandinavian countries as a new pathogen of firs, has not yet been observed in Austria.

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New Blight of *Cupressaceae* in Austria and Croatia

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Abstract – In 2003, foliar blight was reported from several large trees of *Chamaecyparis lawsoniana* growing in an Austrian park. First symptoms had already been observed in 2000, and in 2003 up to 2/3 of tree height was blighted. In May 2003, samples were taken and a fungus fructificating abundantly on dead leaves and twigs was identified as *Stigmina thujina* (Dearn.)Sutton.

In July 2007, similar symptoms were observed in a nursery in Croatia on large *Chamaecyparis lawsoniana* trees and *Stigmina thujina* was again the main fungal species involved.

On both sites trees of *Chamaecyparis* were older than 25 years.

The blight spread outwards and upwards in the crown

The disease affected small twigs, leading to small cankers there, thus the distal parts died off. Infection also occurred on needles.

The lower branches in the crown totally had died off, in the rest of the crown the branches remained with only the outermost parts living.

Up to now, none of the trees died off completely.

There was no indication of root diseases or of collar rot.

The climatic situation on the two sites shows similarities:

Both sites are characterized by high air humidity all over the year.

An analysis of the sporulation dynamics conducted in 2004 revealed the presence of viable spores during the whole year. A striking peak in the number of sporodochia observed in June 2004 is believed to be a consequence of heavy precipitation in the preceding May.

Mycelial growth on MA was slow, showing a maximum rate at 20°C (12mm in 6 weeks). No growth was observed at 30°C and growth below 4mm at 10°C in 6 weeks.

Sporulation started readily at 20°C between 4 and 5 weeks on MA.

The primary source of infection remains unclear. The Austrian site is a public park daily visited by tourists from all over the world, where an unintended introduction is conceivable. The site in Croatia, however is a local nursery. According to observations of aborists, the disease can be spread with pruning equipment.

It seems very probable, that the main triggering factor for the epidemics to develop was high air humidity for many years.

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Incidence of *Anisogramma virgultorum* in Planted and Site-natural Birch Stands in Scotland

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Abstract – In Scotland, *Betula* spp. are the most commonly found native broadleaf species and a major component in woodland plantings. To assess incidence of *A. virgultorum* and its association with crown dieback in planted and site-natural stands in Scotland, three field surveys were conducted. The survey of nine Woodland Grant Scheme plantings (*n*=900) showed that 47% of survey trees had severe crown dieback (40% or greater) and 57% of survey trees had *A. virgultorum*. Incidence and severity of disease were greater in *B. pubesens* than *B. pendula*. Crown dieback was greater in infected *B. pubescens* than non-infected trees, while disease incidence had no effect on *B. pendula*. A survey of six birch provenance trials showed that *A.virgultorum* was present at two of the sites with 3.7% (*n*=830) and 17.3% (*n*=2741) disease incidence. A survey of 90 site-natural birch stands across Scotland showed that *A. virgultorum* was only present at 12 sites with disease incidence ranging between 1 and 5% except for two sites where incidence of disease was 12% and 52%. Although no comparison has been conducted it appears that *A. virgultorum* is more abundant in planted birch than site-natural birch stands.

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Viscum album ssp. *abietis* on Silver Fir in Gorski Kotar (Croatia)

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Abstract – Research on the occurrence of common mistletoe (*Viscum album* ssp. *abietis* /Wiesb./ Abromeit) on Silver fir (*Abies alba* Mill.) in the region of Gorski kotar, Croatia, was carried out in the period 2003-2005. Six sites – fir natural stands were selected, three of soil sub-types on silicate and three of soil sub-types on limestone-dolomite. The aim of research was to reveal the intensity of mistletoes' occurrence in fir trees according to the degree of crown defoliation, and also to found out if there were any differences in mistletoes' presence between sites.

In total of 42 fir trees (7 per each research site) in defoliation categories 20-25, 30-35, 40-45, 50-55 and 60-65%, were felled and analysed. Firs in categories of 30-35 and 60-65% crown defoliation were represented by two trees. The data per sampled tree were collected as follows: tree's height and breast height diameter, fir age on stump, number of mistletoes and their biomass, and the age of the oldest mistletoe obtained in analysed crown.

The research data revealed differences in fir stands according to mistletoes' occurrence. The site average number of mistletoes in the crown of affected fir tree varied from 80.0 (locality Oštrac on silicate) to 160.3 (locality Miletka on silicate), with average biomass of 12.43 kg (locality Oštrac) to 23.68 kg (locality Podvodenjak on silicate). The heavily affected firs were obtained in three localities: Miletka and Podvodenjak on silicate, and Potočine-Crna Kosa on limestone-dolomite.

In all research sites it was found out that with the increased crown defoliation the number of mistletoes and their biomasses increase as well. The average fir tree grown on silicate revealed slightly more mistletoes (3.24%) in the crown than the fir grown on limestone-dolomite, and the mistletoes' average biomass was higher (11,69%) in the fir crown grown on silicate than on limestone-dolomite. On the other hand, the average age of the oldest mistletoe obtained in the fir crown growing on limestone-dolomite had 2.2 years more (15,56%) then the oldest mistletoe in the fir crown growing on silicate.

crown defoliation / number of mistletoes / mistletoes' biomass / silicate / limestone-dolomite

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Gymnosporangium Rust on *Juniperus excelsa* L. in a Nursery and in Forests in South-western Turkey

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Abstract – Juniper forests contribute to about 5.3% of the total forest area of Turkey. We investigated the frequency of *Gymnosporangium* infections on *Juniperus excelsa* both on seedlings in one nursery and on trees in six juniper forests located in southwestern Turkey. In the nursery, 0.7% of 5510 one-year-old seedlings and 29% of 200 two-year-old potted seedlings were infected. In each of the juniper forests fifty trees were chosen within a square of approximately four hundred square meters in size. Between the stands, the average height and diameter of the trees varied from 7 to13.4 m and 20.5 to 50.1 cm, respectively. In general, rust infections were common on the trees. On average 49% of the trees were infected; on 18% of the trees the infections were located on the stem, on 43.3% on the branches, and on 12.3% on both. There were significant differences between the stands, which can be due to different age of the trees, site factors and the presence of alternate hosts.

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Dutch Elm Disease in the Czech Republic

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Abstract – The first occurrence of Dutch elm disease in the Czech Republic caused by later described *Ophiostoma ulmi* (Buism.) Nannf. was noted by professor Peklo who found infected trees in elm alleys in Prague and Poděbrady in 1932. Subsequently the disease was spread to the rest of Czechoslovakia. New wave of wilting occurred at the beginning of 1960's. Probably it was caused by the new species *Ophiostoma novo-ulmi* Bras. mentioned in all countries around. To this time was not explained the identity of the right causative agent of this new wave of DED in the Czech Republic. Our study revealed some new facts about DED in the Czech Republic. *Ophiostoma novo-ulmi* was for the first time recorded on the area of the Czech Republic, so as its both subspecies ssp. *novoulmi* originated in the area of Ukraine and Moldavia and ssp. *americana* originated in North America. Remarkable is the number of hybrids of these subspecies, but non-hybrids ssp. *novo-ulmi* occurs at most. On the other hand *Ophiostoma ulmi* was not identified in any analyzed sample of elm twig. These taxonomic analyses were provided by methods of molecular biology - PCR and RFLP of CU and COL1 gene region. During cultivation of elm samples an endophytic fungus *Phomopsis oblonga* was isolated in a few cases. Elm bark invaded by this fungus is less attractive as a breeding site for the DED vectors - Elm bark beetles (*Scolytus* spp).

Dutch elm disease / *Ulmus, Ophiostoma novo-ulmi* / PCR - RFLP

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Diseases of *Pinus sibirica* Provenances Induced by *Lophodermiun pinastri* in South Central Siberia

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Abstract – A reliable method of studying intraspecific variability of woody plants is by provenance trials.

The *Pinus sibirica* Du Tour provenance trials were organized in 1983 in foot heels of West Sayan Mountains (Yermakovsk district, Krasnoyarsk region) characterized by optimal Siberian pine growth conditions. *P. sibirica* plots were represented by climatypes moved from western Siberia (Kemerovo and Tomsk regions) and Krasnoyarsk region. Korean pine (*Pinus koraiensis* Siebold ETZUCC.) was represented by climatypes from the Russian Far East: Khabarovsk and Primorie regions.

These Siberian pine provenances were studied in 1990, 1995, 2000, and 2006 to reveal that they differed in rate of survival 20 years after they had been moved. The highest survival rate (89%) was exhibited by Siberian pine of Krasnoyarsk and Korean pine of Primorie provenances, followed by 87, 80, and 69% survival for Tomsk, Khabarovsk, and Kemerovo provenances, respectively. The provenances from Kemerovo region were found to be characterized by lower values of biometric parameters compared to other provenances. For this provenance, the needle foliage was observed to turn yellow and brown annually, which gradually lead to tree mortality.

Mycological analysis showed *Lophodermiun pinastri* (Schrad.:Fr.) Chevall to be the major factor accounting for Kemerovo mature Siberian pine needle drying out. This fungus was also recorded on 3-4 year-old *Pinus sibirica* seedlings in a forest nursery. *Aspergillus, Alternaria, Mucor, Trichoderma, Fusarium* saprophytic fungi were noticed to accompany *L. pinastri*.

The role of *L. pinastri* as a needle fall agent for Siberian pine individuals moved from Kemerovo region remains an open question.

Kemerovo provenance was recorded to become less resistant to Krasnoyarsk regional ecological factors with aging compared to other provenances.

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Viruses of *Gremmeniella abietina*

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Abstract – Types and races of *Gremmeniella abietina* species complex cause Scleroderris canker on pine trees in Europe and North America. We have studied viruses of types A and B of *Gremmeniella abietina*.

In type A three different mycoviruses belonging to families *totivirus*, *partitivirus* and *mitovirus* occur. They were even observed to inhabiting a single mycelium. During early analyses of these viruses family *partitivirus* was the most common one. In more recent sampling partitiviruses and totiviruses were rare, but mitoviruses very common.

In type B we observed totiviruses, mitoviruses and a previously uncharacterized virus with high similarity to plant endornaviruses. The difference of viruses in types A and B supports our previous hypothesis that viruses do not move freely between the two Fennoscandian types of *G. abietina*.

A partial sequence, covering about 70 % of the complete genome, of putative totivirus of type B was determined. The dsRNA genome codes for putative coat and putative RNA dependent RNA polymerase (RdRp) and they were most similar to similar proteins of the totiviruses of *Spaeropsis sapinea* and *Helicobasidion momba*, respectively.

A complete sequence of a mitovirus from type B was determined. The putative RdRp was most similar to RdRp of a mitovirus from *Ophiostoma novo-ulmi*. The previously sequenced mitoviruses from type A had a relatively dissimilar RdRp. The putative start codon was in an AU-rich region surrounded by regions with a relatively high GC-content. Several previously observed secondary structures could be deduced from the nucleotide sequence, and sequence variations occurred at both ends.

The previously uncharacterized virus from type B was completely determined independently from two isolates. The dsRNA molecules (10374 and 10375 nucleotides) encoded for putative polyprotein possessing four conserved motifs coding for viral methyl transferase, DEAD/DEAH box helicase, viral RNA helicase and RdRp.

A population structure was determined for type A mitoviruses based on RT-PCR amplification and sequencing. It showed no genetic differentiation between the populations, suggesting that viruses are able to disperse freely between locations in southern Finland.

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Gremmeniella abietina (Lagerb.) Morelet: Distribution in Serbia and Montenegro, Significance and Control

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Abstract – In Serbia and Monte Negro, the study of the most important diseases of Austrian pine and Scots pine has become very relevant, having in the mind the importance of these species which is constantly growing, as new plantations have been established in accordance with the long-time programme of bare land afforestation. In Austrian pine plantations, the greatest damage has been caused by the fungi *Mycosphaerella pini, Sphaeropsis sapinea, Lophodermium* spp.(*seditiosum, conigenum, pinastri*), *Cenangium ferruginosum* and in the mountains regions *Germmeniella abietina.* In Scots pine plantations the main causes for the premature needle casting are *Lophodermium* species and in the mountains regions *Phacidium infestans* and *Lophodermella sulcigena.*

Fungus *G. abietina* is one of the most dangerous pathogenic fungi inhabiting conifer plantations and especially endangered species are the pines. Among the pines, the most susceptible species are Austrian pine and Scots pine, and endangered plantations are aged between 8 and 25 years. The fungus was detected in the plantation of Austrian pine in region of National Park "Durmitor" for the first time at the end of 1979. Already in 1988 this fungus was identified in the plantation of Scots pine on Mt. Kopaonik, and in 1992 in the regions of Vlasina and Goč. During 1998, the fungus occurred in epiphytotic proportions in Scots pine plantations in the region of Bukovica (Mt. Ivica). During 2006.y. *G. abietina* was detected second time in the plantations of Scots pine on Mt. Kopaonik (location "Samohovska River"). In Serbia and Monte Negro the fungus was identified on Austrian pine (*Pinus nigra* Arnold), Scots pine (*Pinus sylvestris* L.) and spruce (*Picea abies* (L.) Karsten). In its development *G. abietina* form both stages. Its picnidial stage is far more significant for the infection process, and mainly all infections are carried by conidia. Infection of trees is possible throughout the year, but the critical period of infection is May-June. Conidia are transmitted by raindrops, and the infection is spread through the buds and bark of young shoots. Incubation period lasts for 9 months. The symptoms of infection are visible at the base of buds, on the needles (orange discolouration at the base of the needles) and on the bark of young shoots. Soon after the symptoms, the fruiting bodies (i.e. pycnidia) appear on the necrotic tissues of the host. Apothecia formed on the bark pine two years after tree dying. In the severely infested plantations, all dead trees should be felled and removed, and remaining trees should be treated with copper fungicides. Previous preliminary investigations (in plantations of Scots pine on Mt. Ivica) showed that the copper fungicides (for example copper oxychloride) have given the best results and protection. The protection is satisfactory of the treatment is carried out twice a year, during the critical period of infection. As this is a quarantine disease, care must be taken to prevent the spread of the disease to the new uninfected region. This measure is imposed by the legal regulations on quarantine disease.

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Tolerance of Scots Pine to Pathogens in the Provenance Trial of Siberia

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Abstract – Results of resistance assessment of provenance trials in Central Siberia to fungal diseases show a genetic heterogeneity of Scots pine by the influence of ecological-climatic factors of places of origin. Dissimilar resistance to pathogens shows itself in the same Scots pine climatypes at growing them in provenance trials at different ecological backgrounds. Pine trees growing on poor dry soils (bear berry pine forest type, sandy soil) are subjected to the common and snowy schutte (*Lophodermium pinastri Chev*. and *Phacidium infestans Karst)*, cenangium necrosis (*Cenangium abietis (Pers) Pehm* in the stage *Dothichiza ferruginosa*). However, on the more humid and rich soils (dark-grey forest soil, rich in herbs pine forest type) the rusts, pathogen organisms of bladdery rust or canker-blister rust have been found (*Cronatrium flaccidum (Alb. Et Schw.) Wint.* and *Peridermium pini (Pers.) Lew. Et Kleb.*).

It is known that phacidiosis (Phacidium infestans) occurs in natural pine forests beginning from the northern timberline. In the northern taiga subzone there are favorable conditions for development and spreading of pathogens. Their negative impact strengthens towards high latitudes as the soil- climatic regime becomes worse, the winter period becomes much longer and snow cover becomes more stable. High resistance of northern Scots pine climatypes (subspecies "northern lapponian" and "siberian" from southern taiga subzone) to pathogens is observed in provenance trial in the Priangarie region. Therefore we can suppose that resistance to pathogens has formed and developed with the time in pine posterities of northern populations. Resistance to fungal diseases has not formed in pine climatypes from western, central and southern regions of the areal (subspecies "kulinda p." and "scots p.") just in places of their origin. Therefore in the test area (under Priangarie region conditions) they are more vulnerable to pathogens. Disturbance of linear growth is observed in pine climatypes not tolerant after snowy Schutte disease. It is connected with falling down more than 50% of needle, with drying up of a terminal bud of the central and sucker shoots and with replacement of a central shoot due to a living shoot of a lower whorl. The plant gains a bushy form in this case. Such a stem form is observed in the bear berry pine forest type (sandy soil) in climatypes from steppe and forest steppe regions.

Literature evidences about relation of bladdery rust to forest site conditions are fragmentary and contradictory. According to one authors the mass fungus spreading in young pine tree stands is observed only under unfavorable growing regimes, mainly, in lichen pine forest. According to other authors – the loss from this pathogen is found in pine stands with a better (more rich) growing regime. This disease is met in our experiment only in the more humid and more rich forest type (rich in herbs pine forest type, dark- grey forest soil). With age increase of trees the number of damaged trees not resistant to this pathogen, mainly, from southern regions of pine areal enlarges too.

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Snow Pocket, a Triggering Factor for Scleroderris Canker Outbreak

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Abstract – Numerous tree diseases develop in the snow or under cold climatic conditions. The best known are the snow blights caused by *Phacidium* spp., and the brown and white felt blights caused by *Herpotrichia* spp. Sclerroderris canker also needs cold conditions for its development. It is caused by the fungus *Gremmeniella abietina*. We report here on observations concerning the North American race. This disease is found mainly on red pine (*Pinus resinosa*) and jack pine (*Pinus banksiana*) in eastern Canada. Snow plays important roles in the development of the disease. First, it provides conducive conditions for the disease to progress into the shoots while trees are in a latent period; this fungus is still active at - 6° C. Another effect of snow is mechanical but relates to the previous one: the weight of the snow brings down branches and even makes whole trees bend down; thus, more shoots are in the snow where the pathogen can develop. Finally, the snow can trigger off an epidemic: it accumulates in greater quantities in topographic depressions, creating conducive conditions for several shoot infections to develop on a larger portion of trees on numerous neighbouring trees. All these infections raise the inoculum rate, creating a centre of infection in the plantation.

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Natural Selection of *Pinus banksiana* Regeneration Through Increase Inoculum of *G. abietina*, North American Race

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Abstract – Scleroderris canker caused by the North American race of *Gremmeniella abietina* induces damage to pine shoots covered with snow. Jack pine (*Pinus banksiana*) is a native species affected by this disease. Most reports of damage concern plantations. Our observations have been on-going since 1989, in an area where Jack pine was seeded over an area of 552 ha in 1979. This stand is located near lac Nippon, 100 km west of Saint-Felicien, Lac-Saint-Jean region. Even if 96% of the Jack pines were infected in 1989, the rate of mortality only increased from 64 to 72% between 1990 and 1994. The maximum height of infected shoots in trees rose from 0.9 to 1.5 m during the same period. In 1994, the mean height of dead saplings was 0.9 m, while the height of surviving Jack pines was 1.9 m. Since 1995, the disease has been at an endemic level, because of the lack of healthy shoots to be infected under the snow cover. Fast growing or older pines survived the disease. Very few residual trees have shown cankers on the main stem in 2004. There were enough stems left in the stand and a thinning operation was conducted in 2000. The disease was an element of the natural selection of Jack pine regeneration.

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Two Pine Species Resistant to *Gremmeniella abietina*, European Race

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Abstract – Under field conditions, we have demonstrated that *Pinus banksiana* (Pb) is resistant to the European race of *G. abietina*. Using microscopy, compartmentalization of the invaded tissues has been reported to explain the limited tip blight observed on shoots. Following this finding, inoculation field trials on *P. contorta* (Pc) have been carried out. Pc is located in western North America and is closely related to Pb. These species even hybridize at the junction of the two populations in Yukon, Canada. The field trial was conducted with Pc seedlings situated in a 20-year-old *P. resinosa* (Pr) plantation infested with the disease. Pb and Pr seedlings were also used as resistant and sensitive species respectively. All Pr seedlings had died of the disease while Pb and Pc had survived. There were similarities between the resistant Pc seedlings and Pb: the infection was limited to a 2-3 cm long tip blight. A suberized defensive zone was initiated at the base of healthy needles. This zone reached the vascular cambium before proceeding downward. Tissue regeneration, formation of traumatic resin canals and accumulation of phenols are also associated with the defense system of Pc against this disease. Formation of ligno-suberized boundaries appears significant in the defense system of Pb and Pc. Using naturally infected samples, further cytochemical and immunochemical characterizations of phenols, pectin and callose were carried out to clarify their involvement in the resistance to this disease. Reacting parenchyma cells, including hyperplastic cells, were rich in polyphenols, particularly catechins and condensed tannins. A strong reaction was obtained for pectin in the affected tissue, except for necrotic cells. Enrichment in polyphenols and pectin is considered to be an expression of resistance whereas callose does not seem to play a role in this process.

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Impact of Climate on Oak Powdery Mildew

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Abstract – Oak mildew, *Microsphaera alphitoides*, is a widespread exotic oak pathogen that arrived in Europe at the beginning of 20th century. The disease has long been considered by foresters as having limiting impact on trees because it arrives late in the vegetation period and does not infect significantly the first oak leaf flush. The Département de la Santé des Forêts (DSF) however has been mentioning those last years that the disease might have an important impact on mature trees in some situations, with a possible role as inciting decline factor. Limiting information exists on this very common oak pathogen and a work was initiated to better understand it possible role in oak decline.

To identify the situations where oak mildew might be a threat to tree, we analysed the DSF database. This database is a compilation of de reports de forest health problems those last 17 years done by a network of foresters trained for diagnostic. The data analysis was done by using methods developed in human epidemiology, i.e. by comparing the distribution of oak mildew reports in time and regions with the distribution of others heath problems of oaks (root rot, decline ands insects defoliators) that are used for standardisation. The analysis shows that many reports concerns seedlings or oak previously defoliated by insects or frost. However, about 25% of the reports concern mature trees that were not previously defoliated, which is unexpected. We analysed more specifically those reports concerning non-defoliated mature oaks. The results show that they occur mostly in southwest France in some specific years. The reports correspond to a very early arrival of oak mildew in those years, with a massive infection of the first oak leaf flush. The analysis show that the year with an early arrival of oak mildew in the vegetation season are year with especially mild winters. Years with such a climate were very infrequent during most of the 20th century and became more frequent those 17 last years.

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Sphaeropsis Blight of *Pines* in Serbia and Montenegro – Plant Hosts, Epidemiology, Importance

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Abstract – *Sphaeropsis sapinea* DYKO & SUTTON is a destructive conifer pathogen of worldwide distribution and importance. However, the greatest damages have been occurred on *Pinus* species. It has been researched intensively in Serbia and Montenegro during late 80-ties and late 90-ties. In this period it was recorded as one of the most important cause of dieback in Austrian and Aleppo pine plantations older than 20 years and also in urban areas.

This pathogen is widely distributed in the continental and Mediterranean parts in these countries. It is recorded on eleven *Pinus* species - *P. nigra*, *P. sylvestris*, *P. halepensis*, *P. jeffrey*, *P. peuce*, *P. pinaster*, *P. ponderosa*, *P. peuce*, *P. pinea*, *P. mugo* and *P. heldreichii* and on species of six coniferous genera - *Abies*, *Cedrus*, *Chamaecyparis*, *Cupressus*, *Juniperus* and *Thuja*.

For the first time *S. sapinea* was registered in Serbia on *Pinus nigra* in 1981 (KARADŽIĆ, 1983). On *P. heldreichii*, a Tertiary relic and a Balkan subendemic, it was first recorded on individual trees near Pećka Patrijaršija in 1993., and after that, near the Monastery Ostrog. It was the first report of *Sphaeropsis sapinea* on this plant host in Serbia and Montenegro.

S. sapinea can infect almost each part of host plant, causing many symptoms. However, the most common symptoms are shoot blight, characterized by stunted dead shoots and needles, bud wilt, stem cankers and branch dieback. It also causes the necroses of the seed cones and their dwarfishness. All these symptoms were observed during this research.

S. sapinea penetrates through buds, bark of young shoots and needles. The critical period of infection is from middle of April till the middle of May, when infections mainly occur through the bark of young shoots, which results in their dying. The very early symptoms on the young shoots were mostly observed at the first part of May. Changing of colour of infected needles can be seen at the beginning of June, while in the middle of June they become yellow-brown.

Infections through the needles occur mainly at the time of their sudden growth or during summer months. Second year seed cones are susceptible to the infection in the second decade of April. Current year seed cones can be infected as well.

Pycnidia have been observed on young shoots and needles, polen and seed cones, buds, current year and second year cones, and in the bark of older branches on *P. nigra*. Pycnidia with mature conidia can be formed during the same year of infection. In the bark of young shoots they were identified at the end of June and on the cones at the beginning of the third decade of July.

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Excised Shoots of Top-pruned Red Pine (*Pinus resinosa*), a Source of Inoculum of the Shoot Blight Pathogen *Diplodia pinea*

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Abstract – *Diplodia pinea* causes shoot blight and collar rot diseases that kill or otherwise render nursery pine seedlings unmarketable. *D. pinea* sporulates abundantly on needles and stems it has colonized and killed. Nursery managers sometimes top prune pine seedlings to reduce their height. In late summer 2005, *D. pinea* pycnidia were observed on excised shoots present in red pine seedling beds that had been top pruned earlier during that third season of growth. This prompted a survey of top-pruned beds at two nurseries to determine incidence and abundance of *D. pinea* conidia from excised shoots. At each nursery, excised shoots were collected from the seedling canopy in two subplots and adjacent alleyway ground surface in each of five beds (plots). A washing and filtration technique was used to quantify conidia extracted from colonized shoots. Excised shoots from both nurseries abundantly bore *D. pinea* pycnidia and conidia. Excised shoots collected from the seedling canopy yielded more conidia than shoots collected from the ground. Most conidia from shoots in the canopy and from the ground germinated on water agar. Species-specific PCR primers were used to confirm the identity of the pathogen. Removal of excised shoots from top-pruned pine nursery seedlings should be considered as a means to reduce inoculum in nurseries where *D. pinea* is present.

Sphaeropsis sapinea/ Diplodia scrobiculata

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Quantification of Conidia of *Diplodia* spp. Extracted from Red and Jack Pine Cones

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Abstract – Jack (*Pinus banksiana*) and red pine (*P. resinous*) are economically and ecologically important conifer species that are hosts of the pathogenic fungi *Diplodia pinea* and *D. scrobiculata* . In Wisconsin, *Diplodia* spp. cause shoot blight and death of understory red and jack pine seedlings. *Diplodia pinea* has been associated with red pine cones, but there is little information about the association of *D. pinea* with jack pine cones or *D. scrobiculata* with either host. Presumably, the inoculum that infects understory seedlings comes from colonized host tissues, including cones, in the overstory. This prompted us to develop a survey method to quantify inoculum in mature pine cones of both jack and red pine. During two consecutive summers, cones were collected from both the ground and the canopy from three sites in Adams Co and Jackson Co respectively. A total of six stands where mature red and jack pines co-occurred in the overstory were sampled. In the laboratory, cones were systematically washed to extract conidia and spore counts were estimated. A PCR assay was conducted to identify *Diplodia* to species level. *Diplodia* spp. were found every year, at each site, in every tree. More conidia were extracted from cones harvested from the canopy than from cones harvested from the ground. More conidia were extracted from red pine than jack pine cones. At least 60% of the conidia extracted from cones germinated in controlled laboratory conditions. *D. pinea* was more frequently isolated than *D. scrobiculata* from both jack and red pine cones although *D. scrobiculata* occurs more frequently in jack pine cones. Both cones harvested from the canopy and the ground can be used to detect the *Diplodia* spp. present at a site. Cones harvested from the canopy can be utilized to detect site differences in the amount of inoculum produced by *Diplodia* spp.

Sphaeropsis sapinea

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Identification of Secondary Metabolites from *Phytophthora alni*, the Cause of Decline of Alder Trees in Europe

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Abstract – Within the genus *Phytophthora* (*Oomycetes, Pythiales, Pythiaceae*) the distinct species *P. alni* has been developed as a pathogen on alder trees (*Alnus* spp.) causing the European alder decline in natural stands since the ninetieth. The pathogen was considered likely to be a hybrid between *P. cambivora* and a *P. fragariae*-like species. Both species are known as producers of toxic compounds causing necroses and wilt on different plant families. Strains of *P. alni* ssp. *alni* isolated from damaged tissue of alder bark were cultivated on the five media FCM, FDM, M1, MEA, and PDA. From the cell extracts of these strains 15 novel cyclic peptides named phytophthoralnins are identified by means of LC-PDA-ESI-Q-TOF-MS, MALDI-TOF- and -TOF/TOF-MS. Structure elucidation of the metabolites produces by *P. alni* has carried out partly.

Compounds from group of cyclic peptides are secondary metabolites which have varied properties acting as toxins or forming pathogenicity and virulence factors resp. within the host-pathogen complex.

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Irrigation Water and Stem Lesions on *Betula pendula*

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Abstract – In 1991 *Phytophthora cactorum* was first time isolated from necrotic stem lesions on *Betula pendula* seedlings growing in forest nurseries in Finland. In this study we baited *P. cactorum* from a pond from which a forest nursery is taking its water for irrigation. Juvenile, detached *Rhodonendron* and *Ledum palustre* leaves and stem pieces cut from the top of *B. pendula* seedlings were used as baits. Direct isolations were also done from birch seedlings. A simple, but useful technique, the Random Amplified Microsatellites (RAMS) analysis was used to test whether isolates from pond were genetically similar to those actually causing symptoms on birch in the nursery. The pathogenicity of two isolates was also tested. The beta tubulin coding region of all *Phytophthora* isolates were amplified and sequenced. Cloning of the beta tubulin gene region and sequencing of the clones were also done using one isolate. The pathogen was present in the pond every year, but in 2005 no diseased seedlings were found. Lesions were formed on all baits, but *P. cactorum* was only isolated from lesions on *Rhododendron* leaves. Although the isolates from pond and from stem lesions were genetically similar, the isolates showed significant variation in both cultural and morphological characteristics as well as differences in pathogenity. Beta tubulin gene sequences were identical in all studied *P. cactorum* isolates. Interestingly, two distinct sequences of beta tubulin gene were detected from all studied isolates. One of them was identical to the most often reported *P. cactorum* allele in GenBank, whereas no exact match was found for the other allele. The result indicates that our *P. cactorum* isolates might exhibit heterozygosity in the beta tubulin gene. Other possible explanation is that the genomes of the isolates contain two separate beta tubulin gene locuses. Either possibility has not been previously reported in *Phytophthora* species.

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Susceptibility of *Cupressus arizonica* and *Pinus pinea* to *Pestalotiopsis funerea* Isolates

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Abstract – *Pestalotiopsis funerea* is a fungus reported mainly as a weak pathogen of conifers, although it has also been found causing severe damage on several hosts. The main aim of the study was to determine the optimal growth conditions *in vitro* for Spanish isolates of *P. funerea* and to evaluate the pathogenic effect of those isolates on *Pinus pinea* and *Cupressus arizonica* under field and lab conditions. Eight isolates of *P. funerea* derived from *C. sempervirens*, *C. arizonica* and *Quercus pyrenaica* were used in the assays. In the growth rate experiment, five culture media (PDA, MEA, WA, PCA and SCALA) and six temperatures (5, 10, 15, 20, 25 and 30 ºC) were evaluated. In the pathogenicity tests, two different experiments were carried out: those in the lab consisted of inoculations in 30 mm long twigs of *C. arizonica*. In those of the field, twigs and needles of *C. arizonica* and *P. pinea* trees respectively were inoculated with mycelia by means of a wound. Four months after inoculations, twigs and needles were transported to the lab where necroses length was measured. The results presented here suggested that the *P. funerea* isolates from Spain had an optimum temperature for growth at 25 ºC on SCALA media. The results also indicated that the fungus showed a virulence on *C. arizonica* and *P. pinea* after inoculating mycelia into a wound but host-preference was not observed. A certain correlation between growth rate in culture media and virulence was also observed on *P. pinea* needles.

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Specific Primers for Detection of Two *Sirococcus* Pathogens of Conifers and Comparison of PCR to Cultural Methods for Detection of *S. conigenus*

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Abstract – A PCR-based method was developed for the specific detection of the conifer shoot blight pathogen *Sirococcus conigenus* and the closely related fungus *Sirococcus tsugae*. Regions of diversity in the internal transcribed spacer (ITS) sequences of *Sirococcus* species were exploited to design primer pairs. Forward primer SirCf and reverse primer SirCr were used for identification of *S. conigenus*, and forward primer SirTf and reverse primer SirTr2 were used for identification of *S. tsugae*. Specificity was tested using multiple isolates of these two species, isolates of *S. piceicola* from spruce, *S. clavigignenti-juglandacearum* from butternut, and isolates of several other fungi obtained from pines. The PCR-based method for detection of *S. conigenus* was tested and results compared to those obtained using a cultural assay using shoots collected at six locations in Wisconsin and Michigan. For needles, bark, and wood of symptomatic shoots, the mean frequencies of detection of *S. conigenus* using the PCR-based methods were consistent ($\geq$ 7.5 out of 10) and always greater than for the cultural assay. For the cultural assays of symptomatic shoots, detection of *Sirococcus* spp. was more frequent from needles than bark or wood. Both the PCR-based method and the cultural assay detected *S. conigenus* in similar frequencies from asymptomatic shoots, though less frequently than from symptomatic shoots. The relative efficiency of our PCR-based method and its utility for direct testing of field-collected host material should make it particularly useful in areas of the western United States and Canada where both *S. conigenus* and *S. tsugae* have been found, and in situations in which other shoot blight pathogens also are commonly encountered.

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Phytophthora Species in Forest Stands in Hungary

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Abstract – Occurrence of *Phytophthora* species and their phytopathological role have been investigated in forest stands since 1999. Trees with specific stem lesions and unspecific top drying symptoms were surveyed in forest stands of different tree species in order to find the causal agents and especially to clarify the role of the *Phytophthora* species in decline.

The isolation of *Phytophthora* was carried out from soil taken from around the diseased trees by baiting with *Prunus laurocerasus* leaves on selective medium PARPNH. The isolates were identified by morphological and molecular way. The morphological characters were observed in the cultures growing on carrot agar medium. The formation of sporangia was induced by flooding of the cultures with soil extract. The molecular identification was performed by sequencing the ITS regions of the rDNA and comparing with the known *Phytophthora* sequences accessible in GenBank database. The pathogenicity of the isolates was tested by wound inoculation in the stem of seedlings and by root infection as well.

Phytophthora species were found in *Alnus glutinosa* with bleeding stem lesions and crown drying symptoms, in *Juglans nigra, Quercus petraea* and *Q. cerris* with crown drying symptoms. The morphological and molecular identification resulted in 8 *Phytophthora* species in *Alnus* (*P. alni, P. citricola, P. gonapodyides, P. inundata, P. megasperma, P*. sp.1, *P*. sp. 2., *P*. sp. 3.), 4 in *Juglans* (*P. cactorum, P. cirticola, P. hedraiandra, P*. sp.1) and 2 in *Quercus* (*P. citricola, P.gonapodyides*). The inoculations caused well-delimited bark necrosis in the stem of seedlings, the largest by *P. alni* in alder and *P. citricola* in black walnut, but generally not exceeding 3-4 cm length. Root infections caused lesion and reduction of fine roots, most pronounced by *P. citricola* in black walnut.

The impact of *Phytophthora* species on the healthy condition of the forest trees in Hungary is most important in *Alnus glutinosa* and *Juglans nigra* stands situated in wet sites and flood areas respectively. A community of *Phytophthora* species occurs in the rhizosphera of these tress causing root and collar rot symptoms in alder and fine root reduction manifesting by crown drying in walnut.

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Distribution of *Diplodia* Blight in France and Determination of Isolates Types

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Abstract – *Sphaeropsis sapinea* (Fr.:Fr) Dyko & Sutton in Sutton seriously damages *Pinus* spp. throughout the world. The disease strongly emerged in the nineties as a serious problem, mainly on *Pinus nigra* and *P. sylvestris*. By contrast, it was reported as a minor problem in the seventies (Lanier, et al, 1976). The aims of this study were to determine the French distribution of *Sphaeropsis sapinea* and in particular which *Diplodia* species are present in the stands. We also wanted to study factors associated with the pathogen presence.

We studied presence of S. sapinea in the stand of European level 1 network, i.e. 73 stands of *P. sylvestris*, *P. nigra* and *P. pinaster* scattered all over France. On each visited plots, crown symptoms on trees were observed, and 10 cones were collected in order to study the frequency of cone colonisation by *S. sapinea* as a measure of the level of the pathogen presence in the stand. *S. sapinea* isolates were obtained from cone scales and were determined to the species using specific PCR primers.

Crown symptoms attributed to *S. sapinea* were recorded in only one plot with *Pinus nigra* in South-western France. In this plot, all pines were infected, with 10 to 100% of crown symptom. For the other plots, the observed crown had less than 10% of crown symptom. Concerning the cone colonisation by *S. sapinea*, the results varied by 0 to 100% of infected cones by stand. The pathogen was detected at least one time on all pine specie studied and was present over all the France, although far less present in SW, on *P. pinaster* and in the Alps.

All fungal isolates of *P. nigra*, *P. pinaster* and *P. sylvestris* were *Diplodia pinea*. Concerning *P. radiata*, we had found the two species, *D. pinea* and *D. scrobiculata*, on Corsica cones, but only *D. pinea* on cones of Metropolitan France. Furthermore, the isolates of the two species were found on the same cone.

We tested the links between the level of pathogen presence and some environmental factors. Some were not linked to the *Sphaeropsis* presence such as stand origin (planted or natural regeneration) and stand age. The best model explaining the *Sphaeropsis* presence in France included the variables pine specie, the rain of summer and the mean of minimum daily temperatures in winter. There was a strong altitudinal effect, *D. pinea* being far less present at higher altitude.

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Significance of Wood Terepnoids and Fenolics in the Resistance of Scots Pine Provenances Against Fungal Diseases

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Abstract – The resistance of trees to herbivores and pathogens consists of both structural and chemical defence mechanism. Carbon based monoterpenes have an important role in plant resistance against herbivores and parasitic ascomycetes. We tested how the variable growing circumstances affect to terpenoid and phenolic concentration and composition of pine needles in provenance trial of Scots pine and to the susceptibility of pine provenances to fungal diseases. The material consists of nine pine provenances representing a 1200-km N-S transect from Estonia to northern Finland. They have grown in three different sites, one in Estonia and two in Finland, one in central and the other in northern part of the country. This material has tested both natural and artificial infections of Scleroderris canker and other disease resistance of pine. The results indicate significant variation in the susceptibility to diseases and in the concentration and composition of secondary metabolites.

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