EXTENDED ABSTRACTS

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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).

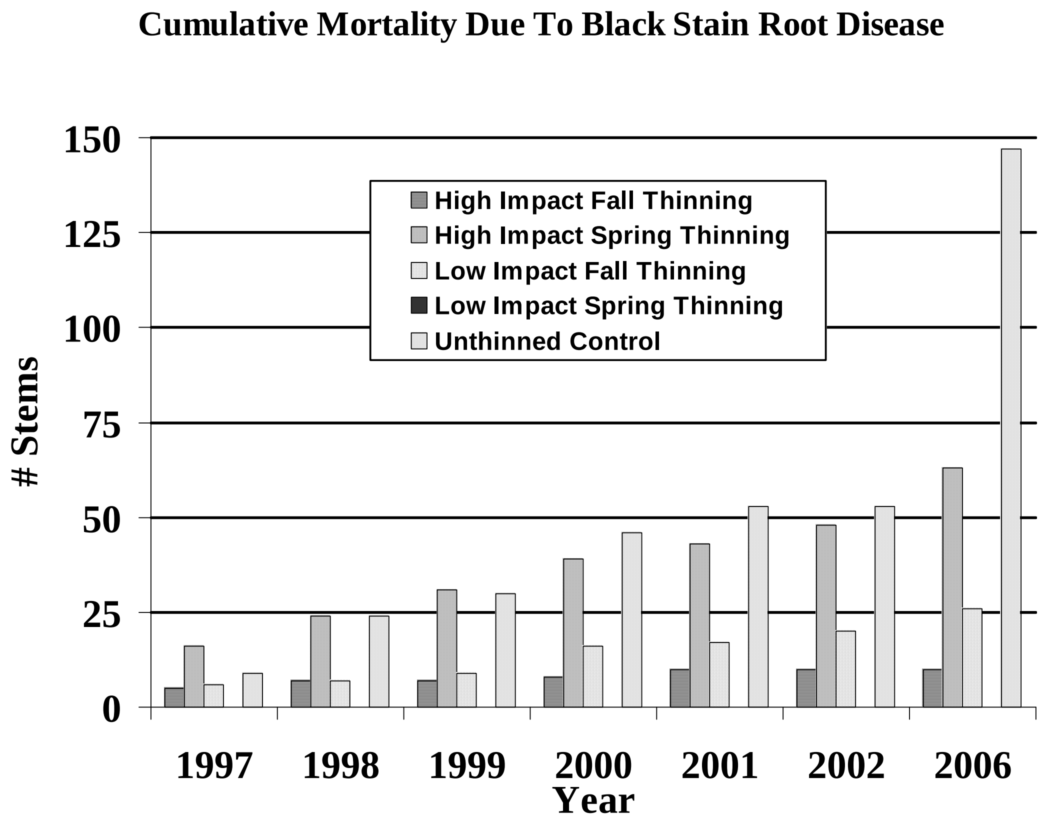

Figure 1. Mortality resulting from thinning treatments over 10 years following four thinning treatments. Note that low impact spring thinning plots never had blackstain root disease due to soil factors.

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.

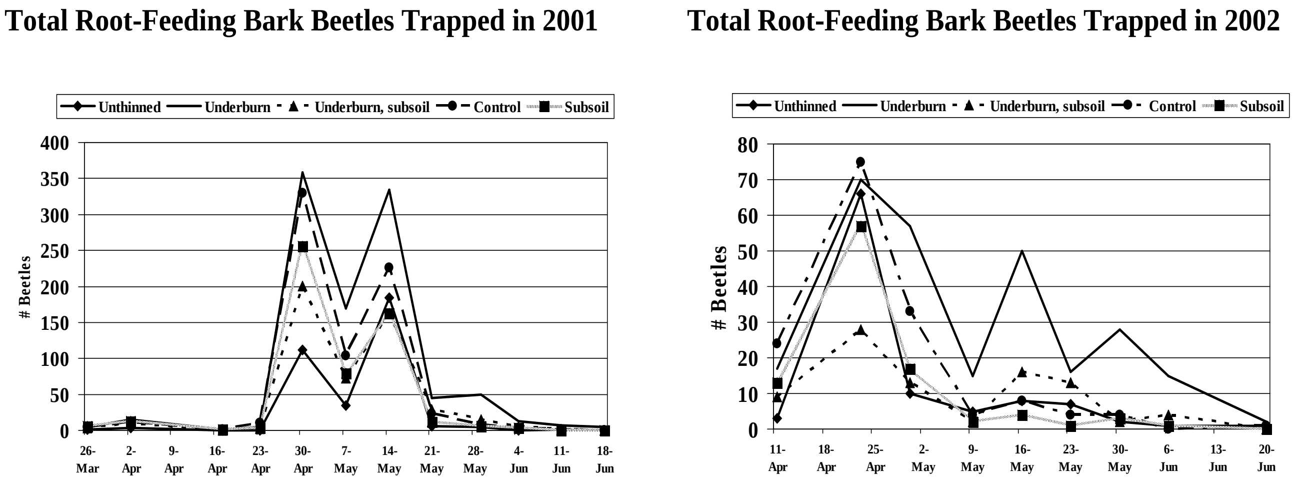

Figure 2. Summary of trap catches of root feeding Scolytids during the 2001 and 2002 trapping season. Note differences in scale between years.

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*.

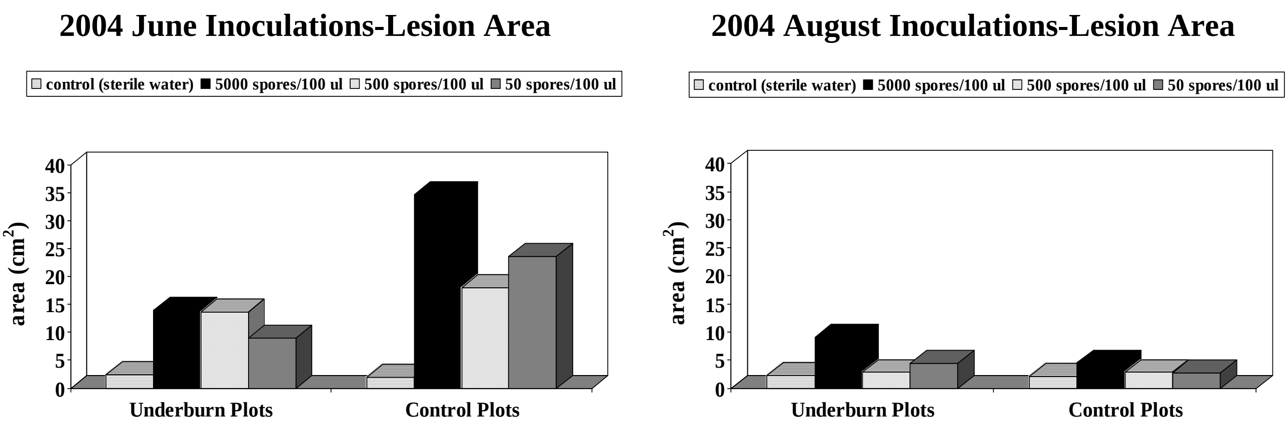

Figure 3. Lesion areas resulting from different L. wageneri spore dosages between underburned and control plots for June and August inoculations.

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.

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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.

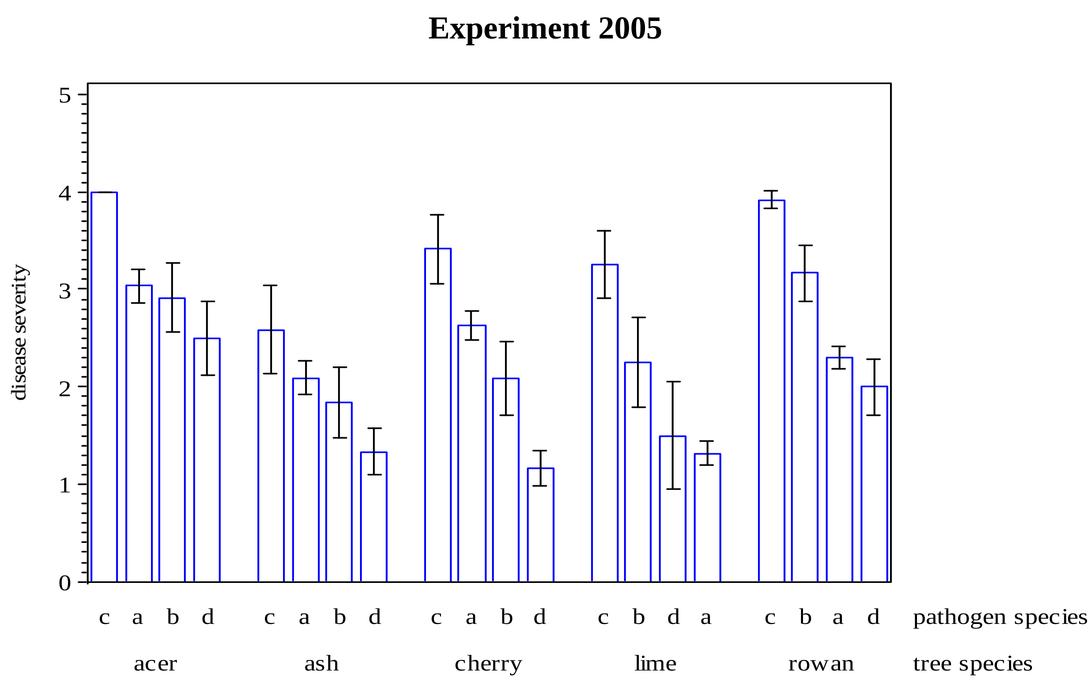

Figure 1. Mean of disease severity of deciduous tree species caused by four different Fusarium species 9 months after artificial inoculation from experiment 2005; the letters are indicating the fungal species: a F. avenaceum (n=54), b F. sporotrichioides (n=12), c F. sambucinum (n=12), d F. tricinctum (n=6).

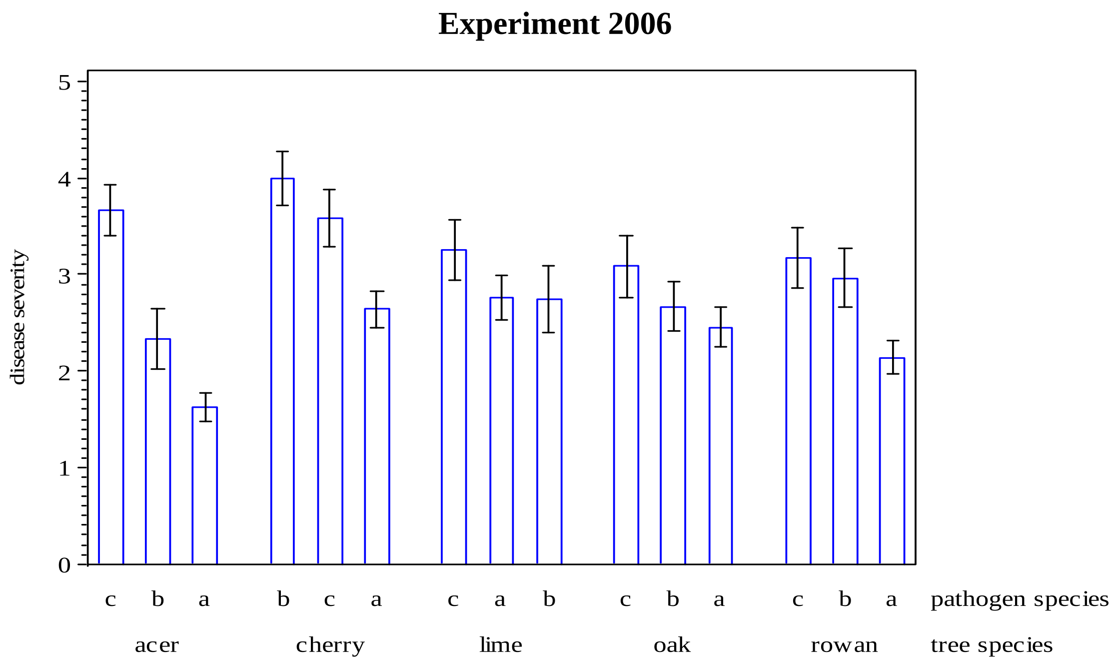

Figure 2. Mean of disease severity of deciduous tree species caused by three different Fusarium species 9 months after artificial inoculation from experiment 2006; the letters are indicating the following fungal species: a F. avenaceum (n=42), b F. sporotrichioides (n=24), c F. sambucinum (n=24).

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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