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IUFRO Working Party 7.03.04

Diseases and insects in forest nurseries

The sixth meeting of the IUFRO Working Party 7.03.04
(Diseases and insects in forest nurseries)
was held from September 11 - 14, 2005,
in Uherské Hradiště, Czech Republic

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PREFACE

The sixth meeting of the IUFRO working Party 7.03.04 (Diseases and insects in forest nurseries) was held from September 11-14, 2005, in Uherské Hradiště, Czech Republic. The meeting was organized by the WP leader Dr. Zdenka Procházková and her colleagues at the Forestry and Game Management Research Institute (FGMRI), Research Station Uherské Hradiště. The FGMRI hosted the event. Participants assembled in Prague on Friday, September 9, and arrived by bus to Uherské Hradiště that evening. All day Saturday and on Sunday morning they enjoyed the local Folk Festival and on Sunday afternoon they visited the nearby Buchlov Castle and the Buchlovice Palace and gardens. That evening an icebreaker was held at the Hotel Slunce, our meeting site, in downtown Uherské Hradiště.

Bright and early on Monday morning participants were given an official welcome by Dr. Karel Vančura, General Member of the IUFRO Board since 1996 and recent head of Honours and Awards Committee. Dr. Jack Sutherland, a founder of the WP, presented an overview of the WP's history since its inception in 1990. Then followed a 45-minute long, invited paper on The Evolution of *Fusarium* Diseases in Forest Nurseries by Dr. Robert James of the U.S.D.A. Forest Service. The remainder of the day was devoted to 20-minute long paper presentations and wound up with an hour-long poster session beginning at 6 p.m.

On the morning of the second day attendees visited the FGMRI Uherské Hradiště Research Station, including the ISTA accredited seed testing laboratory and poplar improvement plots, and then the Kladikov Forest Nursery before returning to lunch at the Hotel Slunce. Paper presentations continued throughout the afternoon. These were followed by the WP business meeting starting at about 6 p.m. At this meeting Mrs. Michelle Cram of the USA was elected to serve as leader of the WP. Too, we accepted the invitation of Mrs. Cram and Dr. James to host our next meeting in Hawaii, USA, most likely in the spring of 2009 with Dr. James serving as local host. The official WP dinner was held that evening at a nearby wine cellar where we were treated to excellent Moravian cuisine and thoroughly immersed in Moravian folk music and dancing.

On Wednesday morning we departed by bus for Prague. Along the way we toured the Budišov Forest Nursery (where we saw several insect and disease problems), and in the historic town of Třebíč we had lunch before visiting the Jewish Town and the Basilica, all on the UNESCO World Heritage list. We arrived in Prague about 7 p.m. that evening where many old and new friends said goodbye before departing for our homelands.

On the statistical side, the 29 participants came from 10 countries (number of participants in parentheses): Bangladesh (1), Canada (1), Czech Republic

(11), Egypt (1), Finland (4), Norway (2), Poland (1), Republic of Slovakia (3), Sweden (3) and the USA (2). During the meeting 24 papers and four posters were presented on a variety of topics including diseases caused by *Fusarium*, *Gremmeniella*, *Phytophthora*, powdery mildew, nematodes and seed-borne pathogens, plus papers on quarantine-related issues, biocontrol of diseases, and papers dealing with both insect and disease pests.

Our special thanks go to the FGMRI for being such gracious hosts and especially Dr. Bohumir Lomský of that institute for doing everything possible to facilitate our meeting. Too, we thank Dr. Karel Vančura and his wife Vlasta for the helping assemble the participants in Prague and for being such wonderful guests at our meeting. We were indeed fortunate that Dr. Vlastislav Jančařík (many would say the modern day father of forest pathology in the Czech Republic) attended at least part of the meeting. Employees of the CeWood Company graciously guided us through the two nurseries tours.

Na shledanou (traditional Czech farewell for 'see you again') until we meet again in Hawaii.

By Zdenka Procházková, immediate past leader of WP 7.03.04
and Jack R. Sutherland, founder of WP 7.03.04

Addendum to the Preface

Dear colleagues,

Coincidental with the proceedings of our Uherské Hradiště meeting going to press (December, 2006) I received a letter from Dr. Peter Mayer, IUFRO Executive Director in Vienna, informing me that on behalf of IUFRO's President professor Don K. Lee, that the Awards Committee (chaired by Dr. Su See) of the Board of IUFRO has bestowed upon me their Distinguished Service Award. This is a great honour for me and I now know that several of our WP members wrote letters supporting this award. Thanks to all of them and all of our other members for your support and help over the years. We all know what a wonderful organization IUFRO is in bringing together forest scientists from around the world. Our WP is a microcosm of all the good features of IUFRO and besides bringing us together as scientists we have seen the formation of many long-lasting friendships worldwide. Once again, thanks and see you in Hawaii.

Jack (Sutherland)

MAJOR DISEASES OF DATE PALM AND THEIR CONTROL

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ABSTRACT

Date palm (*Phoenix dactylifera*) is attacked by many pathogens including 12 fungi, two nematodes and two mycoplasma-like organisms. Non-infectious diseases, i. e. soil and climate factors are also widely known as well as other unknown diseases. Bayoud is incontestably the most serious fungal disease recorded in Morocco, Algeria and Mauritania. Offshoots of date palms are subjected in nurseries and in cultivation at different regions around the world are affected by species of about six genera of fungi i. e., *Botryodiplodia*, *Diplodia*, *Fusarium*, *Pythium*, *Phytophthora* and *Thielaviopsis*. This paper describes the significance and spread of these diseases and their integrated management and some other proposals to increase date production.

INTRODUCTION AND BACKGROUND

Date palm, *Phoenix dactylifera*, a long-living monocotyledon plant belongs to the family *Palmaceae*, is extensively cultivated for its edible fruit. Due to its long history of cultivation for fruit, its exact place of origin is not certain, but it probably originated somewhere in the desert oases of North Africa, and perhaps also southwest Asia. However, there are some postulates that it first originated in Bable, Iraq, while others believe that it is in Saudi Arabia or Bahrain.

It is also believed to have originated around the Persian Gulf, and had been cultivated in ancient times from Mesopotamia to prehistoric Egypt, possibly as early as 6,000 BC. There is archaeological evidence of cultivation in eastern Arabia in 4,000 BC. In later times, Arabs spread dates around northern Africa and into Spain, while the Spaniards introduced dates into California in 1765, around Mission San Ignacio.

Date palm is a medium-sized tree, 15 - 25 m tall, often clumped with several trunks from a single root system, but also often growing singly. The leaves are pinnate, up to 3 m long, with spines on the petiole and about 150 leaflets; the leaflets are 30 cm long and 2 cm broad.

Dates have been a staple food of the Middle East for thousands of years. In some countries such as Egypt, date palm, presenting a source of income to oases inhabitants, is considered of high economical importance. Beside date fruit, date palm gives other products such as date honey, juice from the fresh fruit, date sugar and date sap. The leaf midribs are used for fencing and roofing. The wood from the trunk is cut into planks, used for doors, beams, and rafters.

Date palm trees are grown better in areas having temperature of 32 – 38 ^{o}C, while the trees tolerate conditions up to 52 ^{o}C. Date palms tolerate cold conditions to -11 ^{o}C. However, date palms are highly drought tolerant resistant to windy conditions as well as salty soil. They are so adaptable to soil conditions where their nutritional requirements are moderate.

Date palm production is mainly concentrated in the Near East region, especially in Egypt, Iran, Saudi Arabia, UA Emirates and Pakistan that produce over 60% of the dates of that area.

The most famous date palm varieties in the Near East region are listed in Table 1.

Tab. 1.
Most famous date palm varieties in the Near East producing countries

Country	Varieties
Algeria	Dejlet-Nour, Kesbah, Khars, Maghoul, Arishty, Bostamy, Bo-Ajo, B0-Fakos, Dejlet-Baida, Yateema
Libya	Aabel, Ebel, Haleema, Mgmaget Ayuob, Umeljwary Tagyat, Tamej, Thoory and Zahidi
Mauritania	Bahwa, Akadir, Falha, Dely red, Danka red, Bougira, Bas Breke, Henaweya, Lekhdira, Loted, Lemdina, Mahboula, Amrizka, Om-Arish, Skani, Slemdina, Tigeb, Tefred, Tetrkel
Egypt	Seiwy, Amhat, Bent-asha, El-Amry, Hayany, Kandyla, Malkaby, Pertmoda, Samany, Shamia, Skooty, Zaghlol and others.
Saudi Arabia	Ajwa-Madina, Berny, Hatmy, Anbara, Khalas, Kgar, Makfezy, Maktomy, Nabot-Seif, Reziz, Sefry, Segey, Safawy, Khedry, Sheshy, Kheneezy, Bekeera, Ghorra, Safee, Makaty, Rotana, Shaqrat-Mobarak and Sokary
UA Emirate	Bo-ElOzouk, Bo-Zeid, Barhy, Merzaban, Maan, Gash-Habash, Khesab, Khalas, Khenezy, Ein Bakar, Reziz, Fard, Lolo, Neghal, Helaly, Bo-Qebal, Anwaq, Gabry, Shishy, Maktoumy and Shahla
Iran	Azrak, Petoky, Perzy, Shahany, Shaby, Shokr, Kebkab, Kankar, Porko, Tebrez, Khasoy
Iraq	Breem, Dery, Bergy, Halawy, Khadrawy, Maktoum, Sair, Ashras, Merhag, Taberzal, Berben, Khosab, Gebgab, Khestawy, Khalas and Zohdy.
Kuwait	Bergy, Khalas
Morocco	Yatema, Dejlet Nour, Khars, Maghoul, Bostamy, Bo-Ajo, B0-Fakos, Jehel and Bosklene
Oman	Bershy, Fard, Menzef, Meznaj, Shehl and Zebdah.
Pakistan	Dendery, Jelfy, Jorban Kelry, Kgety, Koro, Mraty.
Tunisia	Dejlet-Nour, Kenta, Manakher, Khars, Maghoul, Lamsy, Bostamy, Bo-Ajo, B0-Fakos, Oleeq, Yateema
Yemen	Gazaz, Feel Manaseb, Medini, Hamraa, Hejry, Megraf, Hashedy, Arqady
Sudan	Barakawy, Mishhriq wed Khateeb, Ibrahemy, Mishhriq wed leqay, Madina, Qarqouda, Bent-Moda, Quandila, Kelma

Date palm trees suffer from many diseases that significantly affect their yield and quality including incidence of fruit rots. Moreover, some of those diseases may kill palm trees. Therefore, in view of the fact that date palms are of great economical importance, this work was done to shed light on the major diseases of date palm and to describe measures for their preservation.

MAJOR DISEASES OF DATE PALM

There are 12 fungal diseases (Table 2), two mycoplasma-like diseases, two nematode-caused diseases, two physiological disorders and three unknown diseases that commonly affect date palm in the Near East countries.

Tab. 2.
Most common fungal diseases on date palm and their distribution in the Near East region

Disease	Causal fungi	Country of disease spread
Bayoud	*Fusarium oxysporum* f. sp. *albidenis*	Morocco, Algeria and Mauritania
Black Scorch	*Thielaviopsis paradoxa* (*Ceratocystis paradoxa*)	Egypt, Algeria, Tunisia, Iraq, Saudi Arabia, Emirate, Bahrain, India and Mauritania
Khamedj	*Fusarium moniliforme* *Thielaviopsis paradoxa*	Egypt, Algeria, Tunisia, Morocco, Libya, Iraq, Saudi Arabia, Emirate, Bahrain, India and Mauritania
Diplodia & Botryodiplodia	*Diplodia phoenicum* *Botryodiplodia theobromae*	
Graphiola Leaf Spot	*Graphiola phoenicis*	all countries growing date palm in Middle East and North Africa
Brown Leaf Spot	*Cladosporium herbarum* *Alternaria alternata* *Drechslera australiensis* *D. biseptata*	North Africa
Balâat	Phytophthora sp.	Morocco, Algeria, Egypt and Tunisia
Wilt	*Fusarium oxysporum* *Pythium* spp.	in date palm nurseries and in areas of high moisture
Omphalia	*Omphalia tralucida* *O. pigmentata*	Mauritania
Basel stem rot	*Ganoderma zonatum* *G. boninese* *G. tornatum*	Saudi Arabia
Fruit Rots	*Thielaviopsis* *Alternaria* *Stemphylium* *Cladosporium* *Phomopsis* *Aspergillus*	all humid areas growing date palm
Stylar-end rot	*Aspergillus niger*	all humid areas growing date palm

1. Fungal diseases of date palm

1.1. Bayoud disease

Origin, distribution: The name bayoud comes from the Arabic word, "abiadh", meaning white which refers to the whitening of the fronds of diseased palms. This disease was first reported in 1870 in Zagora-Morocco. By 1940, it had already affected several date plantations and after one century, the disease had practically affected all Moroccan palm groves, plus those of western and central Algerian Sahara. The causal organism responsible for bayoud is the microscopic fungus *Fusarium oxysporum* forma *specialis albedinis* (Malencon 1934, 1936).

Economical significance: Diseased palms may die within 6 months to 2 years after appearance of the first symptoms. However, bayoud disease destroyed about 13 millions of date palm trees in Morocco, Algeria and Mauritania (Toutain 1967).

Disease transmission: The disease is transmitted through infected offshoots, fronds, and their manufactured products, soil (the fungus is soil-borne), roots of infected palms and other crops such as alfalfa (intercropping).

Disease symptoms: Bayoud disease attacks both mature and young palms, as well as offshoots at their base (Carpenter, Klotz 1966, Djerbi 1988).

External symptoms*:* The first symptom of the disease appears on a palm leaf of the middle crown. This leaf takes on a leaden hue (ash grey colour) and then withers, from bottom to top, in a very particular way. After one side has been affected, the whitening begins on the other side (Fig. 1), progressing this time in the opposite direction from the top of the frond to the base. A brown stain appears lengthwise on the dorsal side of the rachis and advances from the base to the tip of the frond, corresponding to the passage of the mycelium in the vascular bundles of the rachis. Afterwards, the frond exhibits a characteristic arch, resembling a wet feather and hangs down along the trunk. This whitening and dying process of the pinnae may take from a few days to several weeks. The palm dies when the terminal bud is affected.

Fig. 1.
Whitening of one side of the leaf, then start on the other side

Internal symptoms*:* A few disease infected reddish roots are evident when an affected palm is uprooted. The spots are large and abundant towards the base of the stripe. As they advance towards the upper parts of the palm, the coloured conducting fascicles separate and their complicated path inside the healthy tissues can be followed. Palm fronds manifesting external symptoms exhibit a reddish brown colour when cut, showing highly coloured conducting fascicles. There is, therefore, a continuity of vascular symptoms that exist from the roots of the palm to the tips of the palm fronds.

Control of bayoud disease:

Chemical control: Soil treatment of this type of disease is destined, a priori, to fail and should therefore be avoided. Chemical control can, however, be feasible in the event of the discovery of primary sources of infection in a healthy area. In this case, eradication techniques should be used, where diseased palms are uprooted and incinerated on the spot. The soil is then treated

with methyl bromide or chloropicrin and the area closed off with replanting prohibited until further notice.

Cultural control: Since the factors that favour high yield in date palms (e. g. irrigation and fertilization) are the same that favour the growth of the fungus, modification of cultural techniques are not advised. However, a significant reduction in the amount of irrigation can retard the advance of infection, i. e. stopping irrigation between the months of May and October, during the hot season in the northern hemisphere (PEREAU-LEROY 1958). Since the contamination occurs mainly by root contact, disease-free palms can be isolated by digging a 2 m deep trench around them. Water should be provided by a trough bridging the rest of the grove to this isolated plot. Under these conditions these palms can be protected for more than 10 years (DJERBI 1982). As well, pre-plant determination of the suppressive ability of soil (for disease development) is useful in reducing disease spread.

Prophylactic measures: The essential task is to prevent the movement of contaminated plant material from an infected palm grove to a healthy one. This material, as has been previously mentioned, consists mainly of offshoots, palm fragments, manure and infected soil, and artefacts made from these materials. Detection of the date palm pathogen earlier may help in avoiding transferring infected plant materials to new soils (FERNANDEZ et al. 1998).

Genetic control: The only productive means of controlling bayoud disease lies in continuous research of resistant varieties (TOUTAIN, LOUVET 1972). Many resistant cultivars have already been obtained in Morocco from three sources: selection of bayoud-resistant varieties from those already existing (local and introduced), selection of high-quality resistant clones from the natural population of the date palm, and creation of resistant and high quality varieties through a hybridization program (TOUTAIN 1967, FAO 1984, SEDRA, DJERBI 1986). In addition, the present success of date palm propagation by in vitro culture will make it possible to rehabilitate the Moroccan and Algerian palm groves that have been destroyed by bayoud disease.

1.2. Black scorch disease

Black scorch, also called Medjnoon or Fool's disease, is caused by *Ceratocystis paradoxa* (HOHN) which is the perfect form of *Thielaviopsis paradoxa* (KLOTZ, FAWCETT 1932, DJERBI, 1983). Black scorch has been observed on date palm all over the world.

Economical significance: Black scorch is the most serious disease on date palm in many countries such as Egypt. It may cause death of the heavily infected trees, causing about 50% loss in newly planted offshoots in humid growing areas. It causes also fruit rot.

Disease transmission: Black scorch is an air borne disease. Also, it can be transmitted through infected offshoots.

Fig. 2.
Inflorescence blight

Disease symptoms: The symptoms are usually expressed in four distinct forms: black scorch on the leaves, inflorescence blight (Fig. 2), heart or trunk rot and bud rot on palms of all ages. Infections are characterized by partial to complete necrosis of the tissues. Typical lesions are dark brown to black, hard, carbonaceous, and, as a mass, give the petioles, fruit strands and fruit stalks a scorched, charcoal-like appearance. Decay is most serious when it

attacks the terminal bud and heart leading to the death of the palm. Some palms recover, probably by development of a lateral bud from the uninjured portions of meristematic tissue. These palms show a characteristic bend in the region of infection. This is why it is called Medjnoon.

Control: Good sanitation is the first step in the control of black scorch. The affected fronds, leaf bases and inflorescences should be pruned, collected and immediately burned. The pruning cuts and surrounding tissues should be protected by spraying with Bordeaux mixture, lime-sulphur solution, copper sulphate lime mixture, dichlone, thiram or any new copper-based fungicides. When the disease is severe, affected palms should be removed and burnt.

1.3. Khamedj disease

Khamedj disease, or inflorescence rot, is a serious disease affecting spathes of date palm in most growing areas in hot and humid regions or in areas with prolonged periods of heavy rain, 2 to 3 months before emergence of spathes.

Economical significance: In 1983, diseased spathes caused losses in date palm yield in Katif (Saudi Arabia) of 50 to 70%. From 1948 - 1949 and 1977 - 1978, severe outbreaks occurred in Iraq at Basrah, affecting male and female palms and destroying 80 % of the harvest (Al Hassan, Waleed 1977).

Disease transmission: Khamadj disease is an air borne disease mainly caused by *Mauginiella scaettae* Cav. However, *Fusarium moniliforme* and *Thielaviopsis paradox*a may also cause, more rarely, inflorescence rot. Infected spathes remain on the tree from the previous season causing as the source of infection for following growing season. The fungal spores infect pollen. Transmission of the disease from one palm to the next occurs through the contamination of male inflorescences during the pollination period. The infection of the young inflorescence occurs early and happens when the spathe is still hidden in the leaf bases. The fungus penetrates directly into the spathe and then reaches the inflorescences where the fungus sporulates abundantly.

Fig. 3.
Diseased spathes by Khamedj remain closed and their content got decayed

Disease symptoms: The disease appears on the external surface of unopened spathes as they begin to emerge in late winter or early spring. Brownish or rusty areas develop and spread slowly. Severely damaged spathes may remain closed and their contents may be totally infected. The inflorescences become dry and covered with powdery fructifications of the fungus (Fig. 3). The diseased tissues are oily and translucent in appearance. Small drops of water form on the surface and the central part of the affected area is often covered with whitish brown dots.

Control: Good sanitation including collection and burning of all infected inflorescences and spathes is a good approach for disease control. Palms with diseased inflorescences should be treated with fungicides after harvest and 1 month in early spring before the spathes emerge. Bordeaux mixture, copper sulphate-lime mixture, 3 % dichlone or 4 % thiram spray at the rate of 8 litres per palm are effective to protect the date palms from the disease infection.

1.4. Diplodia and Botryodiplodia diseases

Diplodia and Botryodiplodia diseases, caused by *Diplodia phoenicum* (SACC.) and *Botryodiplodia theobromae*, have been recorded all around the world.

Economical significance: The fungus may infect the outside leaves and eventually kill younger leaves and the terminal bud, or the central cluster may be infected and die before the older leaves.

Disease transmission: The fungus is an air borne, where pycnidiospores are produced in a pycnidium. The disease can also be disseminated by the offshoots.

Fig. 4.
Death of immature frond in older trees due to infection with *Diplodia*

Disease symptoms: Symptoms are severe on offshoots and are characterized by death either while they are still attached to the mother palm tree or after they have been detached and planted out. Yellowish-brown streaks extend along the diseased leaf base. On the leaves of older palms, the ventral mid-portion of the stalks is commonly affected, showing yellowish brown streaks, 15 cm to over one meter in length, extending along the leaf base and rachis (Fig. 4). The upper part of the leaves however, may still appear green and unaffected.

Control: Since the fungus usually enters the palm through wounds made during pruning or cutting when removing the offshoots, one precaution is to disinfect all tools and cut surfaces. Dipping or spraying the offshoots with various chemicals (Bordeaux mixture, thiophanate methyl, thiram and other copper-based fungicides) have been found effective against the disease.

1.5. Graphiola leaf spot

Graphiola leaf spot caused by *Graphiola phoenicis* (MOUG.) POIT. is the most common disease wherever date palm is cultivated under humid conditions, mostly marginal date growing areas (Mediterranean coast), but also in the southern most humid regions of Mali, Mauritania, Niger and Senegal and in Egypt (Delta region and Fayum), but not in the less humid oases. In Saudi Arabia, it is abundant in Kattif, Demam and Jeddah, but absent in Iraq. Reports of this disease also originate from Algeria and the USA. Around the world it is the most widely spread and most frequently occurring disease.

Economical significance: The normal 6 - 8 year life of date palm fronds are reduced to 3 years by *Graphiola* leaf spot disease and heavily infected leaves die prematurely which consequently reduce yield of the palm.

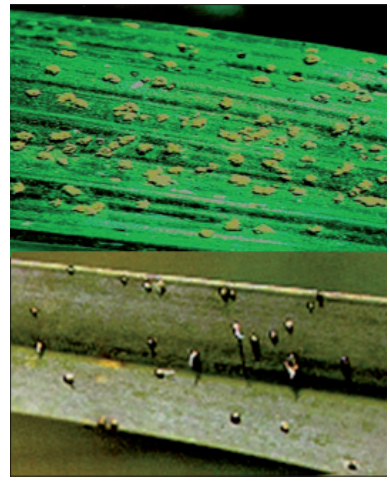

Fig. 5.
Sori containing smut spores due to *Graphiola* leaf spot disease

Disease transmission: Graphiola leaf spot, caused by a smut fungus, is an air borne disease.

Disease symptoms: The fungus develops subepidermal, in small spots on both sides of the pinnae leaves, on the rachis and on the leaf base. The numerous fruiting structures emerge as small-yellow/brown to black sori, 1 to 3 mm in diameter, with two layers (Fig. 5). These sori are abundant on three year-old leaves, conspicuous on two year-old, but absent or infrequent on one year-old

leaves. This is because of the 10 - 11-month incubation cycle for this pathogen. On a leaf, sori are abundant on apical pinnae, less abundant on the middle section becoming even less abundant on the basal section.

Control: Control measures include leaf pruning coupled with treatment with Bordeaux mixture or any wide spectrum fungicide (mancozeb, cupric hydroxide, cupric hydroxide + maneb, or copper oxychloride + maneb + zineb). Three to four applications on a 15-day schedule after sporulation are recommended. Genetic tolerance has been found in some varieties such as Barhee, Adbad, Rahman, Gizaz, Iteema, Khastawy, Jouzi and Tadala.

1.6. Brown leaf spot

Brown leaf spot, as with other common date palm diseases, has also been observed in North Africa and the Middle East (Rieuf 1968). It is caused by *Mycosphaerella tassiana* (De Not) Johns.

Disease transmission: The pathogen is air borne. It could be also transmitted through infected off-shoots.

Disease symptoms: Dark lesions are clearly delimited on green leaves, and on dying leaves the margin of the lesion remains reddish brown as the centre becomes pale. Lesions also occur on the rachis, pinnae and spines.

Control: Because it is a minor disease, no treatment is recommended. However, annual pruning of old infected leaves and their immediate burning is advised. Spraying of cuprous fungicides after pruning help in avoiding infection with such disease.

1.7. Balâat disease

Belâat disease was reported by several authors in several North African countries such as Algeria, Morocco, Tunisia, etc. (Maire 1935, Monciero 1947, Calcat 1959, Toutain 1967). Belâat disease is caused by *Phytophthora* sp. similar to *P. palmivora* (Djerbi 1983).

Economical significance: Balâat is a minor disease; however, when present it causes major damage to the heart of the tree.

Disease transmission: The pathogens are soil borne. Sporangium of the fungus may be spread by air.

Disease symptoms: The entire cluster of young fronds whitens and dies as a result of attack, followed by the infection and death of the terminal bud. The infection progresses downward in the trunk as a conical wet heart rot form (Fig. 6), releasing an odour of acetic and butyric fermentation due to secondary infection with other microorganisms.

Fig. 6.
Heart rot caused by Balâat disease

Control: Efficient maintenance of date palm plantations is highly recommended to avoid attacks by this disease. Spraying with maneb or Bordeaux mixture at the rate of 8 litres/palm could control the disease at its early stages. Offshoots of affected palms usually remain healthy.

1.8. Root rot and wilt disease

Wilt disease in date palm nurseries and in areas of high soil moisture is caused by *Fusarium oxysporum* (Schlect) and *Pythium* spp. and *Phytophthora* sp. but *Fusarium* spp. are the major causal organism (Sedra, Djerbi 1986). Infection occurs through roots, and then systemic infection developed. The disease is common in most growing areas.

Economical significance: Wilting caused by root rot fungi is called a decline disease, where infected tree declined over time. The disease directly affects the yield and the fruit quality.

Disease transmission: The spread of this disease as a soil borne disease depends on the movement of infected trees or offshoots, infested soil, or poor maintenance practices.

Disease symptoms: The roots of diseased plants become dark. Growth of *Fusarium oxysporum* as the main pathogen for wilt disease is observed in the xylem, parenchyma and vascular bundles of the roots, where a discoloured vascular system is observed. The wilt disease is diagnosed by progressive leaf death from the oldest to youngest leaves, and a prominent brown strip on the base of petiole or rachis.

Control: Before outplanting to plantations, planting stock should be inspected to be disease-free prior to purchase. Palms need to be examined on a regular basis for symptoms visually and in the laboratory for verification. If only 1 or 2 fronds have clear symptoms, remove a symptom-less lower and upper frond as well. Infected palms should be carefully eliminated. Avoid scattering infested soil. These trees should be taken to the landfill or incinerated. Clean the tools used in palm removal as well as pruning with chlorine solution 1 : 1 chlorine-water. Replanting with palms is risky in infested soil, where there is no effective treatment or fumigants enough to eradicate this fungus from this soil. Pruning only dead fronds is preferable to minimize the risk of fungus movement.

1.9. *Omphalia* root rot

Omphalia root rot was recorded in California, USA and in Mauritania by Fawcett and Klotz (1932) and Bliss (1944), respectively. Two species of *Omphalia* (*O. tralucida* Bliss and *O. pigmentata* Bliss) cause the disease and are widely spread in date plantations of Coachella Valley, California and in Kankossa (Mauritania) (Djerbi 1983). Sachs (1967) found four Mauritanian varieties (Ahmar, Marsij, Mrizigueg and Tinterguel) to be susceptible to this disease. Unlike other date varieties planted in California, Dejlet-Nour was found to have the lower infection rate.

Economical significance: *Omphalia* root rot is designated as a decline disease because of its association with declining date palms.

Disease symptoms: The premature death of fronds followed by retardation and cessation of growth are the main disease characteristics followed by necrosis and destruction of the roots. A completely non-productive tree is the result of the attack.

Control: The use of Brestan or Dexon as protective fungicides at the rate of one spray every 2 weeks for 8 weeks was recommended by Sachs (1967) as a chemical control measure.

1.10. Basel stem rot

Basel stem rot is a lethal and incurable disease affecting mature palms and is caused by *Ganoderma zonatum* (Murrill), *G. boninese* and *G. tomatum*. *Ganoderma* is a wind-borne fungus.

Economical significance: Palm death may occur within 6 months to 4 years after symptoms are observed depending on the age of the tree and environmental conditions.

Disease transmission: *Ganoderma* fungus produces wind-borne spores from infected palms which result in new infections. No palm species is considered resistant to *Ganoderma*.

Disease symptoms: The disease is normally concentrated in the lower part of trunk. The fungus moves from the centre of the tree to the outside. Old fronds wither collapse and droop parallel to the trunk (Fig. 7). Fronds do not break off but are retained on the palm. New growth slows, and becomes pale green or yellow in colour. Liquid bleeds and stains the trunk followed by formation of a soft white ‘conk’ or bracket fungus. Conks are shelf-like structures that protrude from the tree on the lower trunk. Older conks may be kidney shaped and are usually hard and woody with

rings. When the conks mature, the outer edge becomes swollen where spores are produced. Millions of rust-colour spores are released from one conk. Later symptoms may include trunk collapse or in some cases, the head of the infected palm may fall off. Depending on the point of invasion, the roots may be severely decayed. Outer trunk tissues may seem solid, but affected palms have a hollow sound when tapped.

Control: There are currently no known fungicides that will prevent or cure this disease on the tree or in the infected soil. Injuries to the trunk and roots especially during installation and maintenance activities should be avoided. Preventing the infection from reaching other palms is to immediately cut down the diseased palm. The stump and root system of the infected tree should be removed and destroyed as much as possible. Burning or incineration is the only way to destroy the fungus. If the stump is not removed, conks, produced on the stump, must be removed and burnt as soon as stump starts to form. The infected palm should not be replaced with another palm, where *Ganoderma* fungus present in the soil will probably infect a new palm.

Fig. 7.
Basel stem rot caused by *Ganoderma*

1.11. Fruit rots

Fruit rot damage varies from year to year depending on available humidity and rain from the Khalal stage until fruit maturation. However, losses vary from one country to another and from one variety to another.

Economical significance: Losses can be easily estimated to be 10 - 50 % of the harvest (Darley, Wilbur 1955, Calcat 1959, Sedra, Djerbi 1986).

Disease transmission: The most common fungi causing fruit spoilage are *Thielaviopsis*, *Alternaria, Stemphylium, Cladosporium, Phomopsis* and *Aspergillus* spp. Most of these fungi are air borne. Infected offshoots are also the source of infection for some fungi such as *Thielaviopsis*.

Disease symptoms: While infection with fruit rots occurs on the farm, the rots develop with fruit ripeness. Symptoms vary from brown to black spots or blemishes (Fig. 8). Softness of infected tissues is seen when the fruits got ripe.

Control: Lowering the humidity inside the bunch, by the use of wire rings, or by removing a few fruit strands from the centre of the bunch, will facilitate ventilation and drying of wet fruit. Protection from rain or dew can be achieved by using paper covers in the early Khalal stage to cover the fruit bunch. Fungus spoilage could also be limited by dusting the fruit bunches during the Khalal stage with mixture of 5 % ferbam, 5 % malathion, 50 % sulphur and an inert carrier (40 %) (Djerbi 1983).

Fig. 8.
Fruit rot on immature fruits

1.12. Calyx-end rot

Calyx-end rot is caused by *Aspergillus niger*. Calyx-end rot or black mould is commonly found in soiled dates. A side spot decay is the second common type of fungus spoilage.

Disease transmission: The fungus is air borne. The fungus attacks wounded fruit in the Khalal and Rutab stage, but direct penetration of unwounded fruit occurs only in the Rutab stage.

Disease symptoms: The infected fruits show water soaked tissues followed by develop of black fungal spores on the affected areas (Fig. 9).

Control: Sanitation, avoiding fruit injury, and dusting fruit bunches with sulphur help in controlling *Aspergillus* infection.

Fig. 9.
Aspergillus niger infection and sporulation on mature dates

2. Mycoplasma-like diseases of date palm

2.1. Lethal yellowing

Many palm species are attacked by lethal yellowing disease including *Phoenix dactylifera* L., *P. canariensis* HORT., and *P. reclinata* JACQ.

Economical significance: Lethal yellowing destroyed about 300,000 coconut palms in Miami (Florida, USA) in less than 5 years (MCCOY 1976, HOWARD 1992). The causal agent is a mycoplasma-like organism.

Disease dissemination: It is believed that the pathogen is disseminated by wind-born arthropod vectors.

Disease symptoms: Development of necrotic lesions on inflorescences followed by generalized yellowing of the palm leaves, leading to the death of the palm (Fig. 10). In date palm the fronds become desiccated and grey-brown instead of becoming yellow. A soft rot of the growing point occurs, converting the meristematic area into a putrid, slimy mass. The crown topples from the palm, leaving a naked trunk.

Fig. 10.
Generalized yellowing of palm leaves followed by death of the disease palms

Control: Removal of diseased palms and their offshoots, quarantine measures, the use of tolerant types of palms and the treatment with antibiotics are the main control measures.

2.2. Al Wijam

NIXON (1954) observed this disease in Al Hassa (Saudi Arabia). In Arabic, Al Wijam means poor or unfruitful.

Disease symptoms: The disease is characterized by a reduction in terminal bud growth, and the whole crown of leaves formed after the occurrence of the disease has rosetting symptoms. Newly formed leaves are reduced in size and marked by a faint narrow, yellow longitudinal line on the midribs (Fig. 11). Leaves become chlorotic and their life span is reduced. Death of leaves starts from the distal-end and extends towards the base. Diseased spathes split open before their complete emergence and are reduced in size (Fig. 12). The number and size of the bunches produced are also reduced year after year till the diseased palm fails to produce and dies.

Fig. 11.
Yellow longitudinal stripson diseased midrib by Al Wijam disease

Fig. 12.
Reduced spathes and yield as affected by Al Wijam disease

3. Nematode diseases

3.1. Root knot nematode

Root knot disease is caused by the nematodes *Meloidogyne* spp., e. g. *M. javanica*, *M. hapla* and *M. incognita* affect palm trees in most growing areas (MCSORLEY 1992).

Disease symptoms: No external symptoms appear on the infected palm trees with the disease. The disease affects seriously the offshoots of the date palm.

Control: Avoid cultivation in heavily infested soil with nematodes. However, fumigation of the soil with metam or dazomet before plantation in the nursery is effective to control soil infested nematode. Soil solarization is effective and environmentally safe for controlling the parasitic nematodes plus other soil inhabiting pathogens. Proper effective nematicides, such as fenamiphos oxamyl, ethoprophos or carbofuran should be use in nurseries to protect the offshoots from infection as well as to control nematode diseases of date palm trees.

3.2. Red ring nematode

Red ring nematode, *Rhadinaphelenchus cocophilus,* affects date palm in Saudi Arabia. Date palm red weevil is the main vector for the red ring nematode, where the insects carry the nematode over a distance of 1.5 km/day (or more) and facilitate infection of palm trees.

Economical significance: The infected young palm tress (3 - 10 years) may die within few months after infection occurs.

Disease symptoms: *Rhadinaphelenchus cocophilus* causes yellowing of the old fronds staring from the frond tip to the base. The symptoms progress from the older to the younger leaves. The internal symptoms appear as red discoloration of the vascular ring of roots, trunk or palm tree leaves (Fig. 13). Development of the ring discoloration of the trunk could be detected after the external symptoms show up.

Fig. 13
Red ring nematode internal symptoms in the vascular ring.

Control: Infestation of date palm trees with red weevil should be controlled to avoid the dissemination of infection with red ring nematode. Samples of soil and roots should be investigated periodically in that areas exposed to infection with the nematodes. When nematode is detected, soil drench with the effective above mentioned nematicides should be applied.

4. Physiological disorders (soil and climate factors)

4.1 Blacknose

This fruit problem, especially on Dejlet-Nour and Hayany varieties, occurs at the green maturity stage of fruits and results from high air humidity and high soil moisture. Intercropping or weed, spread increases the disease development due to increase of humidity. Good aeration and good pruning reduce the disease.

4.2 Black scald

Black scald damages fruit of Dejlet-Nour due to high humidity. The black scald appears on the sides or ends of the date fruit. The affected tissues of the fruit got water soaked, soft, deep and black.

5. Diseases of unknown cause

5.1. Bending head

Bending head is a minor but fatal disease. The central cluster of fronds takes the form of an erect fascicle with a bent tip which rapidly dies and falls.

5.2. False Bayoud

Bayoud disease like symptoms are found in Saudi Arabia. The disease attacks adult, young palms and their basal offshoots. The disease symptoms are yellowing of the lanner leaves of date palm. No vascular discoloration is observed, in cross or longitudinal section of rachis.

5.3. Faroun disease

Faroun disease is a rapid fatal, rapid disease. Male and female palms do not produce flowers and fruits for one or two seasons.

Recommended integrated management of date palm diseases

Policies of integrated disease management of date palm diseases are summarized in:

1. Restricted quarantine system
2. Planting of healthy offshoots
3. Spacing between palm trees to allow good growth and good aeration to reduce infection with foliar and fruit diseases
4. Dipping or spraying offshoots with recommended chemical pesticides
5. Good farm sanitation of date growing areas
6. Disinfestations of all tools used for pruning with chlorine solution or other disinfestants
7. Treating the resulted cut surface by Bordeaux mixture
8. Removing, collecting and burning of infected inflorescences and spathes
9. Treating light or moderate infections by Bordeaux mixture
10. Covering of bunches of high quality varieties with paper wraps to protect them from rain
11. Avoiding excessive soil moisture and nitrogen fertilizer
12. Controlling insects that facilitate fungal infection

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MAJOR FOREST NURSERY PESTS AND DISEASES IN BANGLADESH

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ABSTRACT

The Forest Department of Bangladesh has been engaged in large-scale afforestation programmes and raising nurseries mainly to meet their own demand for planting materials. For the last few decades tree planting activities among the general public have been gaining momentum primarily to meet the ever-increasing demand for fuel wood and timber. This leads to the increased demand for planting materials of forest species in the countryside, particularly in the northwestern part of Bangladesh where the tree cover still remains the lowest. Consequently, villagers have raised many nurseries in their areas. Several pests and diseases affect the growth and survival of forest species raised in these nurseries. This paper reports the result of surveys on the pests and diseases of homestead forest nurseries of Bangladesh. The important pests and diseases observed were the general pests and diseases such as cutworm, cockchafer, termite, ant, cricket, mole cricket and damping off disease. Specific pests and diseases recorded were mahogany shoot borer (*Hypsipyla robusta*), albizia defoliator (*Eurema* spp., *Catopsilia* spp.), kadam defoliator (*Arthroschista hilaralis*), chalta defoliator (*Margaronia* sp.), ipil-ipil psyllid (*Heteropsylla cubana*), jam leaf miner (*Acrocercops telestis*), arjun gall insect (*Trioza flecheri*), gamar root rot (*Fusarium solani*), teak root rot (*Pseudomonas solanacearum*), kadam die back (*Rhizoctonia solani*), and rattan leaf spot (*Guignardia calami*). Nursery owners and their associations were interviewed, their perception on forest nursery pest and disease management assessed, problems discussed and management measures suggested.

INTRODUCTION

The total forest land of Bangladesh is 16.7%, of which only 7.7% is covered with forest vegetation (Kibria et al. 2000). It includes the evergreen and semi-evergreen tropical rain forests in the eastern hilly areas, the moist deciduous sal forests in the central and northwestern part, the tidal mangrove forest in the south and the village or homestead forest. The distribution of forest is uneven with the north- and southwest regions remaining mostly sparsely covered.

Though the major forestry activity is entrusted with the government Forest Department (FD), village forests supply 70% of the timber, 90% of the fuel wood and 90% of the country's bamboo requirements (Kibria et al. 2000). The FD has undertaken massive plantation programmes in the country and this has lead to an increased awareness by the general public for tree planting activities, especially during the last two decades. The FD has established forest nurseries mainly to satisfy their own demand for planting materials, and also to sell to the general public at a minimum price. With the ever-increasing demand for planting materials villagers have established many nurseries in their homesteads. There are at least 2,500 such nurseries in the northwest

part of the country which has resulted in a flourishing cottage-based business that greatly helps reduce poverty in this region. These nurseries make planting materials available at the doorstep of the common people. Various NGOs have been involved in improving the livelihood of the common people in the region including facilitating nursery production of quality planting materials and increased tree planting activity. The nursery owners are now well organized and form professional societies in Upazilla (sub-District) and District levels to cater to their need. These nursery owners have been increasingly facing various pest and disease problems in their nurseries. A research study was, therefore, undertaken to help the nursery owners to better manage these pests and diseases. This paper reports the major pests and diseases faced by the nursery owners and gives the suggested management options.

MATERIALS AND METHODS

Several field surveys on the incidence of pests and diseases problems in the homestead nurseries of Bangladesh were undertaken during 2004 - 2005. The nursery owners were interviewed with a pre-structured questionnaire, followed by visiting their nurseries. The pest and disease samples were collected, reared or cultured and identified in the Entomology and Pathology Laboratories of the Bangladesh Forest Research Institute (BFRI), Chittagong. During field visits the nature and extent of damage by each pest and disease were recorded. Office-bearers of Upazilla and District Nursery Owners' Societies met, and via discussions their problems relating to forest nursery pests and diseases were noted.

RESULTS AND DISCUSSION

The homestead nurseries raise mostly fruit and forest tree species and some flower species, the choice of species being in conformity with the local demand. The most demanded fruit species are mango, jackfruit, litchi, Indian olive, jujube, guava, coconut, citrus, blackberry, sapota, pomegranate, amra etc. and the forest species are eucalyptus, mahogany, albizia, raintree, lumbu (unidentified), ghora neem, neem, sissoo, gamar, teak, kadam, ipil-ipil, *Dellinia* sp., *Terminalia* spp., bamboo, cane, etc. Generally, over-aged seedlings somehow got popularity over the recommended age so that some problems normally known to exist in the plantations start affecting the nurseries. Apart from the general nursery pests and diseases, for example, cutworm, cockchafer, termite, ant, cricket, mole cricket and damping off disease, the following specific pests and diseases problems and their management are highlighted below.

A. Pests

1. Mahogany shoot borer, *Hypsipyla robusta* Moore (Pyralidae: Lepidoptera)

   The larvae of this pest bore into the shoot and feeds inside making a central tunnel in the shoot. The attacked shoot ultimately dries and shrivels. Early infestation is marked by the presence of a gummy mass of frass bound with silk at the entrance hole, usually made at the leaf axil (Beeson 1941, Baksha, Islam 1997).

   Management:The larva can be killed manually. Raising seedlings under partial shade can substantially avoid the attack. Alternative hosts (chickrassy and toon) of the borer should not be raised in or near mahogany nurseries. Application of Furadon 5 G @ 4 kg/ha in the soil followed by watering gives effective control of the pest (Baksha, Islam 1997).

2. Albizia defoliator, *Eurema* spp., *Catopsilia* spp. (Pieridae: Lepidoptera)

   The larvae of these butterflies feed on foliage, completely stripping off the leaves, leaving

only the leaf-rachis. In severe cases the seedlings may completely be defoliated resulting in the death and deformation of the leading shoot (BEESON 1941, BROWNE 1968, BAKSHA 2001).

Management: The eggs and young larvae can be collected and destroyed by hand. Foliar application of Diazinon 60 E or Malathion 57 EC @ 25 ml/10 liters of water gives effective control of the pest (BAKSHA 2001).

3. Kadam defoliator, *Arthroschista hilaralis* WALKER (Pyralidae: Lepidoptera)

Young larvae feed on the soft green tissue making shallow depression on the leaf surface under the protection of silk strands spun openly. Older larvae feed on the tissue between the veins. As a result, the leaves turn brown and fall off prematurely. When the supply of leaves becomes limited larvae may bore into the terminal tissues of the shoot (BEESON 1941, BAKSHA 2000).

Management: Hand collection and destruction of the larva can be practiced. Providing overhead or side shade to seedlings can prevent the attack. Foliar application of Thuricide 70 WP @ 12 gm/10 liter of water can give effective control of the pest (BAKSHA 2000).

4. Chalta defoliator, *Margaronia* sp. (Pyralidae: Lepidoptera)

The larvae spin the margins of leaf with silk and live inside. Initially the larvae feed on the surface tissue but later they consume the whole leaf except the larger veins and midribs. Pupation occurs in cocoon formed between the leaves. Larval damage occurs during the monsoon, producing moths at the end of July.

Management: The larva can be collected and destroyed by hand. Foliar application of Thuricide 70 WP @ 12 gm/10 liter of water give effective control of the pest.

5. Ipil-ipil psyllid, *Heteropsylla cubana* CRAWFORD (Psyllidae: Hemiptera)

Both the nymphs and the adults suck sap from young foliage, shoots, and buds. Due to the loss of sap the leaflets turn yellow, curl and wilt. Honey-dew secreted by the insect encourages the growth of sooty mould fungi that hampers photosynthesis (BAKSHA, ISLAM 1994).

Management: Local predators such as ladybird beetles, dragonflies, and spiders should be conserved and encouraged to multiply. Psyllid resistant clones can be used. Foliar application of Malathion 57 EC @ 15 ml/10 liter of water gives effective control of the pest (BAKSHA, ISLAM 1994).

6. Jam leaf miner, *Acrocercops telestis* MEYRICK (Lithocolletidae: Lepidoptera)

The larvae feed on the leaf tissue by mining beneath the upper surface of the leaf, causing a blister-like swelling or blotch on the leaf, which results in distortion of the leaf and premature shedding (BAKSHA 2001).

Management: The miner can be handpicked and killed by hand. In severe cases foliar application of Dimecron 100 EC @ 12 ml/10 liter of water can kill the pest (BAKSHA 2001).

7. Arjun gall insect, *Trioza fletcheri* CRAWFORD (Psyllidae: Hemiptera)

The nymph sucks sap from the upper surface of the leaf, resulting in the formation of a gall that encloses the insect. A leaf usually contains numerous galls (BAKSHA 2001).

Management: Same as for jam leaf miner.

B. Diseases

1. Gamar root rot, *Fusarium solani*

Initial symptoms include reduced seedling growth and dull green leaf colour, followed by gradual dying out of the leaf and death of the shoot tip. Infection occurs in irregular patches

in the nursery. Small light brown spots appear first on secondary roots that turn brown as the decay progresses. At the advanced stage of the rot only the woody tissue of the roots is left (Rahman et al. 1997).

Management: Application of Granosan M @ 8 gm/ 9 liter of water as soil drench in areas of dead, dying and surrounding healthy seedlings gives effective control of the disease (Rahman et al. 1997).

2. Teak root rot, *Pseudomonas solanacearum*

Initially root rot of teak appears as a gradual death of the lowermost leaves. This bacterium-caused rot first appears on fine roots and ultimately spreads to the tap root where brownish to blackish discoloration develops in the pith and surrounding woody tissues (Rahman et al. 1997).

Management: Locating nurseries on well-drained soil can prevent the disease (Rahman et al. 1997).

3. Kadam die back, *Rhizoctonia solani*

The disease starts as rot at one or more spots on the leaves. The rot gradually expands and coalesces to form bigger necrotic areas killing both young and old leaves simultaneously. Seedlings with affected leaves show healthy roots (Rahman et al. 1997).

Management: Foliar application of the fungicide, Dithane M 45 @ 3 gm/liter of water gives good control of the disease (Rahman et al. 1997).

4. Rattan leaf spot, *Guignardia calami*

The disease starts as light brown spots on leaves that later coalesce to form larger irregular spots. In severe cases, most of the leaves are killed leading to ultimate death of the seedlings (Rahman et al. 1997).

Management: At the onset of the disease, foliar application of Dithane M 45 @ 3 gm/liter of water gives good control. Two to three applications at weekly intervals are needed (Rahman et al. 1997).

With increasing nursery activity newer pests and disease problems are emerging, for example, mango scolytid shoot borer, mango malformation, mahogany leaf skeletoniser, and jam leaf stitcher. Though the nursery owners are well acquainted with the horticultural pests and diseases they have very little exposure to forestry pests and diseases. Pesticides are most frequently and excessively used leaving aside all other alternatives. Non-technical pesticide dealers are normally consulted, and usually outdated, persistent and adulterated pesticides were used. In most cases insecticides were used where fungicides were required and vice versa. In many cases, the same pesticide was applied in the whole nursery irrespective of the problem. While spraying pesticides safety precautions were not usually undertaken. Thus, the desired results were not achieved, costs increased, the environment polluted and beneficial organisms killed.

RECOMMENDATIONS

Training to nursery owners on forest nursery pests and diseases is urgently required. Constant monitoring of the nursery is needed to detect any pest and disease incidence. For newer pest and disease problems research backup is required. Wherever possible, non-chemical methods of pest management should be practiced. Only recommended pesticides at appropriate doses and timing should be used so that pesticide usage can be reduced by at least 50%.

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DOTHISTROMA NEEDLE BLIGHT IN THE CZECH REPUBLIC

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ABSTRACT

Dothistroma needle blight, caused by the fungus *Mycosphaerella pini* E. Rostrup, was first observed in the Czech Republic in 1999 on an imported *Pinus nigra* Arnold. In 2000, it was again found on open-grown trees. During the last few years, it has become an important pathogen of pines in the Czech Republic where it has been detected in more than 60 localities, especially in Moravia and Silesia and eastern Bohemia. It has been found on 13 species of pine (*Pinus nigra* Arnold and *Pinus mugo* Turra, *Pinus ponderosa* Laws. (Douglas), *Pinus jeffreyi* Grev. et Balf, *Pinus banksiana* Lamb., *Pinus contorta* Douglas, *Pinus rotundata* Link, *Pinus leucodermis* Ant., *Pinus sylvestris* L., *Pinus aristata* Engelm., *Pinus rigida* Mill., *Pinus heldreichii* H. Christ, and *Pinus cembra* L. var. *sibirica* (Du Tour) G. Don.). The spruces *Picea pungens* Engelm., *Picea abies* Karst. and *Picea schrenkiana* Fisch. & C. A. Mey are also hosts of red band needle blight. In the Czech Republic, *Pinus nigra* is the most common host of *M. pini* (80 % of sampled localities) followed by *Pinus mugo* (27 % localities). On Scots pine, *Pinus sylvestris*, *Mycosphaerella pini* (resp. *Dothistroma septospora*) was found at two localities. In the Czech Republic the critical period for infection is from the second half of May until the end of June. Depending upon the climate the incubation period lasts 2 – 4 months. The first symptoms on the needles infected in the current year appear in the second half of August and are clearly evident from September to November.

INTRODUCTION

The causal agent of Dothistroma needle blight is the fungus My*cosphaerella pini* E. Rostrup apud Munk, syn. *Scirrhia pini* Funk & Parker, *Eruptio pini* (Rostr. apud Munk) M. E. Barr, anamorph *Dothistroma septospora* (Dorog.) Morelet, *Dothistroma pini* Hulbary, *Cytosporina septospora* G. Doroguine, *Actinothyrium marginatum* Sacc., *Septoriella septosporum* (Dorog.) Sacc. The pathogen was first described from Europe, more exactly from Russia, as *Cytosporina septospora* Dorog in 1911 (Doroguine 1911). Saccardo (1920) described Dothistroma needle blight fungus on *Pinus ponderosa* in Idaho as *Actinothyrium marginatum* Sacc. *Cytosporina septosporum* was later transferred to the genus *Septoriella* Oudem. as *Septoriella 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 determined when Gremmen (1968) and Morelet (1968) realized that the fungus described in Europe as *Cytosporina septosporum* was the same as *Dothistroma 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. e. g. Barnes et al. (2004) found, based on phylogenetic studies, that *Dothistroma septospora* and *Dothistroma pini*, make up two distinct phylogenetic lineages. *Dothistroma septosporum* has a worldwide distribution and is the causal

agent of the disease that has severely damaged plantations of *Pinus radiata*, grown as an exotic in the Southern Hemisphere. In contrast, *Dothistroma 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 should be classified as *Dothistroma septospora.*

The anamorphic stage has been divided to three varieties on the basis of differences in the length of conidia. THYR and SHAW (1964) distinguished within *Dothistroma pini* HULBARY a variety *pini* (syn. *Dothistroma septospora* var. *septospora*) with the length of conidia 15.4 – 28.0 (mean 22.4) µm and *Dothistroma pini* HULBARY var. *linearis* (syn. *Dothistroma pini* var. *lineare*) with the length of conidia 23.0 – 42.0 (31.9) µm. IVORY (1967) distinguished another variety *Dothistroma pini* HULBARY var. *keniensis* (syn. *Dothistroma septospora* var. *keniense*) with mean conidia length of 13.0 – 47.5 (28.9) µm. EVANS (1984) reasons that *Dothistroma pini* comes from mixed forests of Central America and that it occurs on isolated mountain "islands" at altitudes over 1,500 m.

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 a study of diversity of the genus *Mycosphaerella,* to a new genus *Eruptio* as the species *Eruptio pini* (ROSTR. and MUNK) M. E. BARR. Subsequent phyllogenetic analyses have proved that classifying into the genus *Mycosphaerella* is much suitable (GOODWIN et al. 2001).

As compared to the anamorphic stage, the teleomorphic stage *Mycosphaerella pini* (syn. *Scirrhia pini*) occurs rather rarely. In the majority of countries with the occurrence of an anamorphic stage of *Dothistroma pini* or *Dothistroma septospora* a teleomorph is 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 *Mycosphaerella pini* is related to *Dothistroma pini* var. *linearis*. In *Dothistroma pini* var. *pini* and *Dothistroma pini* var. *keniensis*, a perfect stage has not been described (IVORY 1967).

Virtually, more than 70 host species of Dothistroma needle blight are mentioned from all continents. Particularly various species of pine are hosts of the needle blight. Dothistroma needle blight is also mentioned from *Picea abies* (L.) KARST. (LANG 1987), *Picea omorika* (PANČIĆ) PURKYNĚ (KARADŽIĆ 1994), *Picea pungens* ENGELM. (JANKOVSKÝ, BEDNÁŘOVÁ, PALOVČÍKOVÁ 2004), *Picea sitchensis* (BONG.) CARR. (GADGIL 1984), *Pseudotsuga menziesii* (MIRB.) FRANCO (DUBIN, WALPER 1967), *Larix decidua* MILL. (BASSETT 1969) etc.

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

The purpose of the work reported here was to determine the host range of *Mycosphaerella pini* on the basis of the distribution studies of Dothistroma needle blight in the Czech Republic.

MATERIALS AND METHODS

During surveys carried out from 2000 – 2005, pine needle samples were examined mainly from southern and central Moravia, Silesia and eastern and central Bohemia, plus individual samples from elsewhere in the Czech Republic. In all, pine needle samples with *Dothistroma* symptoms were collected and examined from over 60 localities.

The presence of the pathogen was always confirmed based on presence of characteristic symptoms such as red bands, dying tips of needles or the occurrence of subepidermal sporocarps, acervuli. All these identities were subsequently confirmed by examining conidia using a compound

microscope. 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 in Brno (BRNL).

HOST RANGE IN THE CZECH REPUBLIC

More than 70 host species of Dothistroma needle blight are mentioned in the literature. These refer specifically to species in the genus *Pinus,* but also species of the genus *Picea, Larix decidua* and *Pseudotsuga menziesii* are also reported as hosts (BROWN, ROSE, WEBBER 2003).

In the Czech Republic, Dothistroma needle blight was identified on 13 species of pine. *Pinus nigra* ARNOLD (Austrian pine) and *Pinus mugo* TURRA (dwarf mountain pine) are the most common hosts. In addition to these species, Dothistroma needle blight was noted on *Pinus ponderosa* DOUGLAS ex LAWSON (western yellow pine), *Pinus jeffreyi* GREV. et BALF (Jeffrey pine), *Pinus banksiana* LAMB. (jack pine), *Pinus contorta* DOUGLAS (lodgepole pine), *Pinus rotundata* LINK (bog pine), *Pinus leucodermis* ANT. (Bosnian pine) and *Pinus sylvestris* L. (Scots pine). Finding the fungus on *Pinus aristata* ENGELM. (Rocky mountain bristlecone pine), *Pinus rigida* MILL. (pitch pine), *Pinus heldreichii* H. CHRIST. (Heldreich pine) and *Pinus cembra* L. var. *sibirica* (DU TOUR) G. DON. (Siberian cedar) is a certain rarity. These species are not anywhere mentioned as potential hosts of Dothistroma needle blight. We also found species of *Picea* to be hosts, i. e. *Picea pungens* ENGELM. (blue spruce) and *Picea abies* L. KARST. (Norway spruce). Dothistroma needle blight was also found on *Picea schrenkiana* FISCH. & C. A. MEY (Schrenk spruce) which is certainly a rarity.

Neither perfect stage fruit bodies nor ascospores were found in any of the samples, i. e. only conidia occurred at all localities. External symptoms of Dothistroma needle blight infection differed by host and the symptoms are not uniform. Red banding on the needles was not noted for all host species. This shows the necessity of confirming the disease using a microscope to view conidia.

Dothistroma needle blight is of particular risk for several pines. In the Czech Republic monoculture of *Pinus nigra* creates ideal conditions for this disease. In case of Dothistroma needle blight attack infection spreads rapidly and if appropriate disease management measures are not carried out, forest stands can suffer severe consequences. *Pinus sylvestris* stands can be mildly infected by heavy infestations of the disease which spreads from neighbouring stands of *Pinus nigra*. As compared to other species of pine, symptoms of the attack on *Pinus sylvestris* are not so obvious. According to GADGIL (1984), *Pinus sylvestris* is highly susceptible, while data from Great Britain, PETERSON (1982), indicate that attack rarely occurs there.

BIOLOGY OF *MYCOSPHAERELLA PINI* IN THE CZECH REPUBLIC

In 2002, starting by mid-March, open acervuli of *Mycosphaerella pini* (resp. *Dothistroma septospora*) were presented in most localities in the Czech Republic. In some areas conidia formation was noticed by late April. The critical period for infection in the Czech Republic occurs from the second half of May until the end of June (beginning of July). However, conidia are also exuded during the autumn. Depending upon climatic conditions the incubation period lasts 2 – 4 months.

The first symptoms on the needles infected in the current year appear in late summer (end of August, beginning of September) as non-specific, yellow spots on needles. Eventually the needle tips become dry and dead tissues are initially straw–brown. During September, at first dark brown and later narrow black strips are formed on dead parts of needles. Acervuli form from October onward and characteristic red strips appear. In acervuli, conidia can be formed until the end

of November (depending on climatic conditions). During this time infection intensifies. In our studies damage was noted to intensify over a period as short as 1 week. Infection is particularly evident in early spring.

Under strong disease pressure needles die during the year of infection, especially early in the season from August until September. During the same year, acervuli can form along with accompanying symptoms such as the occurrence of red strips on needles. Heavily affected trees are weakened to such an extent that only small, current year shoots often form. If the shoots grow, they are stunted („lion tails“) and during the next year, they die.

SYMPTOMS OF INFECTION IN THE CZECH REPUBLIC

In the Czech Republic the disease is characterized by needle dying on the lower part of the crown and the prevalence of red strips on dead needles, especially on Austrian pine *Pinus nigra*. Typical symptoms were, however, also occurred on other pines such as *Pinus banksiana*, *Pinus contorta*, and *Pinus aristata*. Needle tip dying was found, for example, on *Pinus jeffreyi*, and *Pinus ponderosa*.

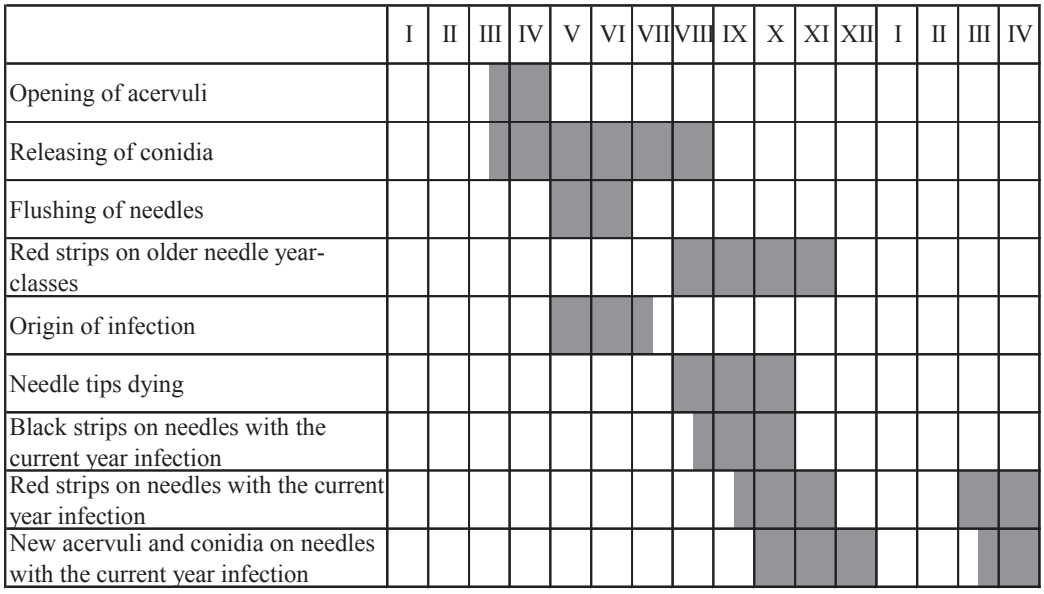

Fig. 1
Phenology of *Mycosphaerella pini* (resp. *Dothistroma septospora*) in the Czech Republic

Since the entire crown of dwarf pine, *Pinus mugo,* frequently dries out, it is impossible to distinguish the attacked lower third of some trees. Characteristic red strips occur only on older, dead needles. Symptoms of this disease are different on each host, so it is very difficult to describe them. Typical red strips are not always evident, but acervuli are always formed (fruiting bodies of imperfect stage). The best thing to do is to confirm presence of the fungus' conidia (typical hyaline and septate) using a microscope.

CONCLUSIONS

Dothistroma needle blight, caused by *Mycosphaerella pini*, and characterized by presence of the conidia of its anamorphic stage *Dothistroma septospora* is one of the most important disease problems in forest and ornamental nurseries and in the forest. In the Czech Republic, the host range includes *Pinus aristata* (Rocky mountain bristlecone pine), *Pinus banksiana* (jack pine), *Pinus cembra* var. *sibirica* (Siberian cedar), *Pinus contorta* (lodgepole pine), *Pinus heldreichii* (Heldreich pine), *Pinus jeffreyi* (Jeffrey pine), *Pinus leucodermis* (Bosnian pine), *Pinus mugo* (dwarf mountain pine), *Pinus nigra* (Austrian pine), *Pinus ponderosa* (western yellow pine), *Pinus rigida* (pitch pine), *Pinus rotundata* (bog pine), *Pinus sylvestris* (Scots pine), *Picea abies* (Norway spruce), *Picea pungens* (blue spruce) and *Picea schrenkiana* (Schrenk spruce). The disease is especially damaging in *Pinus nigra* Christmas tree plantations and nurseries. However, the disease also damages plantation-grown trees. *Pinus mugo* and the three-needle pines *Pinus jeffreyi* and *Pinus ponderosa* are also very susceptible to the disease.

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THE RELATIONSHIP OF *GREMMENIELLA* AND *PHOMOPSIS* TO DAMAGE ON NORWAY SPRUCE SEEDLINGS

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ABSTRACT

In the spring of 2002 extensive damage was recorded in southeast Norway on nursery-grown Norway spruce seedlings that had either wintered in nursery cold storage or had been planted out in the autumn of 2001. The damage was characterized by a top shoot dieback. Two visually distinct types of necroses were located either on the upper or lower part of the 2001-year-shoot. Isolations from the upper stem necroses rendered *Gremmeniella abietina*, while *Phomopsis* sp. was isolated usually from the lower stem necroses. RAMS (random amplified microsatellites) profiling indicated that the *G. abietina* strains associated with diseased nursery seedlings belonged to LTT (large-tree type) ecotype, and inoculation tests confirmed their pathogenicity on Norway spruce seedlings. *Phomopsis* sp. was not pathogenic in inoculation tests, implying that it may be a secondary colonizer. We describe here the *Gremmeniella* - associated shoot dieback symptoms on Norway spruce seedlings and conclude that the unusual disease outburst was related to the *Gremmeniella* epidemic caused by the LTT type on large pines in 2001. The role of *Phomopsis* sp. in the tissue of diseased Norway spruce seedlings is yet unclear.

INTRODUCTION

Damage caused by *Gremmeniella abietina* (Lagerberg) Morelet is well documented and described on large pines as well as on Norway spruce. In Northern Europe *G. abietina* consists of two biotypes, A and B (Uotila 1983), also described as "the small tree type" (STT) and "the large tree type" (LTT), respectively (Hellgren, Högberg 1995). The LTT-type is most common on 15 - 40-year old Scots pine (*Pinus sylvestris* L.) trees in southern Scandinavia and Finland (Hellgren, Barklund 1992, Uotila 1992), where it causes dieback of current year shoots in the entire crown. The STT type occurs on young Scots pine trees in northern Scandinavia and at higher elevations in the south, where it causes perennial cankers on the parts of the tree covered by a persistent snow layer during the winter (Karlman et al. 1994).

On pine seedlings it causes the typical umbrella-like folding of needles on the leader (Nef, Perrin 1999). However, to our knowledge, neither *G. abietina* nor *Phomopsis* sp. infections have been described on Norway spruce nursery seedlings.

In the spring of 2001 a devastating epidemic of *G. abietina* on large pines occurred in the south-eastern part of Norway (Solheim 2001) and in adjacent parts of Sweden (Elna Stenström, personal comm.) which probably was the most damaging outbreak recorded in these areas. The following spring, in 2002, a frequent occurrence of diseased Norway spruce seedlings was noted in forest nurseries in the south-eastern part of Norway. The damage was detected mostly on 2-year old seedlings that were either planted out in the autumn 2001 or removed from cold storage, ready to be planted out in the spring of 2002. The seedlings showed various degrees of top shoot dieback. Surveys made of nurseries with heavy damage also showed that 1-year old seedlings exhibited similar symptoms, but to a lesser extent. A closer examination revealed that prin-

cipally two different types of stem necroses were present. The two types of necroses yielded *G. abietina* and *Phomopsis* sp. respectively.

Here we report on the *Gremmeniella* and *Phomopsis* associated symptoms on Norway spruce seedlings that occurred after the epidemic *Gremmeniella*-outbreak in spring 2001. The objectives of this work were to (i) describe the disease symptoms on Norway spruce seedlings; (ii) to isolate and identify the fungi associated with this damage and further determine their pathogenicity *in vivo* and *in vitro* and (iii) assess survival and development of the outplanted, symptomatic seedlings.

MATERIALS AND METHODS

Plant material and fungal isolation

Norway spruce seedlings (2 years old) were collected from affected nurseries in south-eastern Norway. The length and location of the necroses were measured. Tissue chips were cut out from the necrotic margins, sterilized and plated on the malt (1.25%) agar (2%) medium, incubated at 21 °C in the dark for 3 - 5 weeks, and then the fungi which grew from the diseased tissues were identified.

Pathogenicity test *in vitro* and *in vivo*

The fungi isolated from the diseased seedlings were tested for their ability to induce dieback on fresh living tissue *in vitro* and *in vivo*. For both tests, three isolates of *G. abietina* (02-48/2, 02-26/2, 02-47/1), and *Phomopsis* sp. (02-53/3, 02-117/3, 02-62/1) were chosen. The *in vitro* test compared the ability of the fungi to kill the tissue of freshly detached, aseptic spruce needles. Needles from aseptically-grown spruce seedlings (about 5 weeks old) were detached, placed in a petri plate containing malt agar medium, together with the actively growing culture of the fungus. The needles were positioned in front of the advancing mycelium. Needles on malt agar without any fungal culture were used as controls. The petri plates were incubated in the dark at room temperature. The relative amount of discoloration on each needle was recorded and the percentage of damage for each needle was registered. There were three replicates for each fungal culture with 10 needles in each petri dish. The pathogenicity for each fungal culture was estimated as a time necessary for the fungus to kill 50% of the needles.

To determine the pathogenicity of the isolated fungi *in vivo*, healthy looking 1- and 2-year old Norway spruce seedlings were inoculated with the same fungi as in the pathogenicity test *in vitro*. Ten seedlings of each age class were inoculated with each fungus isolate. A scalpel incision (2 mm) was made in the middle of the stem and a piece of fungus mycelium (about 1 mm^3) on agar medium was placed inside. The wound was sealed with parafilm. Control seedlings were inoculated with agar only. Seedlings were then placed in containers and moved over to a climatic chamber where cold storage conditions (2 - 5 °C, 80% humidity and darkness) were simulated. Eighteen weeks later extend of the necroses and the shoot lengths were measured.

Outplanted symptomatic seedlings

To investigate and follow the development of diseased seedlings, an outdoor outplanting experiment was set up. One-year old Norway spruce seedlings, originating from the nursery with many typical *Gremmeniella*-diseased seedlings, were selected for outplanting. Thirty-six seedlings with the same symptoms were taken to Hoxmark, the experimental garden at the Norwegian Forest Research Institute, and outplanted during the summer of 2002. All seedlings had dead top shoots. In January, 2005, all the seedlings were cut off, their health condition, shoot length and fungus fruit body development was evaluated.

RAMS-PCR-assay of Gremmeniella isolates

Random amplified microsatellite (RAMS) technique was used to further characterize the *Gremmeniella* isolates and determine which biotype they represented. The *Gremmeniella* isolates were grown on cellophane-coated malt and V8 juice agar, and the mycelia harvested were ground with a pestle in liquid N_2 chilled mortars. According to the manufacturer's instructions DNA isolation was performed by using Plant DNA Mini Isolation Kit (Qiagen). The PCR reactions were carried out using the reaction conditions recommended by the manufacturer of the HotStarTaq™ DNA Polymerase by using 2 µM concentration of the degenerate CCA primers described by HANTULA and MÜLLER (1997). The PCR cycling parameters were also as described in that study. Amplification products were separated by gel electrophoresis in 1.5% agarose gels using TAE running buffer and visualized under UV-light after ethidium bromide staining.

Statistical analysis

The data for necrosis length on 1- and 2-year old Norway spruce seedlings in the *in vivo* pathogenicity test were subjected to analysis of variance by using One way ANOVA (JMP, SAS institute)

RESULTS AND DISCUSSION

The symptoms on Norway spruce seedlings became visible during the spring of 2002, a year after the *Gremmeniella* epidemic on large pines. Both 1- and 2-year old plants showed symptoms of desiccated, shoot leaders (Fig. 1) and had necrotic stem lesions on the 2001-year shoot. The first visible signs of stem lesions were a local indentation in the bark, and greyish-green foliage on the lesion area. Later the foliage and branches distal to the lesion area became yellow and brown. Some lesions were located only on one side of the stem, while others encompassed the whole stem, causing top dying of the shoot. Occasionally there were 2 or 3 separate necroses on one stem. Generally, two types of necroses, "upper stem necroses" and "lower stem necroses" could be distinguished.

Upper stem necroses: associated with *Gremmeniella*

Mean 2001-shoot length on 2-year old seedlings with this type of necroses was 25 cm. Necroses on the upper stem were located 14.9 cm (mean distance) above the 2000 - 2001 stem node and their average length was 4.3 cm. The necrotic, dark brown bark was profusely impregnated with resin (Fig. 2A). In this area, the stem was usually girdled, the nearby needles were brown at the base, and the shoots above the necrosis were dead or dying. The edges of the necroses were sharp and distinct. Usually, *G. abietina* was isolated from the advancing edge of the necrotic

Fig. 1
Top shoot dieback caused by *Gremmeniella abietina* on 2-year old Norway spruce seedling Photo: H. Solheim

Fig. 2A
Typical upper stem-necrosis, with brown, resinous tissue, where *Gremmeniella abietina* was isolated. Photo: H. Solheim

Fig. 2B
Typical lower stem necrosis; with light brown and waterlogged tissue. *Phomopsis* sp. was isolated here. Photo: H. Solheim

tissue. *Gremmeniella abietina* alone was isolated predominantly from seedlings sampled in April-May period. In isolations performed later (June and later), *Phomopsis* was occasionally obtained from this type of necroses. No other potentially pathogenic fungi were isolated from the upper stem necroses. Most of the seedlings with upper stem necroses yielding *Gremmeniella* originated from a nursery, where large pine trees were in close vicinity to the nursery.

Lower stem necroses: associated with *Phomopsis*

Mean 2001-shoot length on 2-year old plants with this type of necroses was 21 cm. The mean distance from the lower edge of the necroses to the 2000 - 2001 stem nodes was 3.9 cm. These necroses were often located at the base of the 2001-shoot or partially at the end of the 2000-shoot. Necroses on lower stem were lighter in colour compared to the upper stem necroses, and had a characteristic water-soaked appearance without any resin flow (Fig. 2B). The edges of necroses were diffuse, non-distinct. Occasionally, such necroses were found also on the upper part of the 2001-shoot. The most frequently isolated fungus from these lesions was *Phomopsis* sp., which was isolated from April to December. Apart from two cases where *Botrytis* sp. was obtained, no other potentially pathogenic fungi were isolated from these necroses. Fruit bodies of *Phomopsis* sp. developed readily on plants after storage at +4 °C (Fig. 3A, B). Seedlings with lower stem necroses originated mostly from nurseries, where there were no pine trees in the immediate vicinity.

The stem necroses may have originated from the bark fissures, cracks in the bark associated with rapid growth, usual for plants in nurseries. The damage above the necroses first became visible in 2002. The seedlings were probably infected during the spring or summer of 2001 and the disease was already latent during their moving to cold storage or outplanting, in the autumn of 2001. Presumably, the seedlings at this point had no visible symptoms, which would explain why infected plants were not discarded.

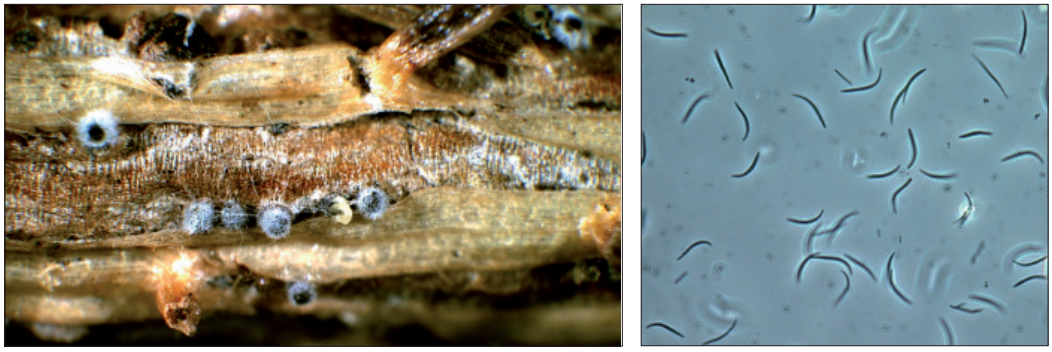

Fig. 3A, B.
Fruitbodies (pycnidia) of *Phomopsis* sp. (A) with beta-spores (B). Photo: H. Solheim

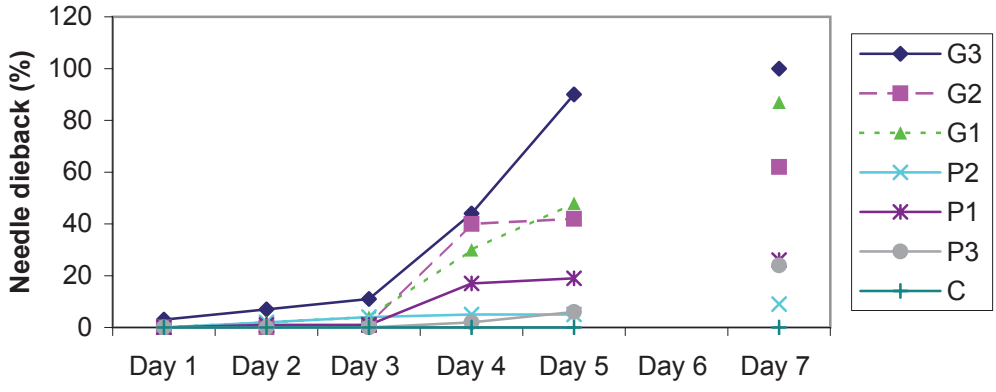

Fig. 4.
Pathogenicity test *in vitro*. Dieback of aseptic spruce needles incubated with three isolates of *Gremmeniella abietina* [02-47/1 (**G1**), 02-26/2 (**G2**) and 02-48/2 (**G3**)] and *Phomopsis* sp. [02-117/3 (**P1**), 02-53/3 (**P2**) and 02-62/1 (**P3**)] all isolated from Norway spruce seedlings with top dieback symptoms

Pathogenicity tests

In the pathogenicity test *in vitro* with needles (Fig. 4), *G. abietina* strains killed 42 - 48 % of the needle tissue within 4 - 5 days, strain 2002-48/2 (G3) being the most aggressive. The *Phomopsis* strains showed no signs of pathogenicity at 10 days after the inoculation (data not shown), when the experiment was terminated.

In the pathogenicity test *in vivo*, seedlings were stored in climatic chambers for 18 weeks from mid November to the end of March. On 1-year old seedlings, *G. abietina* strains 2002-48/2 (G3) and 2002-26/2 (G2) caused significantly longer necroses than the other strains (Fig. 5A). The necroses produced by the other strains were not significantly different from the control. In 2-year old seedlings, the longest necroses were caused by *G. abietina* strains 2002-26/2 (G2) and 2002-48/2 (G3), but only *G. abietina* strain 2002-26/2 (G2) differed significantly from the control (Fig. 5B).

Both pathogenicity tests confirmed the virulence of the *G. abietina* isolates on Norway spruce seedlings. Most of the literature on nurseries reports *Gremmeniella* exclusively as a pathogen on pine seedlings, and if associated to Norway spruce, *G. abietina* is mentioned as a pathogen on saplings (KAITERA et al. 2000) and on larger seedlings in plantations (ROLL-HANSEN 1967). In pine seedlings, the disease is easily recognized by the characteristic umbrella-like folding of needles on the leader shoot (BJÖRKMAN, 1959, NEF, PERRIN 1999), whereas the symptoms of *Gremmeniella* infection on Norway spruce seedlings, necroses and shoot dieback, are rather non-specific and can be caused by several pathogens as well as by abiotic stresses, such as frost, drought or cold storage. Since multiple factors can cause these symptoms in Norway spruce seedlings, incidents of *Gremmeniella*-infection may be misidentified.

Symptomatic seedlings in outplanted plots

In spring of 2003, at the time of the first assessment, 23% of the seedlings (8 seedlings out of 36) had a tendency to develop a double shoot, i. e. two side shoots were competing for dominance. At this time, four dead shoots had pycnidia of *Brunchorstia pinea* (KARSTEN) HÖHN., the anamorph

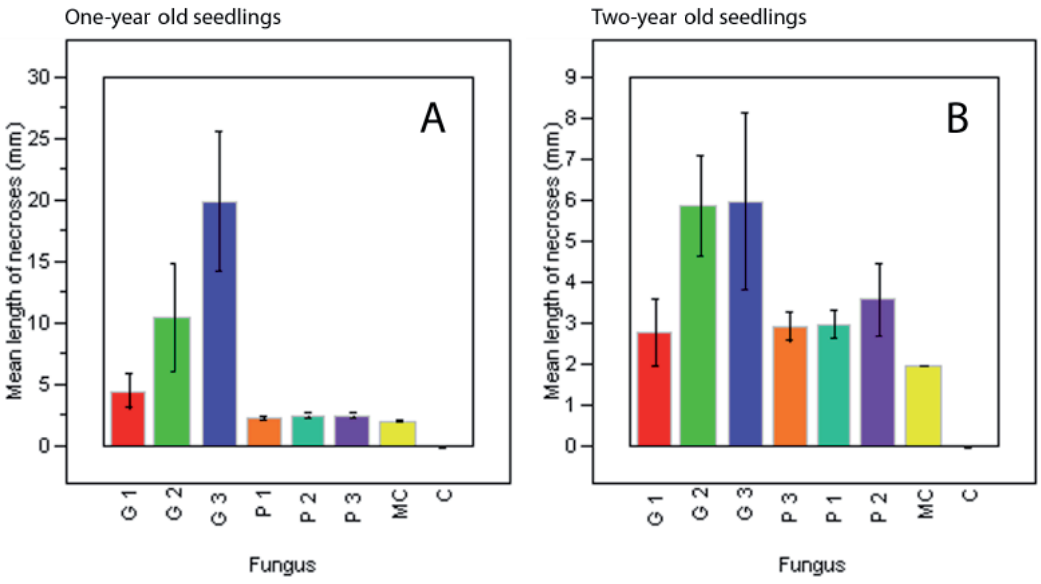

Fig. 5. A, B.
In vivo pathogenicity test. Length of necroses in 1- (A) and 2-year old (B) Norway spruce seedlings 18 weeks after inoculation with different isolates of *Gremmeniella abietina* [02-47/1 (**G1**), 02-26/2 (**G2**) and 02-48/2 (**G3**)], *Phomopsis* sp. [02-117/3 (**P1**), 02-53/3 (**P2**) and 02-62/1 (**P3**)], mock inoculated control (M**C**) and non-inoculated control (**C**)

stage of *G. abietina*, with conidia still present. In January 2005, at the time of final harvesting, 64 % (9 seedlings out of 14) of the seedlings had developed a double stem (unfortunately, 22 seedlings were destroyed by accident before the last evaluation, and thus only 14 remaining seedlings were inspected at the end of the experiment). The seedlings were alive, and showed good growth (Fig. 6). The originally diseased leader shoots had been taken over by a new leader. Out of the 14 dead shoots collected at the last inspection, four had old, empty pycnidia still present, while ten had only visible scars after pycnidia. No apothecia were observed in any seedling.

The outplanting experiment confirmed that the infected Norway spruce seedlings survive the damage. Even if the part above the stem necrosis dies, in young plants usually the side shoot takes over the dead leader. Some of the seedlings develop double leaders after *Gremmeniella* infection.

The RAMS-PCR assay

The RAMS-CCA banding patterns were identical among the *Gremmeniella* isolates from Norway spruce seedlings, while the included reference strains of LTT and STT ecotypes showed type specific banding patterns (Fig. 7). The assay confirmed that the *Gremmeniella* isolates from Norway spruce seedlings belonged to the LTT ecotype, as their banding patterns were identical to those from the reference strains of the type and differed from the STT ecotype reference strains. With the CCA primer, only the LTT type reference strains and the strains from the seedlings had a 1500-bp band.

These data confirm that the strains associated with nursery-grown Norway spruce seedlings belonged to the LTT ecotype of *G. abietina*. Our nursery samples were collected from the geographical area in south-eastern Norway, where a devastating epidemic of *G. abietina*

Fig. 6.
Long-term field performance of damaged 1-year old Norway spruce seedlings. Left: Seedlings were 1-year old (in 2002) when top shoot damage occurred (arrow). One year later (in 2003) the dead shoot was taken over by side-shoots. Right: The same seedling in 2005. Photo: H. Solheim

had occurred on large pines the previous year. This epidemic was a typical LTT type outbreak characterized by dieback of shoots in the entire crown (SOLHEIM 2001). As the *Gremmeniella* strains from diseased nursery seedlings of Norway spruce grouped to the LTT type, we conclude that the unusual disease outburst on Norway spruce seedlings in 2002 was related to the previous year's epidemic on pines. Apparently similar damages in Norway spruce seedlings after the pine epidemic were observed in Sweden (STENSTRÖM, personal comm.) and in Finland (PETÄISTÖ 2003) as well. During periods of high inoculum density the pathogen can also infect the Norway spruce seedlings in the neighbouring nurseries. To avoid infection from the pines it is important to keep the pines away from the forest nurseries and Christmas tree plantations.

Besides *Gremmeniella*, a *Phomopsis* species was frequently associated with the shoot dieback-stem necrosis symptoms in the Norway spruce seedlings now examined. Compatible with our observations on *Phomopsis*, HANSEN and HAMM (1988) report on *Phomopsis* associated with top-kill symptoms of Douglas fir seedlings, where necroses were formed at the base of new shoots. They suggested that the infection takes place during the summer, possibly through the bud scales. In addition to location, also the appearance of necroses associated with *Gremmeniella* and *Phomopsis* differed. Resin flow, a characteristic conifer response upon pathogen attack, was commonly observed in necroses hosting *Gremmeniella*, whereas *Phomopsis*-associated necroses were water soaked and without any resin flow.

Based on the ITS rDNA sequence analysis performed, the *Phomopsis* isolates do not represent any previously characterized *Phomopsis* species associated with conifers (BØRJA et

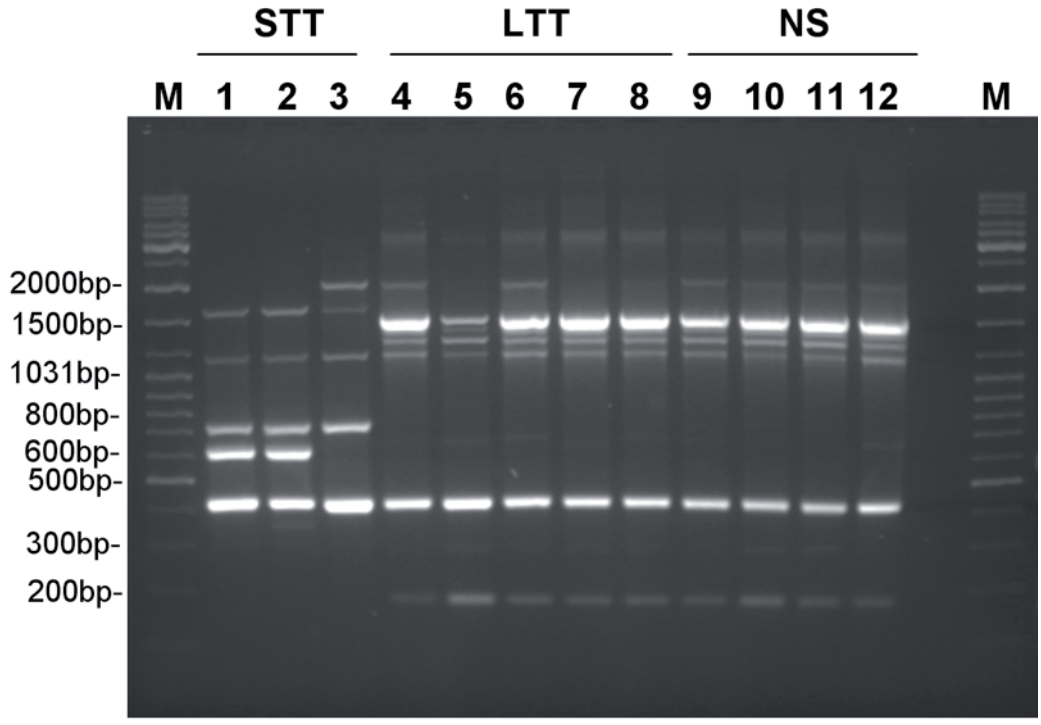

Fig. 7.
RAMS patterns (CCA primer) of three small-tree type (STT) (1988-306/1, 1988-307/3 and 1974-46/1, respectively) and five large-tree type (LTT) (2002-20/4, 2002-47/1, 1985-111/6, 1985-393/16/1 and 1966-163/2, respectively) *G. abietina* reference strains, and four isolates (2002-4/4, 2002-79/2, 2002-107/2 and 2002-124/1, respectively) obtained from diseased Norway spruce seedlings from nurseries (NS) with *Gremmeniella* problems. Lane M: DNA size marker (GeneRulerTM NA ladder mix)

al., submitted). Since the ITS sequence similarity of the *Phomopsis* strains from Norway spruce seedlings to deposits at the NCBI GenBank Sequence Database was also relatively low (≤ 95%), it is likely that these *Phomopsis* strains now studied represent a yet non-characterized species on Norway spruce. This complicates the comparison to other studies. However, *P. occulta* has been associated with stem cankers (Donaubauer 1995, Hahn 1943), while *P. conorum* has been observed in correlation with shoot dieback of young spruce trees in Austria (Donaubauer 1995, Cech, Perny 1995). In British Columbia, *P. occulta* is considered as a pathogen on spruce seedlings in nurseries (Thompson et al. 2002). Cech (personal comm.) confirms the occurrence of *Phomopsis* spp. on spruce, but has the opinion that *Phomopsis* is a secondary fungus, infecting after e. g. *Sirococcus* or *Gremmeniella*. Consistently, Perny et al. (2002) also described *Phomopsis* species as merely a weak parasite of spruce that is favoured only in cases of adverse climatic conditions, the inappropriate provenance or localization. Our data are consistent with the latter two cases since the pathogenicity tests showed that the *Phomopsis* strains were non-pathogenic. Our current hypothesis is that to become pathogenic, our *Phomopsis* strains need specific host-predisposing conditions, such as infection by other pathogens or abiotic stress, or both.

The occurrence of the top shoot dieback on Norway spruce seedlings, associated with *Gremmeniella*, is not new, but overlooked. The unique event of *Gremmeniella* epidemics on large pines which occurred in 2001 allowed us to follow and describe the occurrence of the *Gremmeniella*-disease development on Norway spruce seedlings in nurseries.

CONCLUSIONS

In conclusion:

1. Ours is the first report of a massive *Gremmeniella* infection on nursery-grown Norway spruce seedlings.
2. The incidence of the disease is related to the serious *Gremmeniella* epidemic on large pine and spruce trees the previous season.
3. The resulting extreme infection pressure combined with predisposing weather conditions, cold and high rainfall periods in the summer followed by mild winter account for the atypical outbreak of *Gremmeniella* on nursery-grown Norway spruce seedlings.
4. Removal of the large pines, a source of *G. abietina*-inoculum, from the immediate vicinity of the nursery, may decrease the damage on seedlings.
5. In years with high infection pressure from *G. abietina*, selective chemical treatment of Scots pine and Norway spruce seedlings seems warranted.
6. Our studies found a *Phomopsis* sp., associated with lower stem necroses in Norway spruce seedlings, yet the pathogenicity potential and function of this fungus is unclear.

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BIOLOGICAL CONTROL OF *BOTRYTIS CINEREA* IN *PINUS SYLVESTRIS* SEEDLINGS IN SWEDISH FOREST NURSERIES

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In Swedish forest nurseries, grey mould (*B. cinerea* Pers.: Fr.) is the most prevalent of all fungal diseases occurring on Scots pine and Norway spruce seedlings. At present, only one fungicide (tolylfluanid) is registered for use against the pathogen.

We demonstrated the potential of biocontrol as an alternative control measure for suppression of *B. cinerea* in Scots pine seedlings in growth room co-inoculation assays. In a field trial the commercial products Mycostop®, Binab® TF.WP and GlioMix® reduced *B. cinerea* infections in needles of first-year container-grown Scots pine seedlings by 16 to 57%, and were as effective as recommended fungicidal sprays.

A novel microcosm bioassay was developed to screen microbial isolates obtained from conifer needles for biocontrol of *B. cinerea* in co-inoculation tests on three-week old Scots pine seedlings. One of the isolates with good biocontrol potential was identified as *Fusarium* sp. strain F31. This isolate secretes a spectrum of enniatins when grown in liquid media cultures. Two new enniatins J_2 and J_3 were co-isolated, while enniatins J_1 and K_1 were isolated for the first time without directed biosynthesis. Enniatins are antifungal metabolites and inhibition of germination of conidia of *B. cinerea* was demonstrated.

Based on the screening tests in the microcosm bioassay, nine fungal isolates were selected and evaluated for their control efficacy against *B. cinerea* infections in first-year Scots pine seedlings in a small-scale field experiment. Results will be presented on the impact of applications of spore suspensions of the fungal isolates on *B. cinerea* needle infections and community structure of needle-inhabiting fungi.

BIOLOGY AND MANAGEMENT OF *LONGIDORUS AMERICANUS* IN A SOUTHERN USA NURSERY

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ABSTRACT

A recently discovered nematode, *Longidorus americanus*, caused stunting of *Pinus taeda* seedlings at a forest-tree nursery in Georgia, USA. In growth chamber experiments, *L. americanus* significantly reduced the root size of *P. taeda* and *P. elliottii* seedlings. Although the root systems of *P. palustris* were not significantly reduced, it was found to be a host for *L. americanus*. The field where damage by *L. americanus* occurred was in continuous production of *Pinus* spp. and *Quercus alba* seedlings from 1990 to 1998. Fumigation by MC33 (methyl bromide 67%/chloropicrin 33%) in the spring of 1998 and again in 2000 remedied the problem for only one year; the seedlings were severely stunted during the second production years (1999 and 2001). Growth chamber tests found that *Quercus* spp. (*Q. alba, Q. rubra, Q. falcata, Q. virginiana,* and *Q. nigra*) were hosts for *L. americanus*. The populations of nematodes declined with *Q. acutissima*. Grasses used as cover crops in southern (USA) nurseries, including *Triticum aestivum*, *Secale cereale*, *Sorghum bicolor*, *Avena sativa* and *Panicum ramosum* are not hosts for *L. americanus*. Weeds such as *Cyperus esculentus* and *C. rotundus* that are common in southern (USA) nurseries were also not suitable hosts for the nematode. A fallow study conducted in a field and in growth chambers suggests that *L. americanus* does not survive in soil for extended periods (> 334 days) without a suitable host. Surveys for *L. americanus* have determined that the nematode occurs in oak forests that border the nursery and in an adjacent pine seed orchard.

Key words: *Longidorus americanus, Pinus* sp., *Quercus* sp., cover crops, *Cyperus* sp. fallow

INTRODUCTION

A new species of *Longidorus* discovered in 2000 was associated with stunting of *Pinus taeda* in a forest nursery located in southern Georgia, USA (Fraedrich, Cram 2002). The nematode was recently described and named *Longidorus americanus* (Handoo et al. 2005). Symptoms of seedling damage by *L. americanus* include chlorotic needles, reduced shoot growth and root systems that lack lateral and fine roots. The stunted seedlings were found in field 7S where similar damage had been observed since 1996 on both *P. taeda* and *P. elliottii* crops. The cause of this damage went undiagnosed until 2000 largely due to our reliance on a nematode testing laboratory that processed soil using a sugar flotation method. This technique is more suitable for smaller plant-parasitic nematodes. The adult nematodes of *L. americanus* are large (7 - 8 mm long) and require extraction methods specific for large nematodes (Flegg 1967, Shurtleff, Averre 2000). Over the next 5 years, a series of surveys, dosage response tests, host range tests, and fumigation and fallow studies were conducted to investigate various aspects of the biology of the nematode and determine how best to manage this problem. In all our surveys and studies, nematodes were extracted using a technique developed by Flegg (1967) with minor modifications by Fraedrich and Cram (2002).

Tab. 1.
Field history of fumigation and crop rotations of pine, hardwoods, and cover crops for blocks 1 - 5 in field 7S of a south Georgia forest tree nursery (Byromville GA).

Date	Block 1[+]	Block 2	Block 3	Block 4	Block 5
1989	**MC33**[‡]	**MC33**	**MC33**	**MC33**	**MC33**
1990	*P. taeda*[*]	*P. taeda*	*P. taeda*	*P. taeda*	*P. taeda*
1991	*P. taeda*	*P. taeda*	*P. elliottii*	*P. elliottii*	*P. elliottii*
1992	*P. taeda*	*P. taeda*	*P. elliottii*	**Basamid/** *P. elliottii*	**MC33/** *P. elliottii*
1993	*Q. alba|P. taeda*	*P. taeda*	**Fallow**	**Fallow/ MC33**	**Fallow/ MC33**
1994	*Q. alba*	*Q. alba*	*C. florida|L. styraciflua*	*P. elliottii*	*P. elliottii*
1995	*Q. alba*	*Q. alba*	*C. florida|L. styraciflua*	*P. virginiana*	*P. virginiana|P. clausa*
1996	***P. taeda***|*Q. alba*	*Q. alba*	*L. bicolor*	*L. bicolor*	*S. cereale*
1997	***P. taeda***	*P. taeda*	*L. bicolor*	*S. bicolor*	*S. bicolor*
1998	**Fallow**	**MC33/** *P. elliottii*	**MC33/** *P. elliottii*	**Fumigation study/** *P. taeda*	**Fumigation study/** *P. taeda*
1999	**Fallow**	***P. taeda***	***P. taeda***	*P. taeda*	*P. taeda*
2000	**MC33/** *P. taeda*	**MC33/** *P. taeda*	**MC33/** *P. taeda*	***P. taeda***	***P. taeda***
2001	***P. taeda***	***P. taeda***	***P. taeda***	***P. taeda***	***P. taeda***

[+]**Text** – stunted seedling damage
[‡]MC33 = methyl bromide 67% / chloropicrin 33%
[*]*P. taeda = Pinus taeda; P. elliottii = Pinus elliottii; Q. alba = Quercus alba; P. virginiana = Pinus virginiana; P. clausa = Pinus clausa; C. florida = Cornus florida; L. bicolor = Lespedeza bicolor; L styraciflua = Liquidambar styraciflua; S. cereale = Secale cereale*

FIELD HISTORY

The crop and fumigation history of field 7S, provided in Table 1, is vital to understanding the development of the problem caused by *L. americanus*. Blocks 1 and 2 of this field were used to test rotations of pine and white oak from 1990 to 1996. This crop rotation was experimental and was not part of the normal crop rotation used by this nursery or other southeastern forest-tree nurseries. In 1996 and again in 1997, a few areas of stunted *Pinus taeda* seedlings occurred in block 1. Seedlings were examined and soil was sent to a nematode laboratory, but these efforts failed to find a cause for the damage. Blocks 2 and 3 in field 7S were fumigated in 1998 with 67% methyl bromide/33% chloropicrin (MC33) and planted to *P. elliottii*. Block 1 was too wet at the time of fumi-

gation and remained fallow during 1998. A fumigation study was also established in blocks 4 and 5, and sown with *P. taeda* (CRAM et al. 2002). In 1999, large areas of stunted seedlings occurred in block 2 and extended into block 3 (Fig. 1). Seedlings from the affected areas were examined for fungi and soil samples were sent to a nematode testing laboratory, but again the cause of the damage could not be identified. Blocks 1 to 3 were fumigated with MC33 in the spring of 2000 and sown in *P. taeda*.

Fig. 1.
Stunted *Pinus taeda* in block 2 of field 7S, summer 1999

In 2000, small spots (3 - 9 m long) of stunted seedlings occurred in unfumigated control plots of the fumigation study in blocks 4 and 5 (FRAEDRICH, CRAM 2002). Soil samples were sent to a nematode lab, which reported low levels of *Pratylenchus* sp. and *Criconemella* sp. Evaluations for pathogenic fungi were also inconclusive. Finally, we discovered large (5.4 - 9 mm long) plant-parasitic nematodes associated with stunted seedlings as we were examining unwashed roots; these nematodes were determined to be members of the genus *Longidorus*. We believe that the nematode was moved into these nonfumigated control plots during routine field operations before establishment of the study in 1998.

SURVEYS

1 Nursery blocks

In August and October of 2000, the populations of *L. americanus* were greatest in soil from the centers and margins of stunted areas compared to adjacent areas with healthy seedlings (FRAEDRICH, CRAM 2002). In August, 26 *L. americanus* per 25 g soil were obtained from the center of patches, 13.1 at the margins, and 0.5 and 0.2 at distances of 1.5 and 3 m from the margins, respectively. In October, there was an average of 25.8 *L. americanus* per 25 g of soil at the margins of patches, and only 1.5 *L. americanus* /25 g soil at locations 1.5 m outside the margin.

2 Outside the nursery field

Surveys were also performed from 2001 to 2003 in various locations within the nursery and in locations that border the nursery. These samples typically consisted of 8 - 10 cores at a 6" depth taken at specific locations and along transects. Soil samples were taken in 3 red cedar windrows, an oak (*Quercus* spp.) seed orchard, and oak and pine forests adjacent to the nursery. Sixteen composite soil samples were also obtained from *P. taeda* and *P. elliottii* seed orchards that border the nursery. *Longidorus americanus* was found in 37% soil samples from the pine seed orchards, and in an area of oak (primarily *Q. nigra*) trees that bordered the nursery.

Tab. 2.
Longidorus americanus population densities and root dry weights of southern pine species 26 weeks after infestation (experiment 2)[+]

Pine species	Initial needle nematode	Final needle nematode	Root dry weight (g)[‡]
	Number / container		
Pinus taeda	200	1257	0.159a
	0	0	0.295b
Pinus elliottii	200	1683	0.334a
	0	0	0.556b
Pinus palustris	200	820	0.681a
	0	3	0.825a

[+]Data obtained from FRAEDRICH et al. 2003 (Plant Dis., 87:1129:1132)
[‡]Means followed by the same letter do not differ significantly ($P \leq 0.05$) according to t-test

Tab. 3.
Mean number of *Longidorus americanus* obtained from soil and roots of plant species 13 weeks after infestation with 100 nematodes/container

Plant species	Longidorus sp./400cc soil+	Total estimated Longidorus sp. per container
Pinus taeda	74a	295
Quercus nigra‡	94a	377
Q. rubra	56ab	223
Q. akba‡	42ab	168
Q. falcata	33ab	131
Q. virginiana	32ab	129
Q. acutissima	17bc	69
Fallow	1c	5

+Means followed by the same letter do not differ significantly (alpha = 0.05) according to Tukey's HSD test. Square root transformation of nematode counts performed before analysis. Data analyzed as a randomized complete block design.
‡Means based on three replications

HOST RANGE STUDIES

1 Pine hosts

Population densities of *Longidorus americanus* increased on roots of *P. taeda* seedlings and damaged the root systems of seedlings in growth chamber tests. Root dry weights of seedlings decreased with respect to both the initial *L. americanus* dose, and the final population per container (FRAEDRICH, CRAM 2002). *Pinus elliottii* and *P. palustris* were also found to be hosts of *L. americanus* (FRAEDRICH et al. 2003). Population densities of *L. americanus* increased on roots of *P. elliottii* and *P. palustris*, but at the initial population densities used in these experiments only the root dry weights of *P. elliottii* seedlings were reduced by the nematode (Table 2). This lack of effect of the nematode on *P. palustris* may be related to the unique developmental characteristics of this pine

species. *Pinus palustris* typically remains in a "grass stage" during the first 1.5 or more years of its development, and root system growth is favored during this period.

2 Cover crops and weeds

Small grain cover crops are typically alternated with pine and hardwood seedling production by many nurseries in the southern USA. The suitability of cover crops and weeds as hosts for *L. americanus* were evaluated in a series of experiments (FRAEDRICH et al. 2003). The species (cultivars) of cover crops tested were *Triticum aestivum* (Saluda) *Secale cereale* (Wrens Albruzzi), *sorghum bicolor* (Richardson 9300, SG Ultra), *Panicum ramosum* (DW-01), and *Avena sativa* (FLA 501). The weeds tested as hosts for *L. americanus* were *Cyperus esculentus* and *C. rotundus*. *Longidorus americanus* population densities decreased substantially on roots of all cover crops and weeds, and were not significantly different than populations in the fallow containers (FRAEDRICH et al. 2003).

3 Oak host range

Six species of oak have been evaluated as hosts for the *L. americanus* (unpublished data). The oak species tested were *Quercus virginiana*, *Q. acutissima*, *Q. alba*, *Q. nigra*, *Q. falcata*, and *Q. rubra*. *Pinus taeda* and fallow treatments were also included. Soil (loamy sand) from the nursery field was microwaved for 8 minutes in 2,000 g batches, and containers were filled with 1,600 cc of soil. There were four replications (containers) of each species, and germinated oak and loblolly pine seeds were established in their respective containers. Fallow containers were maintained free of all plants. The containers were infested with 100 adult and juvenile nematodes when the oaks were 15-week old and the pines were 7-week old. Containers were placed in growth chambers at 25 °C with a 14 hr photoperiod. After 13 weeks, *L. americanus* population densities had increased on roots of *Q. virginiana*, *Q. alba*, *Q. nigra*, *Q. falcata*, and *Q. rubra* (Table 3). *Quercus acutissima* was the only species that had significantly less nematodes than loblolly pine, and the population density did not differ significantly from the fallow treatment.

FALLOW STUDIES

Several nurseries in the southern USA have begun to alternate tree seedling production with summer fallow in order to control nutsedge with glyphosate (FRAEDRICH et al. 2003). The effect of fallow on the survival of *L. americanus* was determined in field and growth chamber studies during 2002 (FRAEDRICH et al. 2005). In the field study, the population density of *L. americanus* decreased steadily during the first 101 days in the fallow field, and only a few nematodes were detected between 128 to 220 days (Figure 2; $P \leq 0.0001$). *Longidorus americanus* was not detected in soil samples from any plot on days 263 (January), 325 (March) or 365 (May).

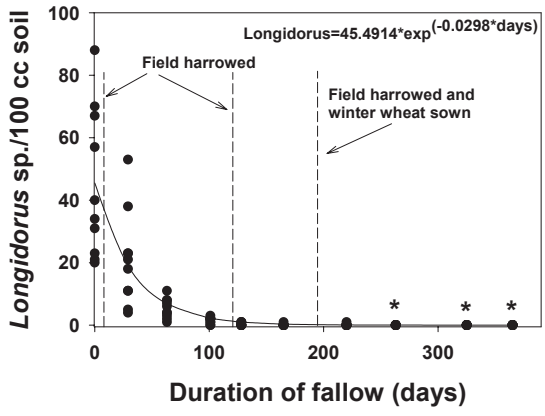

Fig. 2.
Relationship between *Longidorus americanus* population densities and days of fallow in field plots after April 11, 2002
Asterisks (*) at sample days indicate that the needle nematode was not detected in any field plot (FRAEDRICH et al. 2005).

In the growth chamber study, the population of *L. americanus* also decreased exponentially over time ($P \leq 0.0001$) in fallow containers (FRAEDRICH et al. 2005 in press). *Longidorus americanus* was not detected after days 334 and 427. In containers planted with pine seedlings, the population density of *L. americanus* initially declined but subsequently increased after additional pine seedlings were added to containers. *Longidorus americanus* does not survive for extended periods in the upper 15 cm of fallow soil in the field (> 263 days) or in fallow containers under optimal conditions (> 334 days). The use of fallow or use of non-host cover crops should provide control of the *L. americanus* nematode in the southern Georgia nursery.

DISCUSSION AND CONCLUSIONS

Longidorus species have been found in southern pine nurseries and forests (HOPPER 1958, RUEHLE, SASSER 1962); however, there have been no reports of these nematodes damaging pine seedlings in nursery beds. In 2000, *Longidorus americanus* was discovered to cause stunting of *Pinus taeda* seedling at a south Georgia forest nursery (FRAEDRICH, CRAM 2002). *Pinus elliottii* and *P. palustris* were found to be hosts of *L. americanus* (FRAEDRICH et al. 2003).

Damage by *L. americanus* occurred in a field where the nursery had alternated *P. taeda* production with production of *Quercus alba*. Host suitability tests have found that *Q. alba* and other native oak species are hosts for *L. americanus*. The initial development of pine seedling stunting in block 1 of field 7S was most likely due to continuous cropping of pine and oak species over an extended period of time.

Surveys have found *L. americanus* outside the nursery under *Q. nigra* trees (FRAEDRICH, unpublished) and in an adjacent *P. taeda* and *P. elliottii* seed orchards. Locations such as these may provide the sources for reintroduction in nursery fields through soil and water movement (e. g. floods, equipment, wind and animals). It is possible that this nematode has been introduced to the nursery fields in the past, but that the typical rotations of tree seedling crops with grass cover crops has provided adequate control of this nematode.

In 1998 and 2000, the nursery attempted to remedy the problem of stunting in pine seedling crops by using methyl bromide fumigation. Fumigation suppressed disease development in the first seedling crop after fumigation, but the problem reappeared during the second year of production. Although fumigation has been found to depress high nematode populations (DROPKIN 1989), nematode populations can rebound quickly and significantly impact subsequent seedling crops (MCKENRY, THOMASON 1976). The rebound of nematode populations after fumigation was demonstrated by FRAEDRICH and DWINELL (2003). In this study, dazomet and metam sodium reduced the needle nematode to nondeductible levels in the upper 15 cm of soil but populations subsequently increased during loblolly pine production to levels comparable to those in control plots by the end of the growing season (FRAEDRICH, DWINELL 2003).

The nursery where the *Longidorus* problem was found typical rotates from pine production to cover crops every two years. In fact, the majority of southern nurseries rotate their tree seedling production with small grain crops yearly or biennially (CRAM, FRAEDRICH 1997). All of the small grain cover crops used at this nursery were found to be non-hosts for *L. americanus*. These results coupled with the findings that *L. americanus* does not appear to survive extended periods in fallow soil may help explain why this nematode problem has not been a reoccurring problem in other fields at the nursery. We believe that the nursery will not have a serious problem with *L. americanus* in the future if they maintain the practice of alternating cover crops and periods of fallow with pine seedling production on a regular basis.

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EVOLUTION OF OUR KNOWLEDGE OF *FUSARIUM* DISEASES IN FOREST NURSERIES

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ABSTRACT

Fusarium spp. are recognized throughout the world as very important pathogens in forest nurseries. Diseases caused by *Fusarium* spp. are often the most important factors limiting production of high-quality seedlings in western North America. Initial investigations concentrated on disease etiology and development of direct chemical control procedures. Later work focused on disease epidemiology and the role of environmental factors in disease severity. Several different morphologic species of *Fusarium* are routinely encountered in forest nurseries because they commonly colonize soil and seedling tissues. Within seedlings, they may either elicit disease symptoms or exist as endophytes. *Fusarium oxysporum* and *F. proliferatum* are the most important pathogenic *Fusarium* species within western North America nurseries on bareroot and container seedlings, respectively. Successful reduction of disease losses uses an integrated approach involving cultural manipulations and biological and chemical control. Control efforts seek to reduce pathogen inoculum within seedling growing environments and improve seedling vigor to reduce disease expression. Preventing disease is usually much more successful than therapeutic approaches. Recent studies indicated that pathogenic and non-pathogenic isolates of *F. oxysporum* within forest nurseries are probably genetically distinct. Identifying genetic markers and subsequent development of molecular probes may improve disease severity prediction within forest nurseries by quantifying pathogenic *Fusarium* populations and be useful in screening, evaluating, and producing effective biological controls.

INTRODUCTION

Diseases are important factors limiting the production of high-quality seedlings in forest nurseries throughout the world. When serious investigations into forest nursery diseases began early in the 20th century, *Fusarium* spp. were often found associated with diseases. Investigations in North America initially dealt with pre- and post-emergence damping-off and identified several *Fusarium* taxa that were commonly isolated from diseased seeds and young seedlings (Hartley, Merrill 1918, Rathbun 1918). Subsequent work evaluated pathogenic potential of several Fusaria and indicated that several species were capable of causing diseases (Rathbun-Gravatt 1925, Tint 1945a). After identifying the importance of *Fusarium* spp. in nursery disease etiology, steps were taken to ameliorate pathogen effects, particularly drenching diseased seedbeds with chemical pesticides (Warcup 1952). Research into *Fusarium* diseases in the 1940s revisited aspects of disease etiology as well as focusing on environmental factors affecting disease expression and severity (Tint 1945b, c). In particular, temperature and soil effects were carefully evaluated and recommendations formulated to reduce disease losses.

The next important phase of research in North America dealing with *Fusarium* diseases in forest nurseries was the series of outstanding investigations conducted by Bloomberg (1981, 1985), and Vaartaja and others (Vaartaja 1967, Vaartaja, Bumbieris 1967, Vaartaja, Cram 1956)

conducted in the 1950s - 1970s. This work focused primarily on diseases incited by *Fusarium oxysporum* SCHLECHT. on bareroot Douglas-fir (*Pseudotsuga menziesii* FRANCO). Research dealt with infection biology, symptom production, disease epidemiology, pathogen biology, environmental effects on disease severity, investigation of disease control, and formulation of an epidemiological model for the disease. All of this work laid the foundation for later work on *Fusarium* diseases, particularly in North America.

Subsequent to this work, significant contributions to our understanding of *Fusarium* diseases in forest nurseries were concerned with disease descriptions on different nursery crops, environment-host/pathogen interactions, interactions between mycorrhizal fungi and *Fusarium* pathogens, potential use of biological control agents to reduce disease severity, and improvement of disease control, particularly by refining pesticide applications and implementing pre-plant soil fumigation or solarization. Noteworthy important research contributions outside North American included work in Japan and Europe (LILJA et al. 1992, MATUO, CHIBA 1966, PROCHAZKOVA 1991).

When workers first started investigating *Fusarium* diseases, difficulties in taxonomy of the genus became evident. Several monographs (BOOTH 1971, GERLACH, NIRENBERG 1982, JOFFE 1974, SUMMERELL et al. 2003, TOUSSOUN, NELSON 1968) have been prepared that deal with *Fusarium* speciation and acceptance of specific treatises by researchers has varied. Some taxonomy resulted in very few species, whereas other work divided the genus into many different taxa (GERLACH, NIRENBERG 1982). During the course of our work on *Fusarium* diseases, we have consistently used the taxonomy of NELSON et al. (1983), primarily because the morphological characteristics used in this treatise are easily recognizable, consistent (particularly if isolates are processed and maintained in particular ways), and accepted by many *Fusarium* researchers (SUMMERELL et al. 2003).

Morphological differentiation of taxa may not necessarily reflect actual phylogeny (GORDON, MARTYN 1997, KISTLER 1997, O'DONNELL et al. 1998). As a result, new taxonomy of some *Fusarium* sections have been developed using molecular genetics (NIRENBERG, O'DONNELL 1998, O'DONNELL et al. 1998). These probably more accurately reflect true phylogeny of these organisms than traditional morphological characterization. However, molecular techniques are currently not available to all workers dealing with this important pathogen genus. Therefore, reliance on morphology will be necessary until molecular differentiation can be routinely used by most investigators (SUMMERELL et al. 2003).

Our work on *Fusarium* diseases in forest nurseries began many years ago, primarily in response to problems in newly-established container seedling facilities. Container seedling production became more important starting in the early 1980s, particularly within the Inland Pacific Northwest. However, *Fusarium* diseases were devastating in some nurseries, even to the point where nearly entire crops were lost to these diseases (JAMES, GILLIGAN 1984). Although our work initially involved pathogen identification and recommendations for reducing disease impacts, we found that important biological questions regarding behavior of *Fusarium* spp. in container nurseries needed answers. Therefore, we initiated research into these diseases in container nurseries. Most of the remainder of this paper summarizes this work. In addition, we summarize recent work we have been involved in concerning initial findings about molecular characterization of *Fusarium oxysporum* populations and differentiation of pathogenic from non-pathogenic isolates of this species within forest nurseries.

FUSARIUM IN CONTAINER NURSERIES

Types of diseases and symptomotology

Fusarium causes several types of diseases throughout the growth cycle of container-grown seedlings. Pre- and postemergence damping-off are usually caused by *Fusarium* residing on or, less frequently, within seeds (DUMROESE et al. 1988, JAMES 1984c, 1985c, 1986a, b, 1987c, 1999). The most important damping-off species is *F. oxysporum* (JAMES 1984a, 2004b, JAMES et al. 1991), although several other *Fusarium* species are routinely isolated from conifer seeds (JAMES 1986a, 1987d). Most *Fusarium* contaminate seedcoats and infect emerging radicles during germination (JAMES 1986a, JAMES, GENZ 1981, 1982, JAMES et al. 1988a, 1996). In some cases, particularly for ponderosa pine (*Pinus ponderosa* LAWS.), seedcoats may remain attached to cotyledons for extended periods with contaminating fungi attacking cotyledons (JAMES 1992a, 2003a); these fungi can then move down the stem of young germinants causing mortality (JAMES 2003a).

Although *Fusarium* spp. are usually associated with root diseases, in some cases these fungi can cause either stem cankers or top blight of container seedlings (HANSEN et al. 1990, JAMES 2003a, b, JAMES et al. 1991). *Fusarium* sporodochia may be produced above the ground line of infected seedlings (JAMES 1985e, 1992a, 2005a). These produce spores that can readily be disseminated throughout greenhouses via air movements or during irrigation (JAMES 1984a, 2002b). Top blight occurs when susceptible seedlings are kept under very moist conditions and foliage does not readily dry out between irrigations (HANSEN et al. 1990, JAMES 1991a, 2002b). This type of disease can spread very rapidly and must be dealt with aggressively with sanitation and fungicides to preclude extensive losses.

By far, root diseases are the most important diseases of container-grown seedlings caused by *Fusarium*. Although an extremely wide variety of *Fusarium* spp. can be associated with root-diseased seedlings (JAMES et al. 1989), *F. proliferatum* (MATSUSHIMA) NIRENBERG is the most common and probably the most virulent species (JAMES 1997a, JAMES et al. 1997). This species is not commonly seedborne (JAMES 1997a, 1999), nor does it reside commonly either in nursery soil or peat-based growing media (JAMES 1985a, 2005b). Also, *F. proliferatum* does not form long-lived resting structures such as chlamydospores like several other *Fusarium* spp. found in forest nurseries (GERLACH, NIRENBERG 1982, JAMES 1997a, LESLIE 1991, NELSON et al. 1983). However, *F. proliferatum* successfully colonizes organic matter within or adjacent to greenhouses (JAMES 1983, 1984a) and, once established within greenhouses, can spread very rapidly because it produces long chains of microconidia which are readily disseminated via air movements (GERLACH, NIRENBERG 1982, JAMES 1997a, 2005a, NELSON et al. 1983). Seedling infection by this pathogen can occur early in the growth cycle, even though disease symptoms may not initially appear (JAMES 1985e, JAMES et al. 1987). In most cases, the fungus penetrates root epidermis and colonizes cortical cells, often without causing tissue necrosis (JAMES 2004b, JAMES, GILLIGAN 1988a). Disease symptoms often become more noticeable near the end to the growth cycle when seedlings are stress to stop growth (set buds) and harden off (JAMES 2000c, JAMES et al. 1987). At this time, seedling tip dieback and top necrosis becomes evident (JAMES 1987a, 1988c, 2002b, 2005a, JAMES et al. 1988b). Eventually, entire seedlings are killed. In some cases, seedling growth is severely limited by root infection with *F. proliferatum*, even though above-ground disease symptoms are not apparent (DUMROESE et al. 2002). When root disease is severe, losses can be extensive and may result in a large portion of the crop (JAMES 1997a, 2004b, JAMES, GILLIGAN 1984).

Host ranges

Although many different conifer species are affected by *Fusarium* spp. in containers, the most commonly affected species are Douglas-fir, ponderosa pine, western white pine (*Pinus monticola* DOUGL.), western larch (*Larix occidentalis* NUTT.) and lodgepole pine (*Pinus contorta* DOUGL.). All these species respond similarly to infection by *Fusarium*. However, in some cases, root decay of western white and whitebark (*Pinus albicaulis* ENGELM.) pine seedlings can be very extensive without above-ground symptoms becoming evident (JAMES 1985d, 1988a, 1991a, b, 2000b). In such cases, *Cylindrocarpon* spp. (especially *C. destructans* (ZINS.) SCHOLTEN) colonizes roots at high levels along with *Fusarium* spp. (JAMES 1983, 1991a, 1995, 2000b, JAMES, GILLIGAN 1986).

Douglas-fir grown in greenhouses is especially susceptible to *Fusarium*, particularly *F. proliferatum* (JAMES 1985e, 1986b, c, 2005a, JAMES et al. 1986, 1987, 1989, 1995a, 1997). At some nurseries, it is rare if the Douglas-fir crop is not adversely impacted by this pathogen. Unfortunately, by the time disease symptoms become evident, little can be done that effectively reduces losses. Fungicides are usually not effective near the end of the growth cycle (JAMES 1984b, 1988b, JAMES et al. 1991, SHRIMPTON, WILLIAMS 1989), probably because of extensive root colonization by *Fusarium* and limitations in distribution of chemicals in sufficient concentrations throughout root systems growing in containers.

Inoculum sources

As indicated previously, conifer seeds are important sources of *Fusarium* inoculum in container operations (JAMES 1984c, 1986a, b, 1987d, JAMES, GENZ 1982). Level of seed contamination varies widely throughout different seedlots (JAMES 1985c, 1999, 2004b, JAMES et al. 1996) and pathogens can spread extensively during stratification that is usually necessary prior to sowing (JAMES 1987d, 1999). We usually recommend that chemical seed treatments are necessary when level of *Fusarium* contamination exceeds about 10% of tested seeds, even though not all isolates are necessarily potential pathogens.

Another very important inoculum source is reused plastic or Styrofoam™ containers (JAMES 1990, 2001, JAMES, GILLIGAN 1988a, JAMES et al. 1988c). *Fusarium* is often present within or on the inner walls of containers. Inoculum occupies both roots of previous seedling crops and organic matter left after seedling extraction (JAMES 1992b, JAMES, EGGLESTON 1997, JAMES, SEARS 1990). We have found that most inoculum is concentrated near the bottom of container cells (JAMES 1989, 2001, JAMES, GILLIGAN 1988b, JAMES et al. 1988c), probably because conditions are more conducive there for pathogen survival and propagules may naturally move to the bottom of cells as irrigation water drains from the container or other effects of gravity.

Other important pathogen inoculum sources in container nurseries include organic matter residing inside greenhouses, on walls, floors, and benches (JAMES 1984a, 1988c, 2003a, JAMES et al. 1990, 1995b, SUTHERLAND, VAN EERDEN 1980). Some *Fusarium* spp. can probably remain viable for long periods on organic debris within greenhouses. These fungi can also colonize weeds growing within or adjacent to greenhouses (JAMES 1984a, JAMES et al. 1990).

Insects occurring within greenhouses may be important vectors of some fungal pathogens. For example, adult fungus gnats (Diptera: Sciaridae) were shown to carry *Fusarium* and other pathogens such as *Botrytis* (JAMES et al. 1994). It is likely that these fungi are passively carried by insects during their feeding on container seedlings.

We have not evaluated the potential of irrigation water to introduce *Fusarium* propagules into greenhouses. However, it is unlikely that these fungi are commonly spread in irrigation water, particularly when water comes from deep wells.

Peat-based growing media (containing either vermiculite, perlite, or sawdust) from commercial sources are usually not contaminated with *Fusarium* spp. (JAMES 1985b, JAMES et al. 1990).

Although such media are not sterile, in some cases, it is steam treated to reduce chances for pathogen contamination while maintaining populations of desirable organisms such as certain spore-forming bacteria. On rare occasions *Fusarium* contamination of growing media may occur and seriously impact seedling crops (James 2005b, James, Gilligan 1984).

Disease management

Management of *Fusarium* diseases in container seedling crops is most effective through conscientious efforts at prevention. The major goals of disease prevention are to reduce levels of pathogen inoculum within and adjacent to seedling growing environments (James 2004b, James et al. 1988a, 1990) and reduce stress levels on seedlings during production (James 1984b, 1997b, 2004b, James et al. 1990).

Pathogen inoculum can be effectively reduced from seeds, peat-based growing media, reused containers, and greenhouse environments. Seeds may be treated with chemical sterilants to reduce levels of seedcoat contamination by pathogenic fungi (Dumroese et al. 1988, James 1986a, 1987c, James et al. 1996). The most commonly-used sterilants include bleach (aqueous sodium hypochlorite) (Dumroese et al. 1988, James, Genz 1981, James et al. 1995b, 1996, Wenny, Dumroese 1987), hydrogen peroxide (James 1986a, James, Genz 1981), and selected fungicides (James, Genz 1981, James et al. 1996). Major disadvantages of chemical sterilants include potential problems of worker exposure and potential phytotoxic responses by seeds or young germinants (James 1986a, 1988b, James, Genz 1981). These may be overcome by treating seeds with running water rinses (James 1987b, James et al. 1995b, 1996) or hot water (James et al. 1988). Running water rinses are most effective if carried out for at least 48 hrs with treated seeds being periodically agitated (James 1987b, James et al. 1996). It may be important to treat seeds prior to stratification because of the possibility of pathogens spreading throughout seedlots during stratification (James 1999). We have found that placing seeds in standing water for prolonged periods of time prior to sowing is a good way of spreading contaminating pathogens throughout seedlots (James 1999, 2004b, James, Genz 1981).

Plastic and Styrofoam containers are usually reused to grow several crops of seedlings. These reused containers can become contaminated with potential *Fusarium* pathogens that can infect and cause disease on subsequent seedling crops (James, Gilligan 1988b, 1988c, James et al. 1988c). Therefore, growers treat containers to reduce chances of pathogen carryover. High-temperature steam cleaning was previously the major way containers were treated (Peterson 1990), but although most organic matter was removed from steam-treated containers, propagules of potential pathogens often remained (James et al. 1988c). To deal with this, more effective treatments were needed. Chemical sterilants, such as sodium metabisulfite (Dumroese et al. 1993b, Peterson 1990), aqueous bleach solutions (James, Sears 1990, Peterson 1990), and copper solutions (Dumroese et al. 2002) were evaluated. However, problems of worker exposure and disposal of toxic chemicals led to seeking alternative treatment methods that were more innocuous. Immersion in hot water (80 ºC for 30 seconds for styrofoam and 66 ºC for 15 seconds for plastic containers) was found to be effective (James 1992b, James, Eggleston 1997, James, Woollen 1989, James et al. 1990). However, hot water immersion can be expensive due to high energy costs required to maintain high temperatures for prolonged periods. Therefore, other treatments, including radio frequency waves (James, Trent 2001), dry heat (James, Trent 2002), and large-room steam treatment (Trent et al. 2005) were tested and performed comparably with standard hot water immersion.

We have periodically attempted biological control of *Fusarium*-associated diseases. Our choices of test biocontrol agents were limited by those that were commercially available, which were developed from other agricultural cropping systems. As a result, our results were not good as we had hoped. Examples of the organisms we have previously tested included *Streptomyces gris-*

eoviridis L. Anderson (Dumroese et al. 1998), *Gliocladium virens* Miller, Giddens & Foster (Dumroese et al. 1996), *Trichoderma harzianum* Rifai (James 2000a, Mousseaux et al. 1998) and a non-pathogenic strain of *F. oxysporum* (James 2002a). Most of these tests have occurred under tightly controlled laboratory or greenhouse conditions. In general, the tested organisms may have reduced populations of potential *Fusarium* on roots of tested conifer seedlings, but usually not to levels possible with chemical pesticides. Effects on disease levels varied among the tested biocontrol organisms; significant differences between treated and non-treated seedlings were often not found. We suspect that potential biocontrol agents specifically adapted to forest nursery environments would perform more satisfactorily. For example, we believe that specific strains of non-pathogenic *F. oxysporum* obtained from forest nurseries should provide viable biological control of other *Fusarium* spp., particularly pathogenic *F. oxysporum* and *F. proliferatum*.

Outplanting performance

Because of high levels of root colonization by potentially-pathogenic *Fusarium* spp. on seedlings produced in nurseries, forest managers and nursery growers were concerned about possible impacts of this colonization once seedlings were outplanted on forest sites. We have periodically seen high levels of seedling mortality and poor growth of surviving seedlings following outplanting (James 1988a, 1991b). Although *Fusarium* spp. were commonly found on roots of poorly-performing seedlings and could readily be isolated from seedlings during cold storage (James 2003c), their actual role in seedling deterioration was unknown. Therefore, we monitored seedling performance and root colonization by *Fusarium* spp. following outplanting over time (Dumroese et al. 1993a). Our results were similar to previous work (Smith 1967) that showed that *Fusarium* obtained when seedlings are grown in nurseries is gradually replaced by other mycoflora following planting in forest soils. Mortality that might have occurred following planting is usually not related to *Fusarium* on root systems. Environmental conditions, particularly moisture, and animal damage are the most important factors affecting seedling performance following outplanting.

Pathogenicity testing

During the course of our investigations, many different *Fusarium* spp. were isolated from diseased seedlings, containers, and seeds. We expected that some of these species would likely be more virulent on nursery crops than others. In order to test this hypothesis, we developed techniques that could be used to relatively quickly ascertain potential of selected *Fusarium* isolates to elicit disease under both laboratory (James 1996, James et al. 1986, Miles, Wilcoxsin 1984) and greenhouse (James et al. 1989, Miles, Wilcoxsin 1984, Mousseaux et al. 1998) conditions.

In general, we found that isolates of *F. oxysporum* varied widely in their levels of virulence on young, conifer seedlings (James et al. 2000). However, most tested isolates of *F. proliferatum* exhibited capacities to be very aggressive pathogens (James et al. 1995a, 1997). Similar tests found that isolates of *F. sporotrichioides* Sherb., *F. acuminatum* Ell. & Ev., *F. solani* (Mart.) Appel & Wollenw., *F. culmorum* (W. G. Smith) Sacc. and *F. anthophilum* (A. Braun) Wollenw., all of which were isolated from diseased conifer seedlings, had much less virulence potential than either *F. oxysporum* or *F. proliferatum* (James 2000c, 2004a, b, James, Perez 1999, 2000). We concluded that these other *Fusarium* spp. were mostly saprophytes, at least under the conditions of our tests.

MOLECULAR CHARACTERIZATION OF *FUSARIUM OXYSPORUM*

As indicated previously, we have had serious difficulties interpreting the role of many *Fusarium* isolates we find in nurseries in their ability to elicit disease symptoms. Many of the potential pathogens we have studied are within the *F. oxysporum* species complex. This group of organisms,

although appearing morphologically similar, displays wide genetic variability (Gordon, Martyn 1997, Kistler 1997). Some of this genetic variation may be related to pathogenic potential of selected hosts (Gordon, Martyn 1997, James 2004b). Therefore, studies were initiated to quantify genetic population variability and determine if genetic markers were available that could be used to differentiate pathogenic from nonpathogenic isolates of *F. oxysporum* from forest nurseries. Early results of this work indicate that specific genetic markers for pathogenic strains occur (Donaldson et al. 1995, Stewart et al. 2004, 2005b) and it is possible that these may be used to develop molecular probes that could be used for early detection of pathogens in plant tissues and quantification of pathogen populations in nursery soil (Stewart et al. 2005a, b). If such probes can be developed and become available, they will greatly enhance our abilities to predict disease severity before it occurs so that adequate preventative measures can be taken by nursery growers.

CONCLUSIONS

Fusarium spp. are probably the most important group of pathogens causing diseases of seedlings in western North American forest nurseries. Although several different species may be involved, the two most commonly encountered are *F. oxysporum* and *F. proliferatum*. The former is more important as a pathogen of bareroot seedlings, while the latter is most important on container seedlings. Both species are common root endophytes and are capable of becoming pathogenic under certain environmental conditions. *Fusarium* spp. can be introduced into nursery crops via contaminated seeds, container growing media, reused Styrofoam or plastic containers, nursery soil, non-crop plants within or near nurseries, and from dormant propagules occurring on previous seedling crops. The best approach in reducing disease losses due to *Fusarium* spp. is prevention, primarily by reducing potential pathogen inoculum. This can be done by seed, container, growing media, and soil treatments to eliminate or reduce inoculum. In addition, cultural manipulations during crop growth cycles can help reduce seedling stress and abilities of potential pathogens to elicit disease. Biological control shows promise by introducing organisms that compete with or antagonistic toward pathogens. New research using techniques of molecular biology show promise in improving our abilities to detect and manage important *Fusarium* pathogens in forest nurseries.

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THE RISK OF INTRODUCING QUARANTINE PESTS OF IMPORTANCE TO FOREST NURSERIES AND FORESTS IN THE CZECH REPUBLIC

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ABSTRACT

From 1997 - 2005, three, important (to forestry) quarantine diseases *Mycosphaerella pini, M. dearnessii* and *Cryphonectria parasitica* were noted in the Czech Republic. The first report for *M. pini* occurred in 1999 when it was detected on imported plant material. In 2000, it was found again, this time in a Christmas tree plantation. Since then it has become a common pathogen, particularly of European black pine, *Pinus nigra*. *Cryphonectria parasitica* was first detected in 2002 and then again in 2004 and early 2006. Diseases caused by these fungi result in direct economic damage to trees and indirect losses associated with eradication measures and implementation of extraordinary, phytosanitary measures.

Keywords: quarantine pests, phytosanitary service, *Mycosphaerella pini, Mycosphaerella dearnessii*, *Cryphonectria parasitica*

INTRODUCTION

Intensive transport of plant materials necessitates the establishment of administrative barriers to prevent the spread of quarantine diseases of plants. The need is considered to be urgent in agriculture where the spread of a harmful organism is quickly reflected in economic losses. However, the same problems are caused by the spread of new pathogens to forest and ornamental trees. The phytosanitary legislation of EU countries conforms to regulations of the European and Mediterranean Plant Protection Organization (EPPO). Within EPPO, particularly economically important organisms occurring in other geographically separated regions are considered to be quarantine organisms. These organisms either do not occur in EPPO countries or occur only in limited regions with their distribution being regulated by several measures. A law passed in 1996, phytosanitary service no. 147/1996, was the initial step in Czech legislation coming into conformity with European norms. Until then the only quarantine organism considered to be important for trees in the Czech Republic (CR) was fire blight, *Erwinia amylovora.* In 2000, red band needle blight *Mycosphaerella pini* (a quarantine disease) was reported in open plantings for the first time. In 2002, following extensive surveys, the chestnut blight fungus *Cryphonectria parasitica* was detected. In this paper we discuss several quarantine pests important in forest nurseries and forests and the risks of their spread under Central European conditions. Another objective is to present the results of our work to determine the present distribution of quarantine harmful organisms in the CR.

MATERIALS AND METHODS

Starting in 1997 and continuing through to the present (2005) we have been determining the distribution and importance of quarantine harmful pests (thereinafter called QHP) in the CR. Data have been collected on the occurrence and health of the sweet chestnut, *Castanea sativa,* trees in the CR and to determine possible risks from the possible importation of other pathogens for which we have initially relied upon EPPO data.

RESULTS

Regarding pathogens, to date three QHP of potential importance to forestry have been detected in the CR, i. e. the fire blight pathogen *Erwinia amylovora*, the needle blights caused by *Mycosphaerella pini* and *M. dearnessii* and the chestnut blight fungus *Cryphonectria parasitica*.

Bacteria, phytoplasmas

Erwinia amylovora does not represent an important problem from the viewpoint of forestry. It causes local damage to woody vegetation in towns, e. g. to hawthorn *Crataegus* spp. In forest nurseries, systematic phytosanitary inspection is used to minimize losses. The causal agent of the elm phloem necroses Phytoplasma is known only from North America and the risk of its introduction to the CR appears to be minimal provided phytosanitary measures are followed.

Fungi

- Dothistroma needle blight *Mycosphaerella pini* E. Rostrup (syn. *Scirrhia pini* Funk & Parker, *Eruptio pini* (Rostr. apud Munk) M. E. Barr, anam. *Dothistroma septospora* (Dorog.) Morelet, *Dothistroma pini* Hulbary, *Cytosporina septospora* G. Doroguine., *Actinothyrium marginatum* Sacc.)

In Europe, Dothistroma needle blight, caused by *M. pini,* occurs particularly in countries south of the CR, e. g. Croatia, Italy, France, Hungary, Romania, Spain, and Switzerland. In the CR, the disease was first detected in 1999 on black pine, *Pinus nigra* Arnold, imported from Hungary. Subsequently it was found in a *Pinus nigra* Christmas tree plantation near the village of Jedovnice, about 30 km north of Brno in May, 2000 (Jankovský, Šindelková, Palovčíková 2000). At that time, it was already known from several European countries including Austria (Petrak 1961), Slovenia (Macek 1975), Germany (Butin, Richter 1983), Poland (Kowalski, Jankowiak 1998) where it was found in May 1990, Slovakia (Kunca, Foffová 2000) and Hungary (Koltay 1997).

Over recent years, Dothistroma needle blight has become one of the most important diseases of *Pinus nigra* and some other pine species in the CR. It is particularly prevalent in southern and central Moravia and Silesia. In Bohemia, the greatest incidence has been noted in eastern and central Bohemia. Conversely, it has been found only sporadic in southern Bohemia, but it has not been found in northern and western Bohemia.

In the CR, Dothistroma needle blight has been detected on 13 species of pine and three species of spruce (Bednářová et al. 2006). *Pinus nigra* and *P. mugo* Turra are the most common hosts. The disease is a problem in Christmas tree plantations, nurseries and on ornamentals. The most severe damage was observed on afforested spoil bank areas, agricultural and other non-forest land. The State Phytosanitary Administration deals with Dothistroma needle blight in forest nurseries. After disposing of infected seedlings and carrying out other legislative directives, pine seedlings cannot be sold from affected nurseries until the end of the following growing season. At present, Dothistroma needle blight is being considered a ‘domesticated’ species where the use of

phytosanitary measures has only a small effect on its spread. Research is aimed at methods of early and exact diagnostics and the effectiveness of preventive measures.

- *Mycosphaerella dearnessii* M. E. Barr, (syn.: *Scirrhia acicola* (Dearness) Siggers, anam. *Lecanostica acicola*)

In the CR this fungus was first found in 2000 on imported *Pinus nigra* from Hungary. The fungus was also detected on samples of *Picea omorica* from the same nursery. Presently, other possible occurrences are being investigated.

- *Cryphonectria parasitica* (Murrill) M. E. Barr (syn. *Endothia parasitica* (Murrill) P. J. Anderson et H. W. Anderson)

Since 2001, the health of *Castanea sativa* trees has been increasingly monitored throughout the CR (Haltofová, Jankovský 2003a, b). The main objective is to determine the potential spread of the chestnut blight fungus *Cryphonectria parasitica*. In the CR, chestnut blight fungus was first noted in Uherský Brod in 2002 (Jankovský et al. 2002). The infected sweet chestnut tree had been imported as a 2-year old plant from Bratislava, Slovakia, in 1976. The tree is now about 30 years old. At present the disease is confined to isolated, individual trees and the possibility of disease spread to other trees is minimal. In May, 2004, the chestnut blight fungus was found in Kuřim (Brno – province) on a tree planted in 1975. The disease was confined to a single, sweet chestnut tree. Another infected, sweet chestnut was detected in the same locality in January 2006, and at the same time also near Tišnov, about 15 km NW of this locality.

In June 2004, the occurrence of *C. parasitica* was noted on sweet chestnut trees in shelter belts in a nursery at Moravský Písek (district of Hodonín). Futher studies confirmed that the disease was also present on red oak *Quercus rubra* L. (Haltofová et al. 2004). The presence of many sweet chestnut trees in this locality distinguishes this incidence from the other two localities where the fungus was found just on individual, isolated trees. The chestnut trees originated from left over fruits imported in the early 1970s. In these instances the origin of chestnut blight fungus is not clear. Based on a study of Vegetative Compatibility Groups (VCG), different VCGs were detected in all localities. Thus, the origin of infection is not the same (Haltofová 2006). At Uherský Brod, VCG EU-13 was detected while at Kuřim VCG EU-1 was found, but it was not compatible with the first strain. The VCG of the last isolate is now being determined. At Moravský Písek, 20 virulent cultures of *C. parasitica* were isolated. Laboratory detected tests only one vegetative-compatible strain of EU-15. No hypovirulent cultures of *C. parasitica* have been confirmed from any of the localities. No pathogenicity tests have been done with the *C. parasitica* isolates. The occurrence of seed-borne, *C. parasitica* as a potential source of the disease spread has not been determined. As directed by State Phytosanitary Administration measures, all affected sweet chestnut trees have been removed and State Directive Phytosanitary Measures have been applied to nearby oaks.

Other fungus-caused diseases

At present, there is little risk of importing most of the other quarantine species of fungi to the CR. Within protected zones in Ireland and in the UK, *Gremenniella abietina* is also considered to be a quarantine organism on species of *Abies*, *Larix*, *Picea*, *Pinus* and *Pseudotsuga*. This fungus is autochthonous in the CR as it occurs countrywide on pines and spruces. However, distribution of the fungus in forest nurseries is not known and its occurrence is not being monitored.

Cancer stain, *Ceratocystis fimbriata* Ellis & Halsted f. sp. *platani* Walter, could be imported on sycamore, *Platanus* spp., saplings from southern Europe. One of factors preventing the spread of the disease is the minimum amount of pruning done on such trees in the CR. However, an increase in pruning of newly-planted *Platanus* could increase the risks of spreading the disease.

A potentially important problem could arise as the result of importing the causal agent of oak wilt, *Ceratocystis fagacearum* (Bretz) Hunt, from North America. Its importation or the importation of its vectors (e. g. *Pseudopityophthorus minutissimus* and *Pseudopityophthorus pruinosus*) could have a dramatic effect on oaks.

The leaf rust fungus *Melampsora medusae* Thümen (syn. *Melampsora albertensis* J. C. Arth.), could be imported into the CR; however, there is the problem of differentiating it from domestic species of rust fungi. The situation concerning other rust fungus is less clear as the importation of non-European species of rust fungi such as those in the genera *Endocronartium*, *Chrysomyxa*, *Gymnosporangium*, and *Cronartium* would appear to be of minor concern.

Semiparasitic plants

The importation of dwarf mistletoe, *Arceuthobium* spp. is considered to be of minimal concern at present. The only European dwarf mistletoe, *A. oxycedrii,* could be also imported into the CR on host plants; however, an unfavourable climate and the low density of potential hosts would certainly curtail the spread.

Insect pests

Although many insects are of concern for possible importation, only a few are considered important for forestry. The longhorn beetle, *Anoplophora glabripennis* (Motschulsky), coming from China and the Korean peninsula (Beránek 2005), is presently of concern. In 2001 this insect was detected and eventually eradicated near Brannou at the German-Austrian border, about 90 km from the Czech border. Subsequently, until 2003, some 113 occurrences were found in that locality. Too, this insect has been found at Gien, near Orleans, in France, and in Poland. This insect could be imported with wood from Asia, particularly in untreated wooden pallets and packaging. Special attention needs to be paid to localities neighbouring docks receiving goods from eastern Asia as the risk of importation is very important. In 2001, the first occurrence was noted of a related species, *Anoplophora chinensis* (Forster), in Lombardy. In its homelands, i. e. China and Korea, it is an important pest of citrus; however, this polyphagous longhorn beetle has more than 68 host species. At present the situation is being monitored, especially the possibility of its being imported on wood.

An example of a new insect risk is *Cameraria ohridella* Deschka et Dimić, first described in 1989. This pest quickly spread throughout Central Europe. As early as 1993, it was detected in southern Moravia and then it spread throughout the CR. The spread of this mining insect was so fast that the Czech Phytosanitary Service was not able to respond to it by the change of regulations.

Nematodes

Regarding woody plants and specifically pines, the most potentially important nematode is *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nikle, the cause of the devastating pine wilt disease. It affects several pine species. The nematode is transmitted by several species of longhorn beetles (*Monochamus* spp.). It is assumed that the nematode is native to North America from where it was brought to southern Japan, including the Kyushu Islands, at the beginning of the 20th century. From there, it spread further. Presently, occurrence of this nematode is clouded by the fact that a similar, indigenous nematode, *B. mucronatus,* occurs in most territories of Europe. However, recently both the nematode and severe pine wilt disease have been found in Portugal.

CONCLUSION

From 1997 - 2005, three quarantine diseases, viz *Mycosphaerella pini, M. dearrnessii* and *Cryphonectria parasitica* important from the viewpoint of forestry were found in the CR. These pathogens caused important direct economic losses by damaging trees and indirect losses resulting from the measures implemented to eradicate these diseases. Ever growing international trade results in a corresponding increase in the risk of importing other harmful pests. It is imperative that we apply strict phytosanitary legislation to prevent the importation of such organisms.

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OAK POWDERY MILDEW (*MICROSPHAERA ALPHITOIDES*): BIOLOGY, EPIDEMIOLOGY AND POTENTIAL CONTROL IN EUROPE

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ABSTRACT

Oak powdery mildew is caused by the ascomycetous fungus *Microsphaera (= Erysiphe) alphitoides*. The pathogen affects the development of seedlings and young oak trees and decreases their physiological and commercial quality. We compared the morphological features of ascocarps and species diversity of oak powdery mildew collected in France (Pyrenees, Bordeaux, and Nancy), Scotland (Aberdeen), Austria (Vienna) and Northern and the southern Czech Republic. Occurrence of *Phyllactinia* sp. was noted in the Pyrenees populations whereas only *M. alphitoides* occurred in the remaining samples. This study reports preliminary results of a comparison between a few European populations of *M. alphitoides* based on cleistothecia morphology, overwintering of sexual and asexual structures and occurrence of associated species (other Erysiphales and hyperparasites). French samples frequently contained natural mycoparasites such as *Ampelomyces quisqualis* and *Phoma glomerata.* Ascocarps of *M. alphitoides* varied in size according to geographic origin. Cleistothecia collected in the Pyrenees were significantly smaller compare to other collections. The experiments with overwintering cleistothecia showed that only 1.4 % of the Pyrenees population survived the winter of 2004/2005 in South Bohemia whereas 7 - 8 % of the population from Southern Czech Republic survived the 2004/2005 winter. All overwintered samples released ascospores and resulted in infection of emerging leaves during April, 2005. Samples from northern localities (Aberdeen, Scotland and northern Czech Republic) caused intensive infection when compare to other isolates.

Control of pathogen is based on repeated sulphur treatment during the growing season in most of European nurseries. Ideally, control should be based on integrated management that combines technology and biological control. The combination of mycoparasitic fungi and Natural Neem Oil was found to be as good as sulphur treatment.

Key words: oak powdery mildew, *Microsphaera alphitoides*, epidemic, control

INTRODUCTION

Oak powdery mildew, caused by *Microsphaera alphitoides* Griff. & Maubl. (Erysiphales: Ascomycota) is one of the major diseases that occurs on the two main oak species in Europe, *Quercus petraea* and *Q. robur*. Oak powdery mildew is the generic name of the diseases caused

by the erysiphaceous fungi *Microsphaera alphitoides* (BUTIN 1995), *Microsphaera hyphophylla* (NEVODOVSKIJ 1952) and *Phyllactinia guttata* (BUTIN 1995), but *M. alphitoides* prevails in the present. The origin of the pathogen and its spread from South Europe to Scandinavia via Central and northern Europe during the 20th century remains unclear in spite of several existing theories. The biology and epidemiology of the causative agents, especially *Microsphaera alphitoides*, lack details that are crucial for epidemiological studies and potential control. Generally, infection on oaks starts in April when the first leaves emerge from buds. The source of primary inoculum can be ascospores released from overwintered ascocarps (cleistothecia) or mycelia overwintered in the buds. Asexual propagules, conidia, multiply and spread infection during the growing season. The ascocarps are produced in August - September. *Microsphaera alphitoides* is a biothrophic pathogen that parasites on the leaves, stems and leaf petioles of *Q. petraea* and *Q. robur* and produces parasitic structures (appressoria and haustoria) to obtain nutrients from plant cells. Damage differs according to tree age, season and ecological factors. The most severe damage is observed on young seedlings in nurseries because of host density and the microclimate in seedbeds. The leaves infected by the fungus are covered with a white mass of mycelia and conidia. They can show discoloration and then necrosis. The fungus induces irregular shoot and leaf production during the growing season. Such shoots are thin, pale green, with deformed leaves and they show reduce cold hardiness. As well, irregular branching and unbalanced growth of seedlings plus poor site adaptability after outplanting result from powdery mildew attacks. Control of oak powdery mildew in forest nurseries is limited because of the potential impact of fungicides on the forest environment. Only sulphur fungicides are legally authorized for use against oak powdery mildew in Czech nurseries. The use of systemic fungicides such as strobilurins is risky because of the development of fungicide-resistant strains.

In this paper, we report some differences between populations of oak powdery mildew collected across Europe, based on morphological characteristics and their ability to overwinter. We were interested in how many cleistothecia survived the 2004/2005 winter in the Czech Republic and if the overwintered cleistothecia released ascospores and caused infection in the spring. Biological control based on application of a mycoparasitic fungus and Neem oil was tested in nurseries in the Czech Republic.

MATERIALS AND METHODS

Microsphaera alphitoides collections and morphological study

Oak powdery mildew was collected in: France – Pyrenees (St. Giron), Bordeaux (south-west France), Nancy (north France), Scotland – Aberdeen, Austria – Vienna, and northern (Jizerské hory – Hutě) and southern (České Budějovice, Šumava) Czech Republic. Teleomorphic stage specimens of oak powdery mildew were gathered when cleistothecia occurred in the brown or brownish black stage of development in October 2004. Three hundred cleistothecia from each site were examined using light microscopy to determine species and size of cleistothecia using a binocular (Olympus SZX 7) and light microscope (Olympus BX41). The developmental stage (open vs. close), presence of ascospores and occurrence of natural mycoparasites in populations were noted.

Fungus survival and spring infection

Cleistothecia were brushed onto filter paper and then transferred in mesh bags (mesh size 80 μm). Ten bags with 1,000 cleistothecia were prepared for each population. The bags were hung on branches of young trees in nature to be exposed to winter conditions in the Czech Republic. Five bags per population each containing 100 cleistothecia from selected sites were hung out in the autumn on the terminal shoot of 3-year old oak saplings growing in pots. Five oak plants

were used per population including three replicates (7,500 cleistothecia per population were tested on 15 plants). Whole plants were covered by bonded fabrics and exposed to natural conditions. The occurrence of natural infection from ascospores was observed within 4 weeks from the beginning of April. Control plants were inoculum free and were covered by unwoven fabrics (C1) over winter period and the plants exposed to natural infection (C2). Three control bags per population were collected at the beginning of April and examined microscopically to determine the status of the cleistothecia. The effect of overwintered inoculum was excluded by treatment with SYSTHANE 12 EC (systemic fungicide) applied in October 2005. We noted if cleistothecia were closed with developed ascospores, open without ascospores, or damaged by mites or microfungi.

Control experiments

Trials combining preventive and curative controls were done in forest nurseries and in experimental field plots at the University of South Bohemia. The following biochemicals and fungicides were tested: Natural Neem Oil® (Bio-Logics) (0.05%) – neem oil, BION 50 WG ® (NovArtis) (0.06%), SULIKOL K® (SpolChema, CZ) (0.5%) – sulphur treatment, SYSTHANE 12 EC® (NOVARTIS) (0.06%) – systemic fungicide. *Verticillium lecannii*, *Paecilomyces fumosoroseus* and *Trichoderma harzianum* were applied as mycoparasitic fungi alone and combined with Neem Oil. Mycoparasitic fungi were obtained from the collection of the Biology Faculty in České Budějovice. Isolates originated from natural infections occurring in colonies of powdery mildew and rust from collections made from 2002 – 2004. The fungi were cultivated in vitro in petri dishes with PDA agar (Sigma, Aldrich) to obtain sporulating colonies. A suspension of conidia ($1*10^7$ in 1 ml of suspension) in sterile water with 0.01% Tween 20 added was used for the treatment. The concentration of conidial suspension was determined using an Improved Neubauer haemocytometer. The final concentration was adjusted before use.

The experimental plots were established in nurseries. Curative treatments started when the first symptoms occurred. The patches with seedlings were subdivided into 2 meters long experimental plots separated by control plots (2 meters long). Each treatment was replicated in four plots randomly located in each nursery. The treatments were repeated monthly. The last treatment was applied in September 2004.

RESULTS

The population of oak powdery mildew collected in the Pyrenees produced smaller cleistothecia (about 95 μm in diameter) than all other populations, the difference being significantly different from the populations from Cestas, Aberdeen and northern Czech Republic. Cleistothecia from Nancy (north France), Aberdeen (Scotland) and southern Czech Republic were the largest, being approximately 95 μm in diameter. Most populations contained only one type of cleistothecia, presumably those of *Microsphaera alphitoides*. Leaf samples from the Pyrenees were infected by at least two species of erysiphaceous fungi, i. e. *Microsphaera* sp., presumably *alphitoides* and *Phyllactinia* sp., presumably *roboris*. *Phyllactinia roboris* also occurred in samples from northern France (Nancy) but sporadically. Based on microscopic observations, 1.6% of the *Phyllactinia roboris* ascocarps occurred per 1,000 samples of cleistothecia in Pyrenees population whereas only 0.06% of *Phyllactinia* ascocarps were found in samples from Nancy (T test, $t = 3.98$, $p \leq 0.003$).

Samples from Fontainebleau, the Pyrenees and the Bordeaux area contained the mycoparasitic fungi *Ampelomyces quisqualis* and *Phoma glomerata* associated with the cleistothecia whereas samples from Czech Republic, Austria and Scotland did not. Cleistothecia from northern France (Nancy) were highly infested with the microfungi *A. quisqualis, P. glomerata,* and *Cladosporium herbarum*. Most of the ascocarps were destroyed and only the larger cleistothecia survived (Figure 1).

The results from the winter studies showed that only a very small percentage of cleistothecia overwintered without damage or destruction. Severe damage occurred from naturally occurring mycoparasitic fungi. Although on average less than 10% of the cleistothecia survived, significant differences were found between test populations. The Pyrenees population overwintered very poorly as did ascocarps from Fontainebleau, Bordeaux and Nancy (none were significantly different from one another). Significant differences were found among the populations from the Pyrenees and Cestas, Aberdeen (Scotland) and populations from the Czech Republic and Austria (Figure 2).

Figure 3 shows the course of infection in April 2005, obtained for the samples of cleistothecia hung above oak saplings. Control plants marked as C1 were covered by unwoven fabrics and no infection occurred during the experimental period. The second set of control plants was naturally exposed to infection in the experimental plots where disease occurrence was very low.

The overwintered cleistothecia (Figure 3) from northern Czech Republic produced ascospores that caused the most extensive infection of oak plants by the end of April 2005. The amount of infection was significantly different from all other test populations and the control. Cleistothecia obtained from the Pyrenees infected oak seedlings to the same extent as populations from Cestas

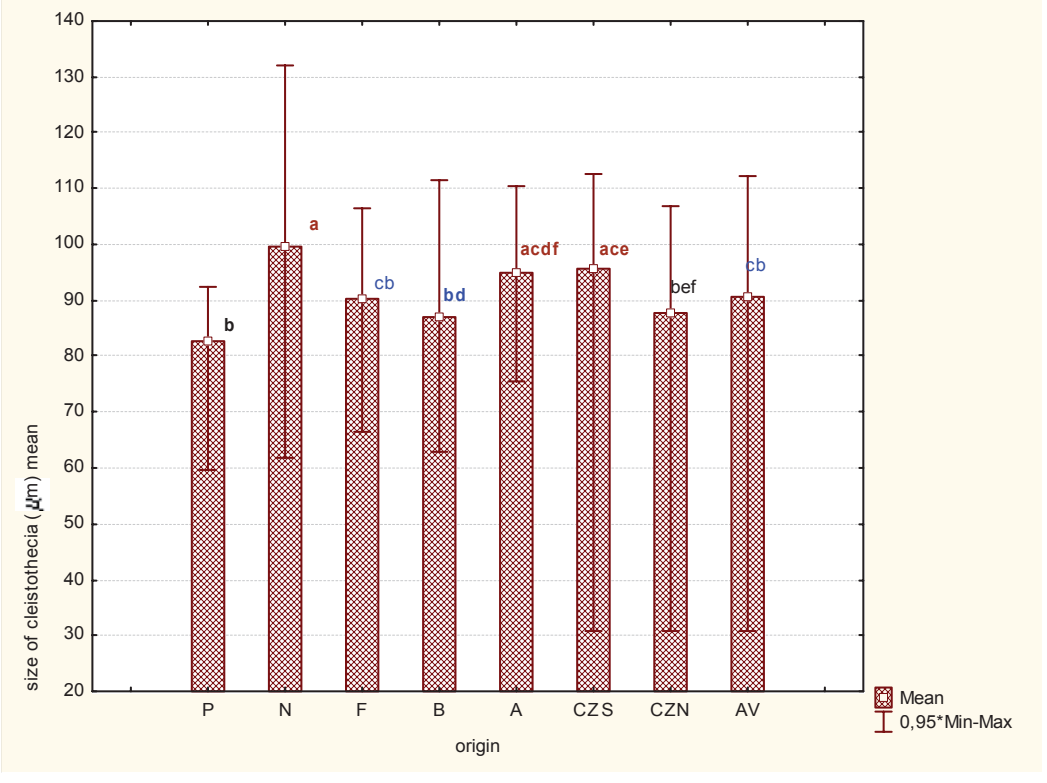

Fig. 1.
Morphological characteristics of cleistothecia (diameter of ascocarp)
Index values above bars indicate the homogenous groups among test variants.
ANOVA (Statistic Software, 7.0 version), $F_{(1,7)} = 17232.65$, $p \leq 0.05$, PostHoc Tukeys Test

P - Pyrenees (Geronde, south-west France)
N - Nancy (north-west France)
F - Fontainebleau (central France)
B - Bordeaux (south-west France)
A - Aberdeen (UK)
CZS - Czech Republic - south
CZN - Czech Republic - north
AV - Austria (Vienna)

and Fontainebleau. Samples from northern France produced a similar amount of infection as populations from Aberdeen and south Bohemia.

The fungicides treatments in the three forest nurseries during the 2004 season showed the effect of the systemic fungicides Systhane and Discus compared to the control. The treatment with a suspension of Neem oil combined with the mycoparasitic fungus *Paecilomyces fumosoroseus* was nearly as effective as the chemicals. Plants treated with sulphur (Sulikol) were more diseased than those treated with the systemic fungicides. The effect of sulphur was comparable with the effect of Neem Oil and with mycoparasitic fungi alone, i. e. *Trichoderma viridae* and *Paecilomyces fumosoroseus*. BION and *Verticillium lecanii* were the least effective.

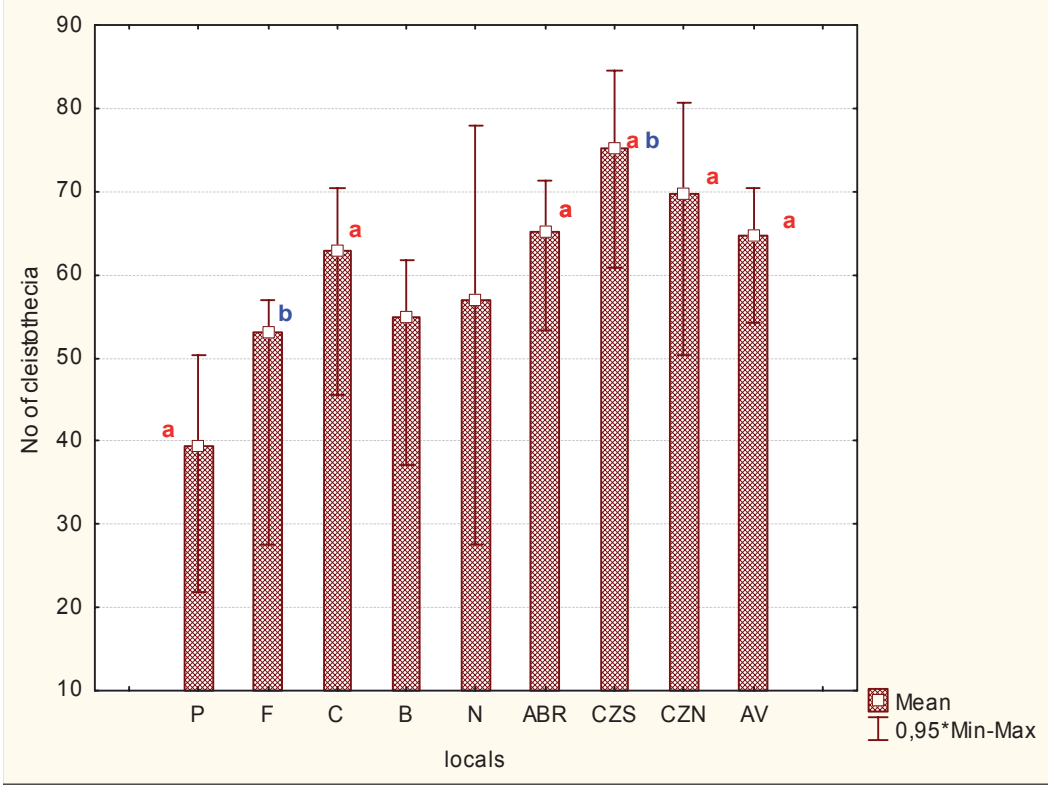

Fig. 2.
Average number of overwintered cleistothecia in the Czech Republic, inter 2004 - 2005. Statistica Software 7.0 Version, ANOVA, PostHoc Tukeys HSD Test $F_{(8,145)} = 5.34$ $p \leq 0.0001$
Index letters mark significantly different groups (non-homogenous group)

P - Pyrenee
C - Cestas
F - Fontainebleau
B - Bordeaux
N - Nancy
A - Aberdeen (UK)
CZS - Czech Republic -south
CZN - Czech Republic - north
AV - Austria (Vienna)

DISCUSSION

The existing literature considers oak powdery mildew as a disease caused by *Microshaera alphitoides* or *Microsphaera hypophylla*. Some references note the occurrence of *Phyllactinia roboris* as the original pathogen in Central Europe and that it was replaced by *Microsphaera alphitoides* at the beginning of last century (Nevodovskij 1950, Černý 1972, Jančařík 1992). One of the theories about introduction of *Microsphaera alphitoides* into Europe suggests that it came from Portugal to southern Europe (Neff, Perrin 1999, Johansson 2001, Butin 1999). Morphological observations of samples collected across Europe demonstrated that *Phyllactinia roboris* does not occur in samples from the Czech Republic, Austria or Scotland. Based on morphological features we found that it was only present in samples from forest nurseries. The samples from France showed species diversity in southern localities close to the Pyrenees. Morphologically the features of the Pyrenees isolates such as appendages and the number of asci correspond to those for *Microsphaera alphitoides*, but the size of the cleistothecia appeared to be smaller (60 - 92 µm) compared to cleistothecia from Cestas (62 - 132 µm). Ascocarps from Cestas had the largest diameter compared with other samples from France. Similar values were noted for cleistothecia from Scotland and south Bohemia. Brown (1987) in his monograph described the variation in the diameter of cleistothecia from 70 to 180 µm. Variability in diameter might

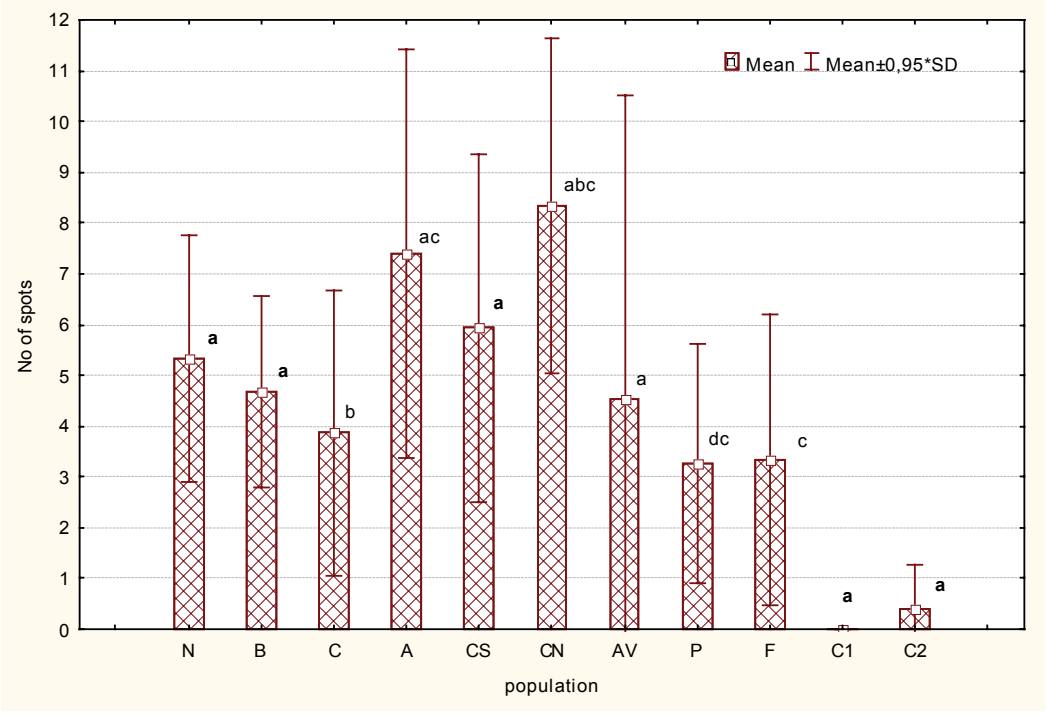

Fig. 3.
Infection from ascospores by the fourth week in April 2005
Ascocarps overwintered on experimental seedlings. MANOVA Statistica Software,
Version 7.0: $F_{(10,298)} = 9.1$, $p \leq 0.005$. Index letters marks significant differences between populations.

P - Pyrenee
N - Nancy
CZN - Czech Republic - Northern part
C - Cestas
A - Aberdeen (UK)
AV - Austria (Vienna)
F - Fountainbleau
B - Bordeaux
CZS - Czech Republic - Southern part

be related to the host plant (*Q. robur* or *Q. petraea*) (BROWN 1985). Competitive microflora in the phylloplane may also affect maturation and development of ascocarps (KAVKOVÁ 2003). Occurrence of *Phyllactinia roboris* was frequently noted on leaves from the Pyrenees and from northern areas in France, but rarely, whereas Czech, Scottish, and Austrian samples contained only *Microsphaera alphitoides*.

Overwintering trials showed the ability of the fungus to survive and release ascospores under different environmental conditions. The preceding references mention that the main source of initial spring infection is from mycelia that overwinter in buds and that ascospores play little or no role in disease epidemiology. Although the survivability of cleistothecia in nature is very low compared to the large numbers produced on leaves in the autumn we conclude that domestic isolates overwintered more successfully than introduced isolates. Cleistothecia from Scotland, Austria and the Czech Republic had a survival rate of 6.5 to 9% whereas only about 1.4 % of cleistothecia from the Pyrenees survived.

The number of lesions caused by ascospore release also showed that domestic isolates can infect seedlings in the second half of April depending upon temperature and rain fall. Recent studies showed that infection from ascospores can start in April when the average temperature is about 20 – 23 °C and rainy (KAVKOVÁ 2004). Unfortunately April 2004, was very cold in the Czech Republic; however, there was a good release of ascospores. Based on data from the life

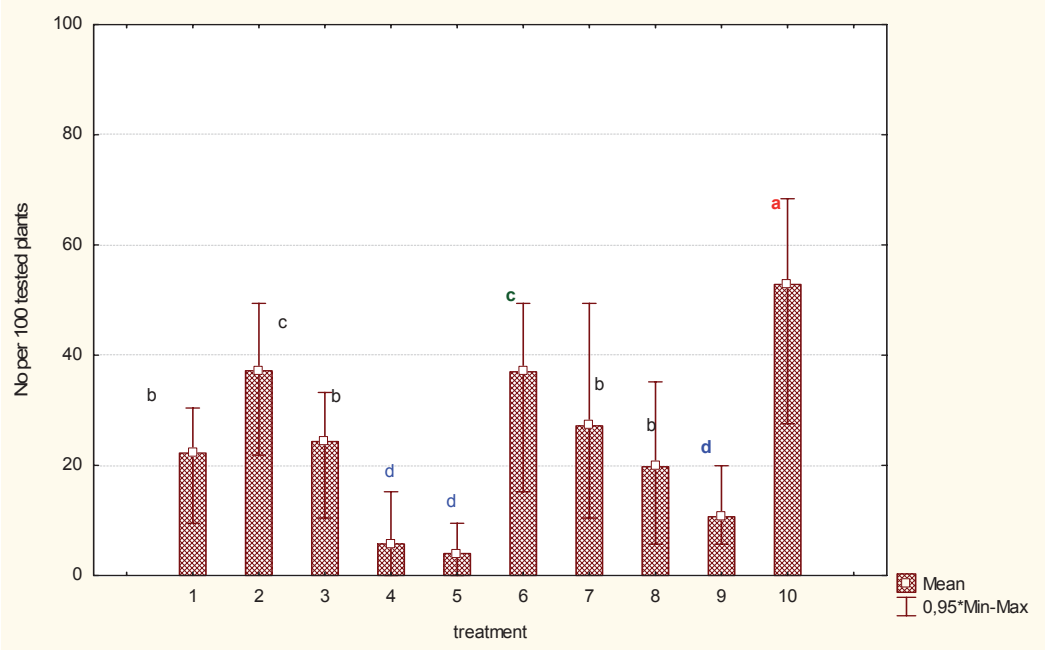

Fig. 4.
Final effect of the treatment in nurseries during the 2004 growing season. The values are the numbers of affected plants by treatment at the end of the 2004 growing season.
Index letters indicate significantly grouped samples (homogenous group).

1 - Natural Neem Oil
2 - BION
3 - Sulikol
4 - Systhane
5 - DISCUS
6 - *Verticillium lecanii*
7 - *Trichoderma viridae*
8 - *P. fumosoroseus*
9 - *P. fumosoroseus* + Natural Neem Oil
10 - Control

cycle of grape powdery mildew we (Jailloux et al. 1999) concluded that synchronism between ascospore release and oak phenology can occur.

Cross sections of buds failed to reveal the presence of mycelia in buds (Kavková 2004), but we cannot confirm the relation to *Microsphaera alphitoides*. Timing of initial infection in the spring depends upon weather conditions then and winter conditions. Rain plays an important role because of dehiscence of cleistothecia and ascospore release (Jailloux et al. 1999). Studies are needed to explain the relation between populations according to their origin, e. g. altitude, average temperature, and humidity and environmental factors. Also phenology of the oak host is crucial to understanding the life cycle and epidemiology of oak powdery mildew.

Control of oak powdery mildew in forest nurseries is restricted by environmental regulations. In the Czech Republic sulphur is the only recommended treatment for its control. Use of systemic fungicides for disease control, while effective, run the risk of increased resistance of strobilurins used against rust and powdery mildews on cereals (McGrath, Shishkoff 2003, Hollomon et al. 1999, Wilcox et al. 2003).

Treatment with Natural neem oil and Neem oil combined with mycoparasitic fungi shows promise for use in forest nurseries. Treatment with the combination of Neem oil and fungi was as effective as systemic fungicides. One disadvantage of such treatments is that the production of the fungi is time consuming and expensive, a common problem with biological control agents. Neem oil is also used against mites, aphids and other insects (Landa, Bohatá 1999, Bruce et al. 2004). Our knowledge of biocontrol agents for control of powdery mildew has increased tremendously in the last 20 years. Over 25 biological products, including mycoparasitic fungi and bacteria, have been incorporated into integrated control programs (Bélanger, Labbé 2002).

Fig. 5.
Symptoms caused by oak powdery mildew: necroses and young shoot induction in late summer 2004

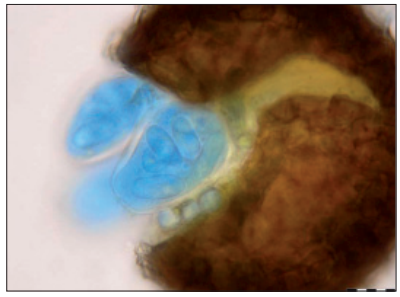

Fig. 6.
Dehiscent cleistothecium of *Microsphaera alphitoides* containing ascus with ascospores; bar = 20 µm

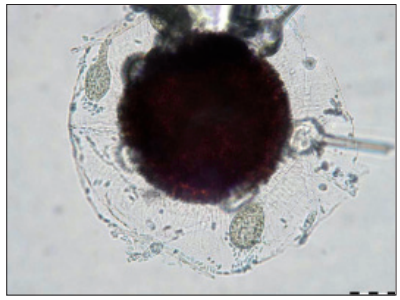

Fig. 7.
Cleistothecium of *Phylactinia* sp. colonized with *Ampelomyces quisqualis;* bar = 20 µm

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EFFECT OF INOCULATION OF OAK SEEDLINGS WITH *PAXILLUS INVOLUTUS* (BATCH.) AND FR. AND *LACCARIA LACCATA* (SCOP. EX FR.) CKE

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ABSTRACT

The overall vigour, morphological features and stress resistance of oak seedlings improved following inoculation with the ectomycorrhizal fungi (ECM) *Paxillus involutus* and *Laccaria laccata* in oak forest soil, a peat and perlite mix, and sterile peat perlite. Qualitative assessments were made using both molecular characterization and morphotyping. Morphological features fully require a literature description, but RFLP analyses showed differences in the *L. laccata* complex when fruit bodies, ectomycorrhizae and cultured mycelia were compared whereas the *P. involutus* complex of fruit bodies, mycelia and ectomycorrhizae showed no such complex. The morphological parameters of seedling root collar size, leaf area, height, fresh and dry weight were used to compare the seedlings on the end of growing season. Seedlings grown from acorns generally achieved parameters of 3-year old seedlings. The measurements of the various parameters of inoculated seedlings significantly exceeded those of control plants. The aggressiveness of inoculum was evaluated by using the PT and LL indices required for each soil type. Although the frequency of *L. laccata* ECM tips per 1 cm of root length was significantly higher than the frequency of *P. involutus* tips, inoculum of *L. laccata* showed less ability to colonized seedling roots in non-sterile soils.

Key words: ectomycorrhiza, inoculum, *Paxillus involutus*, *Laccaria laccata*

INTRODUCTION

Sessile oak (*Quercus petraea)* and pedunculate oak (*Q. robur*) are the most common broadleaf species grown in nurseries in the Czech Republic. The seedlings are grown in seedbeds according to nursery conditions. Most nurseries produce bareroot seedlings as 2 or 3 years old seedlings providing their morphological parameters meet state requirements (see materials and methods). Based on the growing technology, the root system is pruned to encourage root development during the growing season. Generally, the nurseries are established in forest zones and ectomycorrhizal fungi are widespread in the soil. Consequently, a mycorrhizal association is established on oak seedlings during the first year after planting. Establishment of fungus root contact depends on growing methods, use of fertilizers and manures and pesticide usage.

Ectomycorrhizae require the intimate association of plant roots with specialized fungi. Seedlings of forest trees depend upon ectomycorrhizal fungi for adequate nutrient uptake and resistance to fungal pathogens. Ectomycorrhizal fungi also produce plant hormones that stimulate root branching and elongation, thereby increasing root absorptive capacity.

The species distribution of ectomycorrhizal fungi naturally occurring in nurseries varies from that of natural forest for several reasons. The soil management and pesticide usage mentioned above is one of the important limiting factors in nurseries. The succession of ECM fungi occurs naturally according to the R-K strategy theory. Some ECM fungi, such as the ascomycetous *Coenococcum geophilum* is widespread and colonizes roots of tree seedlings in the early stage of development and is well known also for its drought resistance. The ECM fungi colonizing roots of seedlings are different from maternal trees relevant to age, soil type and ecosystem characteristics (O´Dell et al. 1996).

Paxillus involutus is a widespread basidiomycetous fungus (Boletales) forming ectomycorrhizae with oak, beech, spruce. Mycorrhizae formed by this fungus can be easily identified by their outer appearance and sclerotia formation (Agerer 1997). The rapid fine root colonization with *Laccaria* and *Paxillus* sp. and their easy cultivation have resulted in the use of both genera for inoculation (Garbaye, Churin 1996, Herrmann et al. 1992). Suitability of *P. involutus* as inoculum for bareroot seedlings of oak and beech was tested and recommended by Marx et al. (1991), Garbaye, Churin (1997), Kottke, Honig (1998). *Laccaria laccata* is a common basidiomycetous species (Agaricales) occurring in broadleaf, conifer and mixed forests. Utilisation of *L. laccata* as inoculum was tested on spruce seedlings and Douglas fir (Le Tacon et al. 1992, Villenueve 1991, Battista et al. 2002.). The direct effect of *L. laccata* on oak seedlings has not yet been tested. Good growth in culture and performance of vegetative inoculum, ecological adaptation, competitive ability, host range and ability to improve seedling performance in plantations are the main criteria for selection of inoculum.

The description of ectomycorrhizal tips is based on morphological and anatomical features of the ectomycorrhizae (Agerer 1995) and can be performed rapidly, however, the technique has its limitations since the morphology of a particular fungal talon can change with hosts and environments or more than one fungus species can establish ectomycorrhizae (Wurzburger et al. 2001). Molecular methods such as PCR -RITS RFLP can give precise identification of inoculum (Battista et al. 2002, Wurzburger et al. 2001). Inoculation index and the number of ectomycorrhizal tips formed on feeder roots are important criteria for quantitative evaluation of mycorrhization (Marx et al. 1995, Battista et al. 2002). Growth and development of seedlings including height, root collar diameter, fresh weight and other morphological features indicates the influence of introduced ectomycorrhizae (Parladé et al. 2004, Warburton-Egerton et al. 2001, Khasa et al. 2001).

The purpose of the present work was to evaluate and compare different fungus inoculum applied in three types of substrate and to determine their effects on the quality of oak seedlings.

MATERIALS AND METHODS

Plant material

The acorns were stratified in sand over winter and sown at the early spring in pots with various substrates. Twenty acorns were sown in each pot containing 11L of peat-perlite mix (1 : 0.5); twenty acorns in forest soil obtained from oak forest and twenty acorns into sterile peat-perlite (1 : 0.5).

Fungus inoculum

The isolate QR5 obtained from fruit bodies of *P. involutus* and QR2 *L. laccata* collected in an oak forest were maintained on modified Melin–Nokrans medium at 5 °C in the collection of ECM fungi deposited in the Department of Botany, Faculty of Biology, University of South Bohemia. The fungi were grown on Hagem medium (Honig et al. 2000). The nursery inoculum was prepared on Perlite/peat/Hagem medium in 2L Erlenmayer flasks and grown for 2 months at 22 °C

in the dark. The inoculum of *L. laccata* and *P. involutus* was mixed into the top layer of substrate at the time of sowing at approximately 1 $L.m^{-2}$.

Design of the field experiment

Ten pots each with sterile peat, 10 with non-sterile peat and 10 with oak forest soil were inoculated with *L. laccata* inoculum. Each treatment was replicated three times. No inoculum was added to the corresponding control treatments. The same design was used in case of the *P. involutus* inoculum. Plants were planted from March 2004, until the end of October 2004. The experimental plots were watered weekly and treated against oak powdery mildew using Neem Oil in May and June and kept weed-free. The inoculation experiment was done in experimental plots at the University of South Bohemia. The seedlings were gently removed from the pots on the end of October 2004, the roots were washed to remove the substrate and quantitative and qualitative evaluations of mycorrhization were done as described below. The aboveground parts of plants were analysed for length (mm), fresh weight (g), leaf area (g), dry weight and (g), root-collar diameter (mm).

Quantitative assessment of ectomycorrhizae

The fungus aggressiveness, inoculum efficacy and field performance were assessed using the PI index and LL indices according to Marx (1991) and Le Tacon et al. (1997). The PI index and LL index are computed using the modified formula **a x b / c** where a = percent of seedlings with any percentage of the introduced ectomycorrhiza, b = average percent of feeder roots with inoculated mycorrhiza (including 0%), and c = average percent of all feeder roots including contaminants and senescent mycorrhiza. Direct counts of entire root systems were used. The total number of ectomycorrhizae per cm of lateral roots was also determined.

Qualitative evaluation of ECM

The morphotypes of the ectomycorrhizae were described on basis of fresh ECM root tips as recommended by Agerer (1991, 1995) Morphological characters such as colour, shape, branching patterns, and surface structure were examined under a dissecting microscope. The anatomy of the hyphal mantle and Hartig net was described based on tangential sections through the mantle and using a light microscope BX 41 at magnifications of 400x – 1,000x. Photographs were taken using a microscope camera and PhotoQuick Camera 2.1.software.

Characterization of morphotype by PCR-RFLP method

Extraction of genomic DNA for PCR was performed from single ECM roots by using a Qiagen Plant Mini Kit based on CTAB extraction (Karen et al. 2000, Peter et al. 2001). The amount of DNA 50 µl per sample was stored in a freezer at -20 °C. The PCR assays contained 5 µl 10xTaq polymerase reaction buffer, 4 µl 25 mM $MgCl_2$, 10 nmol each of deoxynucleotide, 50 mmol of each primers ITS1 and ITS4 (White et al. 1990) and 1 µl template DNA in total volume 50 µl. After 10 min of denaturation at 95 °C, PCR was started by adding 2 U Taq DNA polymerase (Takara). The PCR program comprises 32 cycles using a thermocycler. The PCR products were cleaned, in single enzyme digestion, with AluI, EcoRI, HinfI. The length of the amplification products and restriction fragments were determined by electrophoresis in 2% agarose gels run at 10V/cm. The gels were stained by using ethidium bromide and photographed under U.V. light (Karen et al. 1997, Bruns et al. 1991, Battista et al. 2002). The length of the polymorphism fragments were analysed using Bioprofile Software.

Statistical analyses

Data obtained from quantitative assessment of ECM roots were subjected to ANOVA and ANCOVA (Statistica Software Version 7.0).

RESULTS

The inoculation index ranged from 0 - 100 according to fungus isolate, soil quality and environment. The PI index represents the ability of inoculum to colonize feeder roots of seedlings and establishment of the ectomycorrhizal association on the experimental plants. The results showed that all containerized seedlings were successfully colonized by inoculum. The type of substrate effected development of the roots, including lateral roots, plus the ability of inoculum to colonize lateral roots. *Paxillus involutus* colonized more lateral roots than *L. laccata* (Table 1) (Factorial ANOVA, $F_{(2, 268)} = 13.33$, $p \leq 0.05$) although the density of ectomycorrhizal tips per root length was less abundant than that for *L. laccata* in the sterile and non-sterile peat substrates. Morphotypes of *P. involutus* and *L. laccata* occurred naturally on the roots of seedlings planted in the forest soil without inoculum added. The ratio of lateral roots containing morphotypes of *P. involutus* and *L. laccata* to the total number of feeder roots was significantly low as opposed to that in inoculated forest soil (Factorial ANOVA, $F_{(2,268)} = 13.3$, $p \leq 0.05$). The natural content of contaminants, i. e. naturally occurring ectomycorrhizal fungi, proved to be relatively high (CFS in Figures 1 and 2), nevertheless, such substrate was suitable for *P. involutus*. The value of the PI index approached 90% and the number of tips per root length attained maximal values whereas the LL index reach about 65% and the amount of ECM tips was about 2.5 per 1 cm of root length in forest soil.

Sterile substrate did not prove suitable for development of feeder roots and colonization by ectomycorrhizal fungi was poor in comparison with other substrates (Factorial ANOVA, $F_{(2,268)} = 96.71$, $p \leq 0.05$). *Laccaria laccata* colonized roots less than *P. involutus*, but the density of ectomycorrhizal tips per 1 cm of root length was significantly higher (Figure 1). Non-sterile peat substrate supported the development of ectomycorrhizal association. PI index in this substrate is close to value obtained from forest soil. *L. laccata* achieved maximal value 76.6% in non-sterile peat.

Tab. 1.
PI and LL index: Efficacy of inoculum in different substrates expressed as the ratio between lateral roots with inoculated ectomycorrhizae and total number of lateral roots including non-mycorrhizal roots and contaminants

Substrate used	PI index (%)	LL index (%)
non-sterile peat	85.5[a]	76.6
sterile peat	74.5	60.8
forest soil	89.4[a]	64.9
control forest soil*	28.9[b]	32.1[b]

*No inoculum added

The quantification of ectomycorrhizal tips (Figure 1) partially confirmed the results of the inoculations. The highest number of root tips formed by *P. involutu*s occurred on lateral roots of seedlings planted in forest soil whereas there were more morphotypes for *L. laccata* in sterile and non-sterile peat-perlite mix substrate.

The natural occurrence of ectomycorrhizal fungi in all the substrates is presented in Figure 2. Other ectomycorrhizal fungi such as *Coenococcum geophillum*, and individual species of *Scleroderma*, *Lactarius* and *Russula* were identified by using morphotyping and supported by the PCR-RFLP method. The competitiveness of *L. laccata* towards other fungi was significantly lower as compared to *P. involutus*. *L. laccata* produced more ectomycorrhizal tips per 1 cm of feeder roots than *P. involutus* when other ECM fungi were absent (sterile peat and non-sterile peat-perlite substrate).

Fig. 1.
Final effect of mycorrhization: Average number of ectomycorrhizal tips formed per 1 cm of root length by the inoculated fungi *Paxillus involutus* and *Laccaria laccata* Factorial ANOVA, $F_{(2,270)} = 11.25$, $p \leq 0.05$, n = 6

PI - *Paxillus involutus*
LL - *Laccaria laccata*
NP - non-sterile peat
SP - sterile peat
FS - oak forest soil
CFS - forest soil –control
CNP - non-sterile peat control
CSP - sterile peat control

Plant response to inoculation

Multivariate analysis of oak seedling productivity showed the significant effects of mycorrhizal inoculation on seedlings ($F_{(2,269)} = 2,807$; $p \leq 0.0001$) as well as significant seedling response to the planting medium ($F_{(3,268)} = 90.81$; $p \leq 0.0001$). All measurements were significant indicators of seedling productivity in the multivariate analyses when control plants were included. In all measures of plant productivity, mycorrhizal colonization enhanced growth with each substrate. Non-mycorrhizal plants produced significantly less fresh and dry mass (g); leaf area was reduced as well

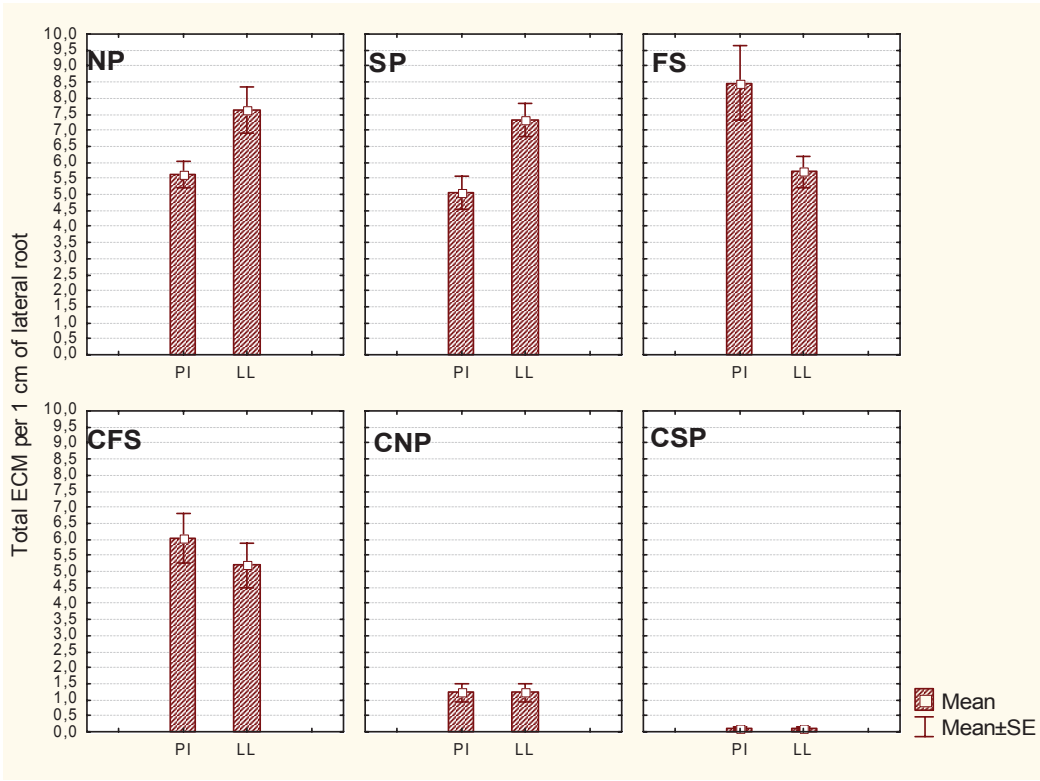


Fig. 2.
Final effect of mycorrhization: Average number of ectomycorrhizal tips formed per 1 cm of root length by all ECM fungi present in the substrates
Factorial ANOVA, $F_{(2,270)} = 5.7065$, $p \leq 0.004$, $n = 6$

as height (Figure 3) and root collar diameter (Figure 4). Inoculum of *L. laccata* and *P. involutus* by themselves, benefited root development almost equally. The comparison of inoculum and substrate showed that the combination of inoculum with sterile peat provided significantly lower fresh root weigh values ($F_{(4, 178)} = 12.7$; $p \leq 0.0001$) and dry weight ($F_{(4,178)} = 16.17$; $p \leq 0.0001$) than combinations with non-sterile peat or forest soil.

Forest soil was found to be a good substrate for mycorrhization as opposed to sterile peat and non-sterile peat. The impact of substrate was slightly effective when the fresh mass of root (g) was compared ($F_{(3,268)} = 90.8$; $p \leq 0.045$). Although both fungi benefited the development of oak seedlings significantly when compared to non-mycorrhizal plants, *P. involutus* increased measurements significantly. The fresh and dry mass of stems was significantly higher in case of combination *P. involutus* and forest soil as opposed to *L. laccata*. The fresh mass of roots ($F_{(2,269)} = 112$; $p \leq 0.001$) and dry mass of roots ($F_{(2,269)} = 75$; $p \leq 0.001$), both increased significantly when inoculum was added to forest soil regardless of the used species used. Inoculation enhanced leaf area (cm^2) including the number of leaves and leaf size. Seedlings treated with *P. involutus* had the largest leaf area when planted in sterile peat and those inoculated with *L. laccata* achieved similar values in forest soil.

Tab. 2.
Comparison of mycorrhizal and non-mycorrhizal seedlings grown in different substrates

	Mycorrhizal			Non-mycorrhizal		
	NP	SP	FS	NP	SP	FS
Dry mass stem PI	5.67 ±2.21 [ac]	6.51 ±1.44 [a]	8.10 ±4.2 [a]	3.42 ±0.82 [b]	2.25 ±0.82 [b]	0.57 ±0.13 [b]
Dry mass stem LL	4.69 ±3.00 [c]	4.58 ±2.87 [c]	6.91 ±2.45 [a]	2.31 ±1.05 [b]	2.62 ±1.12 [b]	3.08 ±1.45 [b]
Dry mass root PI (g)	16.33 ±4.1	13.15 ±6.01	20.44 ±3.18[a]	5.72 ±1.2 [b]	6.13 ±2.06 [b]	2.29 ±2.06 [b]
Dry mass root LL (g)	14.11 ±2.62	12.72 ±4.01	21.23 ±7.22 [a]	4.12 ±0.51 [b]	3.18 ±0.64 [b]	0.87 ±0.14 [b]
Fresh mass root PI (g)	44.02 ±5.71 [a]	36.31 ±6.44	41.10 ±2.71 [a]	19.8 ±1.17 [b]	16.51 ±4.3 [b]	14.67 ±1.34 [b]
Fresh mass root LL (g)	35.12 ±3.26	30.55 ±4.77	40.62 ±6.4 [a]	18.2 ±0.74 [b]	11.62 ±1.4 [b]	15.07 ±1.07 [b]
Fresh mass stem PI (g)	11.68 ±4.15	11.08 ±4.21	13.45 ±3.81	6.87 ±1.11 [b]	3.77 ±1.51 [b]	0.96 ±0.24 [c]
Fresh mass stem LL (g)	7.69 ±1.53 [b]	8.05 ±2.02 [b]	11.91 ±4.2 [a]	7.76 ±1.53 [b]	7.91 ±2.02 [b]	12.35 ±4.2 [a]
Leaf area (cm^2)PI	16719.802 ±4711.048	21404.996 ±5488.191 [b]	19833.8 ±14995.52	6187.135 ±102.32 [a]	7524.124 ±516.24 [a]	2860.405 ±817.35 [a]
Leaf area (cm^2) LL	11955.12 ±3503.52	15555.366 ±1774.30	22846.193 ±2635.79 [b]	9016.381 ±213.26 [a]	8241.995 ±3082.89 [a]	2154.405 ±214.22 [a]

Means in rows within the main effect followed by the same letter are not significant different (homogenous group).

Statistical parameters of the ACONOVA analysis

Morphological parameter	Statistical value	
dry mass stem	$F_{(4,176)} = 12.46$	$p \leq 0.001$
dry mass root	$F_{(4,176)} = 16.17$	$p \leq 0.001$
fresh mass root	$F_{(4,176)} = 12.67$	$p \leq 0.001$
fresh mass stem	$F_{(4,176)} = 13.28$	$p \leq 0.001$
leaf area ratio	$F_{(4,176)} = 18.54$	$p \leq 0.001$

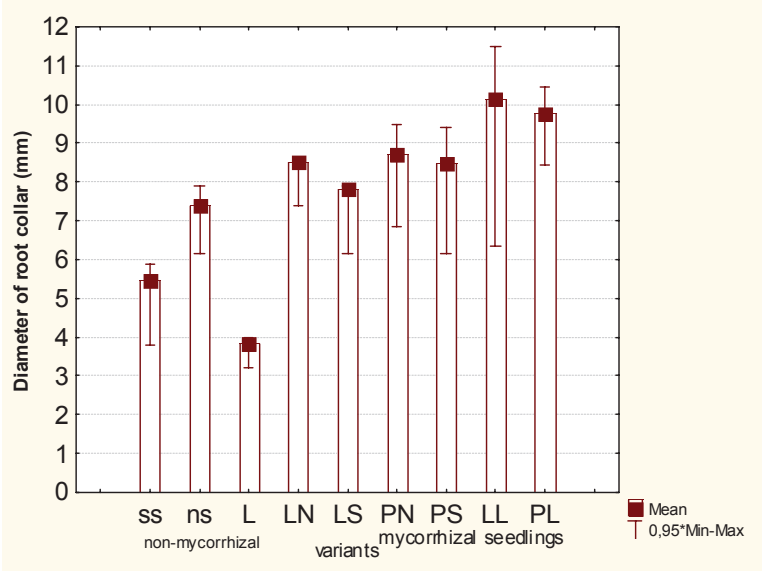


Fig. 3.
Root collar of inoculated and non-inoculated oak seedlings
MANOVA. $F_{(4,178)} = 1142.75$, $p \leq 0.038$, $n = 12$
ss - sterile substrate control, ns - non-sterile substrate control, L - forest soil control, LN - *Laccaria laccata* non-sterile, LS - *Laccaria laccata*-sterile, PN - *Paxillus involutus* non-sterile, PS - *Paxillus involutus* sterile, LL - *Laccaria bicolor* forest soil, PL - *Paxillus involutus* forest soil

Commercially the most important parameters are root collar diameter (Figure 3) and seedling height (Figure 4). Seedlings planted in forest soil (LL and PL) with inoculum added had well-developed root collars comparable to 3-year old seedlings or older plants ($F_{(2,269)} = 74.0$; $p \leq 0.001$). The influence of fungus species on root collar size was slightly apparent only when combined with substrate ($F_{(4,176)} = 3.1$; $p \leq 0.023$). Seedlings planted in sterile peat with *L. laccata* were not significantly different. Although inoculated seedlings grown in forest soil produced the best results, the influence of substrate was only slightly significant ($F_{(2,269)} = 3.35$; $p \leq 0.042$). Surprisingly, control seedlings planted in forest soil were very thin and their root collar diameter was significantly smaller compared to inoculate and control plants in sterile and non-sterile peat. The height of plants showed similar effects as the root collars. Seedlings grown in forest soil with inoculum were significantly taller compared to control seedlings, *L. laccata* inoculation resulted in better overall growth of seedlings in forest soil compared to *P. involutus* ($F_{(2.268)} = 32$; $p \leq 0.001$) in the same substrate. Seedlings planted in sterile and non-sterile peat with *P. involutus* were taller than those grown in forest soil. *Laccaria laccata,* when added to sterile and non-sterile peat, only had a minor impact on the growth of seedlings although the height corresponded to that of 2- and 3-year old plants used for reforestation. Control seedlings on all types of substrate were significantly smaller than inoculated seedlings.

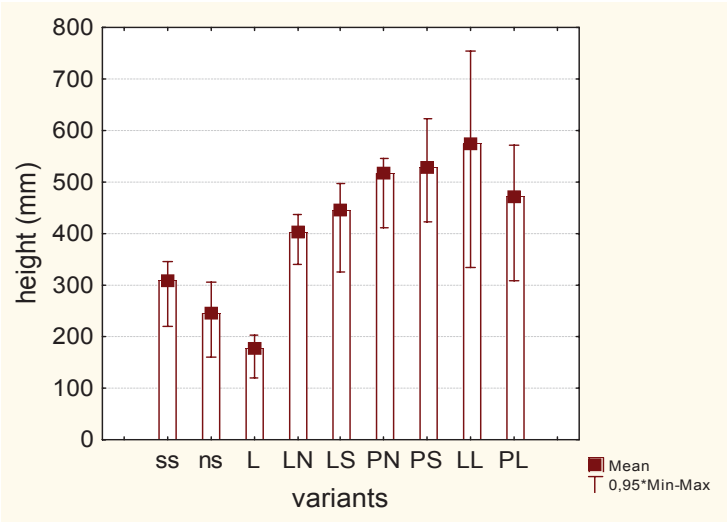


Fig. 4.
Height of inoculated and non-inoculated oak seedlings
ACONOVA. $F_{(4,176)} = 942.75$; $p \leq 0.001$, $n = 12$

ss – sterile substrate control, ns - non-sterile substrate control, L – forest soil control, LN – *Laccaria laccata* non-sterile, LS - *Laccaria laccata*-sterile, PN – *Paxillus involutus* non sterile, PS - *Paxillus involutus* sterile, LL - *Laccaria bicolor* forest soil, PL - *Paxillus involutus* forest soil

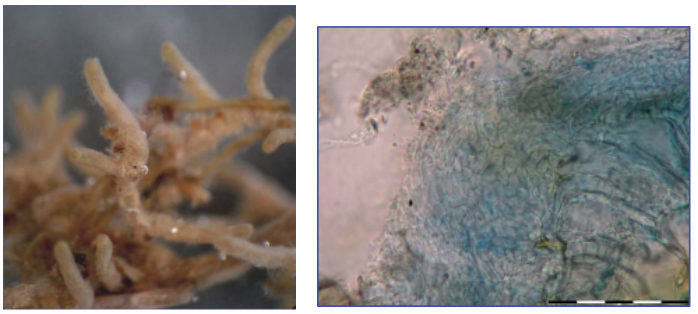


Fig. 5.
Laccaria laccata morphotype*:* unbranched. Ectomycorrhizae, white to beige, mantle plectenchymatic. Emanating hyphae with clamps

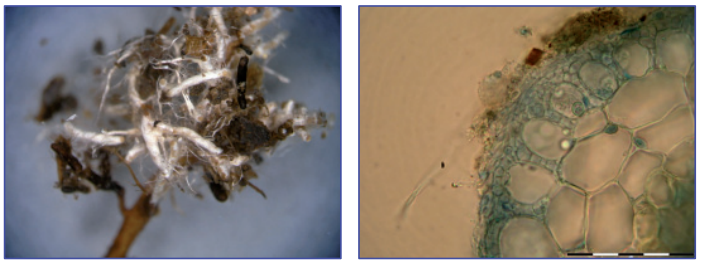


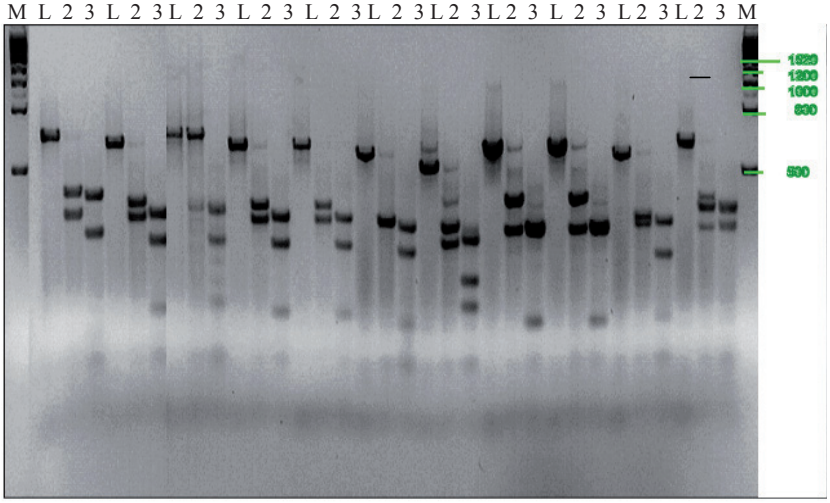
Fig. 6.
Paxillus involutus morphotype: tortuous. Silvery, unbranched with conspicuous rhizomorphs

Verification of ectomycorrhiza

Qualitative assessment of ectomycorrhizal samples confirmed the relationship to inoculum according to the morphological features described in AGERER (1995) (Figs. 5 and 6). The other ectomycorrhizae which occurred naturally in forest soil were determined to be: *Coenococcum geophillum*. *Lactarius quietus, Russula ochroleuca, Scleroderma* sp. and *Tomentella* sp. The homogeneity of morphotypes conforming to *L. laccata* and *P. involutus* was confirmed by PCR-RFLP pattern when PCR products were digested by EcoRI a HinfI.

Laccaria laccata

M L 2 3 L 2 3 L 2 3 L 2 3 L 2 3 L 2 3 L 2 3 L 2 3 L 2 3 L 2 3 L 2 3 L 2 3 M



Laccaria laccata *Paxillus involutus*

M L 2 3 L 2 3 L 2 3 L 2 3 P 2 3 P 2 3 P 2 3 P 2 3 P 2 3 P 2 3 P 2 3 M

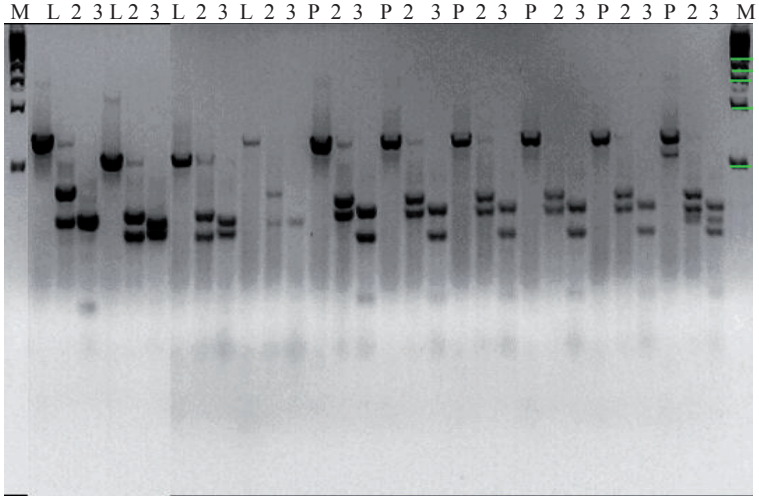


Fig. 7.
RFLP profile of the ITS1 and ITS 4 digested by EcoRI (2) a HinfI (3) of the 14 *Laccaria laccata* (L) ectomycorrhizal tips and one sample of mycelia-isolate QR2 (Fig.6) and six samples of *Paxillus involutus* (P) ectomycorrhizae including the sample of isolate QR5

The primers ITS 1-ITS 4 successfully amplified the ITS region of DNA samples extracted from the ectomycorrhizal tips of inoculated oak seedlings. According to the total length of ITS results of restriction can be grouped into several groups. The products of restriction by EcoRI grouped into mostly two bands, except for samples 8, 11, 2, 3 where more than two bands were noted. Variability of restriction is discussed in the discussion.

The length of polymorphism of the PCR products and the RFLP patterns obtained by digestion of ITS1 and ITS 4 regions with EcoRI and HinfI were sufficient to determine the success or failure of fungal species used as inoculum in forest soil. Morphotypes belonging to *Laccaria* sp. showed higher variability compared to *P. involutus*. The PCR products of *P. involutus* morphotypes restricted with EcoRI and HinfI resulted in homogenous group that confirmed the presence and stability of inoculum.

Tab. 3.
Molecular characterization of the interspecific spacer (ITS) of *Laccaria laccata* (1 - 15) and *Paxillus involutus* samples*(1 - 6) digested by using EcoRI and HinfI

Laccaria laccata

	PCR product			**EcoRI**				**HinfI**							
bp	720	634	580	290	310	340	350	390	360	330	300	270	230	100	10
1	+			+	+			+	+	+		+			+
2*		+			+		+				+		+	+	+
3	+			++		+					+		++	++	+
4		+		+		+					+		+	+	+
5		+		+			+				+		+	+	+
6				++								+	+	+	+
7		+		++			+	+	+			+	++	+	+
8				+			+					++		+	+
9				+			+		+	+		+		+	+
10		+		+	+						+		+	+	+
11				+		+		+		+		+			+
12		+	+	+			+					++		+	
13			+	++								+	+		
14			+	++								+	+		+
15		+		+			+				+	+			+

Paxillus involutus

	PCR		**EcoRI**			**HinfI**			
	644	370	340	320	300	320	280	251	24
1	+	+			+	+	+		+
2	+		+		+	+		+	+
3	+		+		+	+		+	+
4	+	+			+	+		+	+
5	+	+		+		+		+	++
6*	+	+		+		+	+	+	+

Samples marked with an asterisk are the macrocarps used for mycelia production.

DISCUSSION

Paxillus involutus isolate QR5 and *L. laccata*. isolate QR2 provided suitable inoculum for oak plantlets. Both fungi established mycorrhizal associations on all oak seedlings. Contrary to expectation mycorrhizal colonization enhanced seedlings performance regardless of planting substrate. Sterile peat-perlite substrate affected the development of plantlets because of insufficient microbial activity and decreased water absorption. Heat-treated peat can contain phytotoxic compounds released from organic matter during sterilization. Root systems in such a substrate were poorly developed and lacked branches. Ectomycorrhizal root tips were abundant when *L. laccata* was used as inoculum but the rate of colonized roots to total lateral roots was lower. *Paxillus involutus* colonized more lateral roots but the density of ectomycorrhizal tips per 1 cm of root length was less compared to *L. laccata*. Nevertheless, application of mycorrhizal inoculum in sterile peat-perlite showed that both of the fungi used grow and colonize seedlings when microbial activity of the soil is limited. The fresh mass of stems was the only parameter that was not enhanced when *L. laccata* was applied to the sterile peat-perlite substrate. Leaf area of seedlings grown in sterile substrate inoculated with *L. laccata* increased for all parameters. The induction of leaf area and intensive consumption of assimilates at the expense of fresh weight can be result of hormonal imbalance caused by ectomycorrhizal fungi (Podila 2002), irregular water uptake and low microbial activity in sterile substrate.

The direct effect of *L. laccata* on broadleaf seedlings has not yet been described. Many studies have been done on Douglas fir or spruce seedlings. Based on experiments in bareroot nurseries and containerized seedlings *Laccaria* sp. is recommended as suitable fungus when mixed with other ectomycorrhizal fungus such as *Hebeloma* sp., *Paxilus involutus*, *Rhizopogon* sp. and *Suilus tomentosus* (Khasa et al. 2001). Mahony (2005) used *Laccaria proxima* and other ectomycorrhizal fungi to inoculate aspen seedlings. Height and root collar diameter of seedlings treated with *L. proxima* increased significantly compared to plants inoculated with *Paxillus vernalis, Hebeloma* sp. and other fungi. Garbaye et al. (2002) stimulated growth of urban trees and delayed leaf yellowing when inoculum of *L. laccata*, *P. involutus* and *C. geophillum* was added to soil. Martin (2001) confirmed that *L. laccata* was less effective during root colonization of *Pinus banksiana* than *P. involutus* and *Rhizopogon* sp.

Non-sterile peat-perlite substrate was shown to be a suitable medium for inoculum. *Paxillus involutus* colonized lateral roots intensively as well as *L. laccata*. The inoculation index for *L. laccata* was ten times smaller than the PI index but the density of ectomycorrhizal tips formed per 1 cm of root length was significantly higher. The height and other parameters, such as fresh mass of roots and stems and leaf area, decreased when seedlings were colonized with *L. laccata,* i. e. in comparison to *P. involutus*. Nevertheless, inoculated seedlings in non-sterile substrate achieved size equal to that of 2 or 3-year old seedlings in bareroot nurseries.

The forest soil used here originated from an oak forest where the oaks have grown for more than 200 years. This soil contained natural inoculum of *Coenococcum geophilum, Tomentella sublilacina, Paxillus involutus, Laccaria laccata, Scleroderma* sp.*, Lactarius quietus* and *Russula ochroleuca*. *Paxillus involutus* proved to be very suitable for this forest substrate. The inoculation index and number of ectomycorrhizal tips were significantly higher than in case of *L. laccata*. *P. involutus* competed well with native mycorrhizal fungi and formed abundant ectomycorrhizae. In spite of this, *L. laccata* increased the height of containerized seedlings markedly better than *P. involutus* and also slightly increased root collar diameter when compared to *P. involutus*.

We conclude that ectomycorrhizal fungi were quite effective as inoculum. Although the production of containerized seedlings is limited because of cost, application of suitable inoculum can reduce growing time from 2 - 3 to 1 year. Plant analyses confirmed that height and root collar

diameter of inoculated seedlings fully respond to the standards for 2 and 3 years old seedlings as recommended by the Ministry of Agriculture, Czech Republic. Another advantage of the fungi that we used is that *L. laccata* and *P. involutus* can form ectomycorrhiza at higher fertilizer levels (HUNT 1992, KHASA 2001). The ultimate objective of ectomycorrhizal inoculation is higher quality of nursery stock and improved field performance by seedlings as exemplified by increased survival, growth and probably wood quality (HUNT 1992). Containerized seedlings have well developed root systems and ectomycorrhizae can be sustained well when seedlings are used for afforestation of arable soil or restoration of urban and industrial areas and mine spoils. PRASAD (1999) concluded that *L. laccata* and *P. involutus* are able via ectomycorrhizal association ameliorate the toxicity of heavy metals to trees. *Laccaria bicolor* was maintained on Douglas fir trees for 4 years after outplanting and it acted as a pioneer fungus (VILLENEUVE et al. 1991).

Regardless of the forest soil used as substrate we used molecular methods for verifying the presence of inoculated mycorrhiza versus morphotyping. ECM tips of the same morphotype collected from seedlings growing in forest soil produced the same patterns as for *P. involutus*. However, ectomycorrhizal morphotypes were not always discrete ITS types when samples of *L. laccata* were tested. Inconsistencies between morphotypes of *L. laccata* were observed by KAREN and NYLUND (1997), GARDES et al. (1991) and HORTON (2002). Discrepancies obtained by restriction pattern can be explained by theories that not only one species of ectomycorrhizal fungi is fully responsible for a particular morphotype. ITS1 and ITS4 are universal fungal primers and can amplify the ITS region on all fungi present on root tips although results of morphotyping sub-ordered the root tips to *L. laccata* and *P. involutus*. Samples of *P. involutus* showed to be homogenous when restricted. Utilization PCR-RITS pattern should be effective practically when presence of inoculum need to be confirmed. The effectiveness of method can be improved when the selective primers are applied (HENRION 1992, HONIG 2000). Restriction and sequencing analyses of initial inoculum in the form of basidiocarps, mycelia and root tips can be kept in the molecular library as the source of useful information for practical use.

ACKNOWLEDGEMENTS

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OCCURRENCE OF PESTS IN SLOVAK FOREST NURSERIES FROM 1990 TO 2004

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ABSTRACT

Each year the occurrence of pests in forest nurseries is listed in report L116. The report is completed by the owner of a forest nursery and sent to the Forest Protection Service Centre of the Forest Research Institute in Banska Štiavnica by the following February 20. The data are compiled using computers and results are published in the report "Occurrence of Pest Agents in Slovak Forests in the Last Year and Prognosis for the Next Year". This report gives information on pests in forest stands, forest nurseries as well as pesticide usage in forest stands and forest nurseries. The present paper summarizes information on the occurrence of pests and pesticide applied in forest nurseries between 1990 and 2004.

INTRODUCTION

The Republic of Slovakia contains about 4.9 mil. ha. The state is divided into 79 administrative districts which are grouped into eight administrative regions (Fig. 1). The forested area covers about 1.9 mil. ha, which is 40 % of the total area. State forests own 40% of all forests in Slovakia.

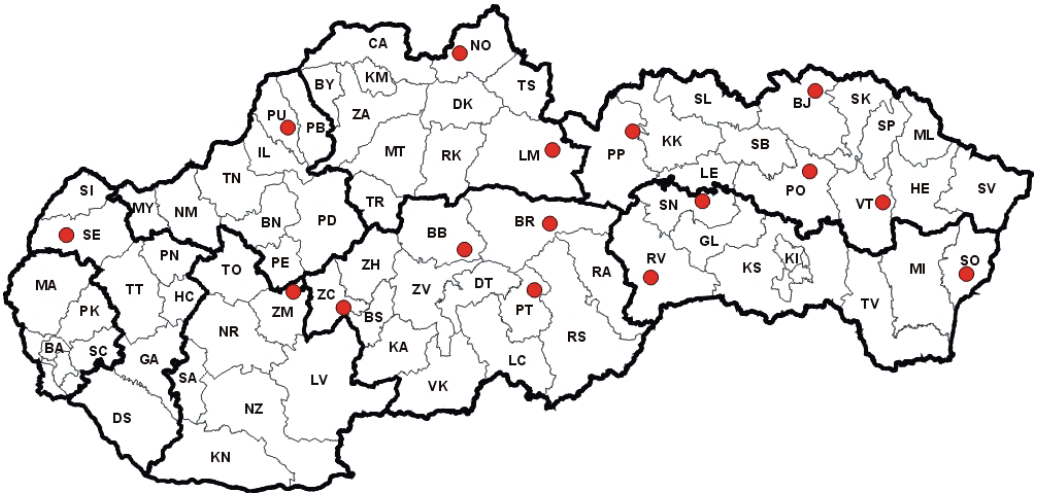

Fig. 1.
Larger forest nurseries are distributed in different administrative districts and regions

Forest nurseries are owned by both state and private organizations (Tab. 1). Their area reached more than 500 ha in 2005. There are 16 bigger forest nurseries (Fig. 1) which cover about 300 ha (more than half).

Tab. 1.
Area of Slovak forest nurseries from 2001 to 2005

Year	Total area [ha]			Area actually used for production of plants [ha]		
	State	Private	Total	State	Private	Total
2001	484	142	626	329	106	435
2002	593	195	788	376	148	524
2003	521	163	684	360	116	476
2004	520	161	681	350	118	468
2005	373	138	511	279	100	379

The production of forest trees varies each year (Tab. 2) and it depends on several factors such as seed production, large forest calamities, and seedling demand. Seedling production consists of 2/3 conifers and 1/3 broadleaved trees. The most common tree species is Norway spruce and the less common are silver fir and European beech (Table 3).

Tab. 2.
Seedling production in Slovak forest nurseries from 2001 to 2005

Year	Conifers [mil. plants]			Broadleaved trees [mil. plants]			Total production [mil. plants]		
	State	Private	Total	State	Private	Total	State	Private	Total
2001	79	29	108	35	45	80	114	74	188
2002	88	28	116	51	33	84	139	61	200
2003	82	27	109	38	23	61	120	50	170
2004	71	35	106	41	25	66	112	60	172
2005	65	40	105	26	20	46	91	60	151

Tab. 3.
Production of seedling species in Slovak forest nurseries in 2005

Tree species	State nurseries [millions plants]	Private nurseries [millions plants]	Total production [millions plants]
Norway spruce *Picea abies* (L.) H. KARST.	33.5	18.3	51.8
European larch *Larix decidua* MILL.	4.8	4.5	9.3
Silver fir *Abies alba* MILL.	17.3	12.3	29.6
Scots pine *Pinus sylvestris* L.	8.6	4.4	13.0
Other conifer species	1.3	0.3	1.6
Conifers together	65.5	39.8	105.3
European beech *Fagus sylvatica* L.	14.6	13.5	28.1
Pedunculate and sessile oak *Quercus robur* L. and *Q. petraea* (MATT.) LIEBL	6.2	3.5	9.7
Sycamore *Acer pseudoplatanus* L.	2.0	1.3	3.3
Ash *Fraxinus excelsior* L.	1.6	1.0	2.6
Other broadleaved tree species	1.6	0.6	2.2
Broadleaved tree species together	26.0	19.9	45.9
TOTAL	91.5	59.7	151.2

The production area decreased in 2005, mostly as the result of reorganization in the state forests which have concentrated their production to 13 larger forest nurseries and consequently they have stopped supporting small (up to 2 ha) nurseries.

Seedlings and transplants production have been decreasing as well. However, this was mainly due to lack of seeds (mostly acorns and beechnuts) in 2004. The second factor is reduction of production area in state forests and increasing area of natural regeneration, as mentioned above (SUŠKOVÁ et al. 2001, 2002, 2003, 2004, 2005).

PESTS

Pests occur on the area of about 63 ha each year (KUNCA et al. 2005a, b). Each year seedlings losses reach 12 million and 4 million transplants are lost to pests (Tab. 4). These amounts vary and it is hard to find the main reason. Pest agents occurred on a large area in 1996 and from 2000 to 2002. Severe dieback of seedlings occurred from 1993 to 1996 and 2000 - 2002, as it was with occurrence of pest agents. The situation is different with transplants. The worst years occurred between 1997 and 1999, when seedlings were not damaged so severely (Fig. 2).

Tab. 4.
Damage resulting from pests (Source: Report L 116)

Year	Pest agents incidence [ha]	Seedlings loss [million]	Transplants loss [million]
1990	63.0	6.5	2.3
1991	63.0	9.3	2.4
1992	57.0	9.0	2.5
1993	62.0	16.3	2.7
1994	60.0	14.0	2.7
1995	57.5	18.2	2.9
1996	82.7	15.7	3.2
1997	69.7	7.9	8.7
1998	68.2	10.6	5.7
1999	38.7	9.4	5.9
2000	77.6	17.5	3.2
2001	87.4	15.9	3.8
2002	82.1	12.2	2.5
2003	49.4	9.5	4.6
2004	31.8	5.6	4.6
Average	63.3	11.8	3.8
s_x	15.2	4.0	1.7
Min	31.8	5.6	2.3
Max	87.4	18.2	8.7
N	15	15	15

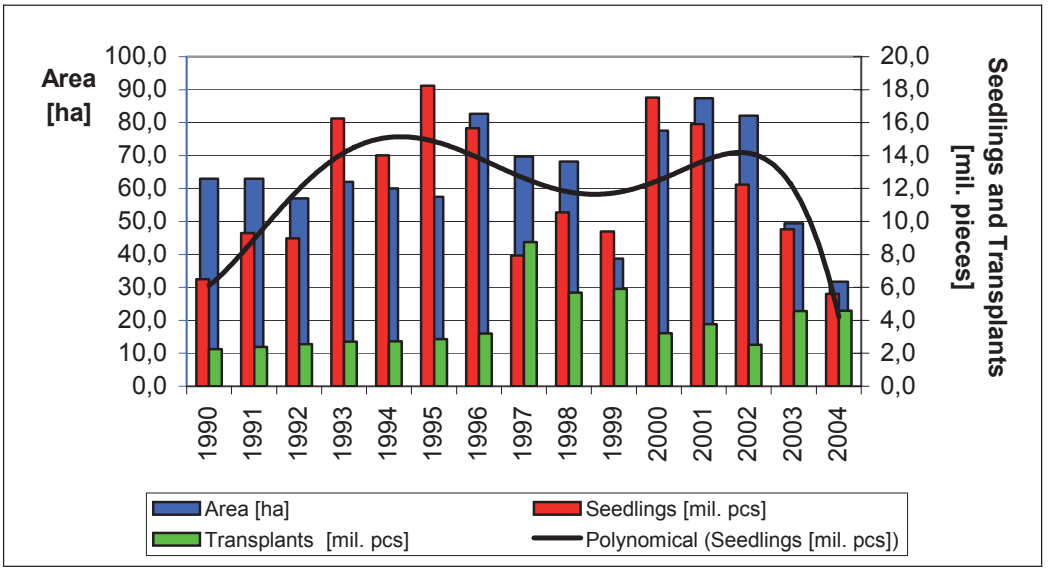

Fig. 2.
Occurrence of pests, dieback of seedlings and transplants

The most important losses occur as the result of abiotic agents (Table 5), especially drought, frost and water logging. Fungi are the second most important cause of losses. Damping-off and needle blight are the most important diseases causing dieback of seedlings and transplants. Powdery mildew is the most common disease, but dieback does not occur so frequent. As for insects, the most dangerous pests are *Melolontha* white grubs and *Grylotalpa grylotalpa*.

Tab. 5.
Pest agents: average from 1990 to 2004

Pest agents	Incidence		Seedlings		Transplants	
	[m^2]	%	[thousands]	%	[thousands]	%
Insects	81 031	12	1 081	9	758	20
Nematodes	1 096	0	13	0	2	0
Rodents	36 423	5	408	3	304	8
Fungi	241 134	36	3 516	30	695	18
Weeds	44 969	7	57	0	44	1
Abiotics	263 802	39	6 767	57	2 041	53
Total	668 454	100	11 841	100	3 844	100

PEST CONTROL

Common pests are controlled with pesticides. On average these are applied to 437 ha (Tab. 6). Fewer pesticides were in 1999 and the most in 2002. Herbicides are the most common pesticide, far less are insecticides.

Tab. 6.
Pesticides applied from 1996 to 1998.

Year	Insecticides [ha]	Fungicides [ha]	Herbicides [ha]	Pesticides [ha]
1996	50	248	237	535
1997	25	200	179	404
1998	13	124	234	371
1999	14	130	178	322
2000	28	152	194	374
2001	43	185	231	458
2002	71	278	316	664
2003	21	121	219	361
2004	51	193	195	439
Average	35	181	220	437
s_x	19	52	40	100
Min	13	121	178	322
Max	71	278	316	664

Pesticides application depends both on the biotic pests occurrence and the total area used for seedling production. The area to which some pesticides do not apply, however, does not increase permanently (Fig. 3).

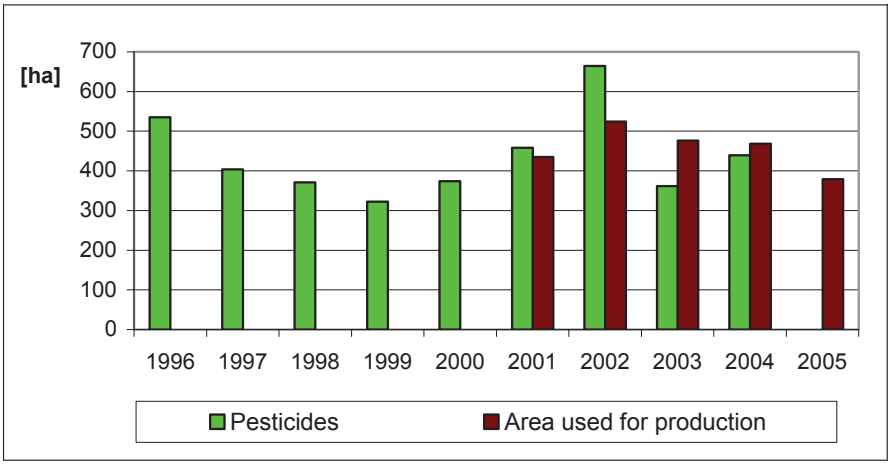

Fig. 3.
Chemical control of pests versus the area of forest nurseries used for production

Application of pesticides depends on climatic conditions as well. In hot and warm years (Tab. 7, Fig. 4), as in 2000 and 2003, pesticides were applied much less than e. g in 2002 which was quite wet (Fig. 4). This likely results from using better watering technologies than ventilation or shading technologies.

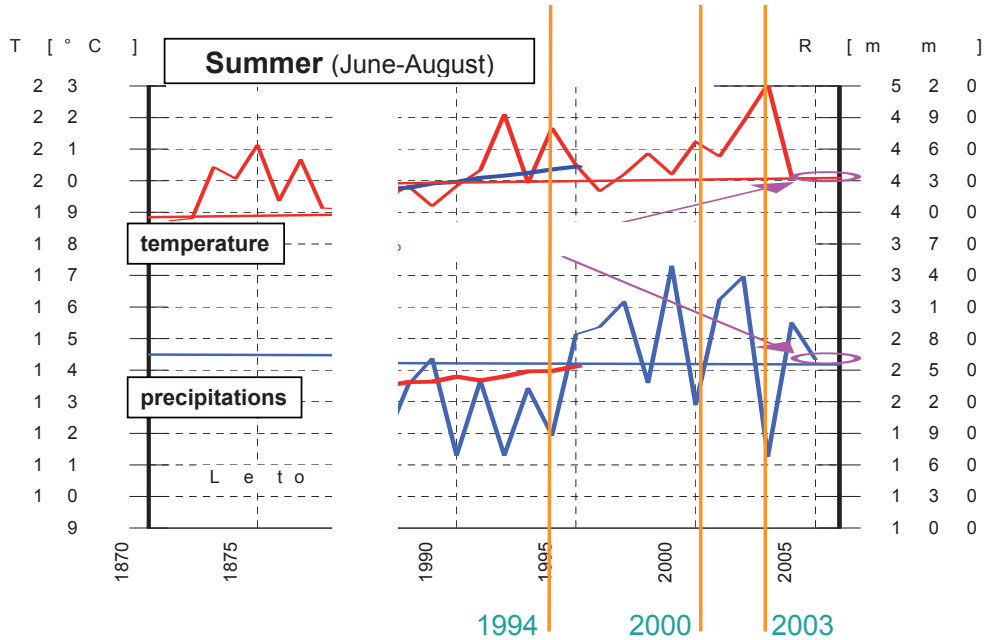

Fig. 4.
Summer conditions from 1990 to 2005 (courtesy Professor Lapin)

Tab. 7.
Climatic excesses since 1990 in Slovakia

Drought and hot in excess	Year
Hot	1992, 1994, 1998, 2000, 2001-2003
Drought	1990, 1992, 1994, 2000, 2003
Both drought and hot	1992, 1994, 2000, 2003

Wet and cold in excess	Year
Wet	1995-1997, 1999, 2001, 2002, 2004
Cold	-
Both wet and cold	-

CONCLUSIONS

The amount of registered area affected by pests depends on several factors:

1. The amount of seedlings being grown,
2. Climatic conditions,
3. Secondary pest agents,
4. The control measures used.

Foresters can determine the amount of cultivated plants or the control measurements, but they cannot influence climatic conditions. Pesticides can save seedlings and transplants, but there are still losses due to biotic pest agents.

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IDENTIFICATION AND DETECTION OF *PHYTOPHTHORA* SPP. AND DISEASES CAUSED BY *PHYTOPHTHORA ALNI*, *P. RAMORUM* AND OTHER NEW *PHYTOPHTHORA* ON TREES AND SEEDLINGS

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ABSTRACT

At least 60 - 80 *Phytophthora* species has been described, most of which are soil-borne pathogens, causing damping off, root rots, collar and stem rots and foliar blights on many plant species. These microbes are sometimes difficult to isolate and even more difficult to identify. Studies made over the past decade have shown that many new *Phytophthora* species are associated with diseased trees. Most *Phytophthora* pathogens are not native in the area where they are a serious problem; e. g. *P. ramorum*, the cause of sudden oak death, is introduced separately to North America and Europe. Typical for *Phytophthora* are also hybrids, a new combination produced by parents representing two different *Phytophthora* species, as was in the case for the *P. alni*-complex, which has altered riparian ecosystems throughout Europe. This article gives a general review of isolation and detection techniques and some newly identified species, including the *Phytophthora alni* complex and *P. ramorum*. The disease symptoms, host species and geographical range are also briefly described.

PHYTOPHTHORA

Phytophthora and other oomycetous microorganisms have long been considered as being fungi, but today because of evolutionary phylogeny and structure of their biflagellate zoospores, they are placed in the kingdom Chromista, which includes, for example, the brown algae (Erwin, Ribeiro 1996, Baldauff et al. 2000). *Phytophthora* is a genus that is mainly parasitic on plants including trees and tree seedlings. Tsao (1990) felt that most crown diseases of woody plants can be attributed to *Phytophthora* although in most cases proper techniques have not been used to demonstrate that they are responsible for the diseases. In plant tissues *Phytophthora* spp. produce mainly diploid hyphae, oospores and chlamydospores. Although oospores can survive in soil organic matter for a long time, the asexual chlamydospores are the main resting stage of oomycetes. The asexual, biflagellate, swimming zoospores are responsible for plant infection under wet conditions and they are produced in a vessel called a sporangium (pl. = sporangia). Some homothallic species are self-sterile and they produce oospores after union of the oogonium and antheridium. Heterothallic species are self-sterile and oospore production requires the presence of two mating types called A1 and A2. Sexual recombination or somatic fusion might create new races which are more virulent than the parents. Typical for *Phytophthora* are also hybrids, a new combination produced by parents representing two different *Phytophthora* species as is the case of the *P. alni*-complex (Brasier et al. 1999, 2004).

Identification

At least 60 - 80 *Phytophthora* species has been described. Most of them are soil borne pathogens causing damping off, root rots, collar and stem rots and foliar blights on different woody plant species (Erwin, Ribeiro 1996). The traditional identification of *Phytophthora* spp. is based on the morphology of sporangia, oogonia and antheridia, presence or absence of chlamydospores and the growth and colony characters of cultures on special agar, culture media (Waterhouse 1963, Stamps et al. 1990). Morphological grouping segregates the species into six main groups based on 1) the structure of the sporangium apex and the width of the exit pore, 2) the caducity of sporangia and the length of pedicel and 3) the antheridial attachment. (A sporangium may be papillate, semi-papillate or non-papillate, caduous sporangia shed at maturity and an antheridial attachment may be paragynous, amphigynous (Fig. 1) or both). However, these morphological features are not distinct and stable and might differ within a species or be similar between species. In addition, the traditional taxonomic grouping does not reflect true phylogenetic relations (Kroon et al. 2004).

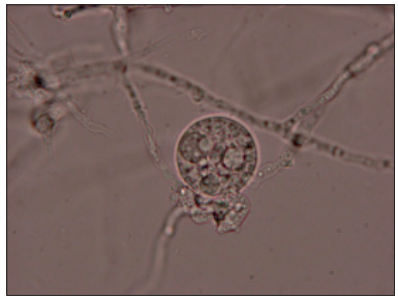

Fig. 1.
Amphigynous antheridium on ospore

Many molecular techniques such as protein electrophoresis, isozymes and PCR-based methods such as DNA fingerprinting and direct sequencing, have been tried in the search for more effective and rapid identification of species within the genus *Phytophthora*. (e. g. Bielenin et al. 1988, Oudemans, Coffey 1991, Cooke et al. 2000). Today, the internal transcribed spacer (ITS) sequence of most *Phytophthora* species is available in the GenBank, and thus this information can be used to determine the identity of unknown isolates.

Fig. 2.
Phytophthora 'rot' in apple baits after incubation. Before inoculation small cores were made in raw, green fruits and they were stuffed with tissue taken from a necrotic lesion on diseased plants.

Detection

Most *Phytophthora* spp. are more difficult to isolate directly from diseased plants, soil or water than are many other pathogens. The affected material should be in a stage of active infection since the ability of *Phytophthora* spp. to compete with other microbes is limited (Erwin, Ribeiro 1996, Martin et al. 2004). A common reason for the failure of isolation procedures is also dry weather or overly dry samples (Kox et al. 2002, Garbelotto 2003).

The overall assumption of baiting is the activation of the pathogen. In general, those baits consist of unripe fruits (e. g. apples and pears) or seedlings (e. g. lupine and alder) of highly susceptible hosts. Small cores of tissue are removed from the fruits and the resulting tubes in the tissue are filled with soil or small fragments of woody tissue taken from necrotic lesions on roots or bark. After incubation a *Phytophthora* 'rot' will develop on the host's exterior (Fig. 2)

Fig. 3.
Baiting of *Phytophthora* from diseased material with fruit baits

and isolation, e. g. plating on agar medium (with or without selective chemicals) can be done from this 'fresh', active infection (Jeffers, Martin 1986, Ervin, Ribeiro 1996). Another option is to add water to the samples and then float a suitable living plant tissue on the surface or place fruits in the water as baits (Fig. 3) (Streito et al. 2002, Themann et al. 2002).

Thus, the need for more reliable techniques has resulted in the search for new methods. For example, PCR-techniques used in studies on many *Phytophthora* spp., take advantage of the sequence in the ITS region of the ribosomal DNA or are based on the sequences for other nuclear genes such as β-tubulin or mitochondrial genes such as cytochrome oxidase subunits coxI and coxII and NADH dehydrogenase subunit 5 nad5 (Schubert et al. 1999, Nechwatal et al. 2001, Grote et al. 2002, Ivors, Garbelotto 2002, Kox et al. 2002, Garbelotto 2003, Martin et al. 2004).

ALDER *PHYTOPHTHORA*

Symptoms and distribution

During 1993 and 1994 an unique *Phytophthora* was consistently isolated from bark lesions at the stem bases of dying *Alnus glutinosa* along riverbanks, in orchard shelter belts and in woodland plantations in southern Britain (Brasier et al. 1995, Gibbs 1995). Typically affected trees were abnormally small, with chlorotic, sparse leaves and they had tarry or rusty coloured exudations on the stem lesions. Subsequently, the disease was also found on *A. incana* and *A. cordata* and now it has been reported from many countries in Europe: Austria, Belgium, France, Estonia, Germany, Hungary, Italy, Lithuania, Netherlands and Sweden (Gibbs et al. 2003). Field studies showed that it might be locally very damaging and an easily disseminated disease.

Origin and variants

The pathogen belongs to a group of heteroploid hybrids. Nucleotide sequence of the ITS-region and amplified fragment length polymorphism (AFLP)-analysis of total DNA showed that the parents of these hybrids are probably *P. cambivora* and *P. fragariae* (Brasier et al. 1999). The hybrid variants (standard, Swedish, German, Dutch and UK) differ in their chromosome numbers (n = 11-22), oogonial and antheridial morphology, oospore viability and colony characters. The origin of different variants may be the breakdown products of the first isolated standard hybrid or products of subsequent back-crosses or inter-crosses (Brasier et al. 1999, 2004). However, all variants seem to be relatively host specific pathogens of alders (Gibbs et al. 2003). The most aggressive are the standard- and Dutch-type variants. Recently the standard-type has been described as *P. alni* subsp. *alni* and the Swedish variant as *P. alni* subsp. *uniformis*. Although the German, Dutch and UK variants have shown phenotypic diversity, they have identical ITS-profiles and thus they have been grouped together as *P. alni* subsp. *multiformis* (Gibbs et al. 2003, Brasier et al. 2004a).

PHYTOPHTHORA RAMORUM

Morphology and distribution

In 2001 a *Phytophthora* associated with a twig blight disease on *Rhododendron* and *Viburnum* in Germany and Netherlands was described as a new species, *P. ramorum* (Werres et al. 2001). This heterothallic *Phytophthora* was first characterized by abundant production of chlamydospores and elongate, ellipsoid, deciduous sporangia. Oogonia with amphigynous antheridia were produced by parings with *P. chryptogea* representing mating type A2 (Werres et al. 2001). Later the same pathogen was found to be responsible for the Sudden Oak Death disease (SOD) of *Quercus* and *Lithocarpus* spp. in California, USA (Rizzo et al. 2002). The disease was first discovered on *Lithocarpus* spp. near Mill Valley in 1995. Since then it has spread throughout coastal counties

around the San Francisco Bay area where many *L. densiflorus*, *Q. agrifolia*, and *Q. kelloggii* have been killed (Rizzo et al. 2002, Davidson et al. 2002, 2005). Later the pathogen was found in Oregon, Washington, and British Columbia (Anon 2003, Davidson et al. 2005, Hansen et al. 2003a).

Subsequently, *P. ramorum* has been found in many European countries including Germany, Netherlands, Belgium, Denmark, Ireland, Italy, France, Norway, Slovenia, Spain, Sweden, Switzerland, the UK and Poland (Werres et al. 2001, Delatour et al. 2002, Moralejo, Werres 2002, Orlikowski, Szkuta 2002, De Merlier et al. 2003, Heiniger et al. 2004, Zerjav et al. 2004). In 2004 the Finnish Plant Production Inspection Centre (KTTK) found *P. ramorum* on *Rhododendron* in one Finnish nursery producing horticultural plants. It was detected by species specific PCR and identified morphologically (Fig. 4).

Symptoms and hosts

P. ramorum invades susceptible trees through the bark on which cankers with tarry or rusty coloured exudations develop. Later the leaves of infected trees may turn brown over a short period (Garbelotto et al. 2001). Non-lethal foliar infections on woody shrubs or other hosts in the understorey can serve as a source of inoculum for trees. Today over 40 plant genera have been found to be susceptible to *P. ramorum* (Rizzo et al. 2005). These include in North America besides *L. densiflorus*, *Q. agrifolia*, *Q. kellogii* and *Q. parvula* var. *shrevei* species such as *Q. chrysolepis*, *Umbellularia californica, Sequoia sempervirens, Pseudostuga menziesii, Acer macrophyllus* and *Aesculus californica*. The pathogen has also been found on *Vaccinium ovatum, Arbutus menziesii, Arctostaphylos manzanita, Heteromeles arbutifolia, Lonicera hispidula, Maianthemum racemosum, Rhamnus californica, Rosa gymnocarpa, Toxicodendron diversilobatum, Rubus spectabilis, Rhamnus purshiana, Corylus cornuta, Pittosporum undulatum* and *Trientalis latifolia* (Davidson et al. 2002, Goheen et al. 2002, Rizzo et al. 2002, Knight 2002, Hong 2003, Hüberli et al. 2004, 2005, Murphy, Rizzo 2003, Maloney et al. 2005). In Europe, *P. ramorum* was first found on *Rhododendron* and *Viburnum*, but later it was isolated a variety of plants, e. g. *Arbutus, Camellia, Hamamelis, Kalmia, Leucothoe, Pieris* and *Syringa* (Werres, De Merlier 2003, Beales et al. 2004a, b). In 2003 the pathogen was found on *Quercus falcata* in the UK, and shortly afterward on *Fagus sylvatica, Quercus ilex, Q. cerris, Castanea sativa, Taxus baccata* and *Aesculus hippocastanum* (Anonymous 2004a, Brasier et al. 2004b, Lane et al. 2004). In the Netherlands the disease has also been found on *Q. rubra* near diseased *Rhododendron*s (Anonymous 2004b).

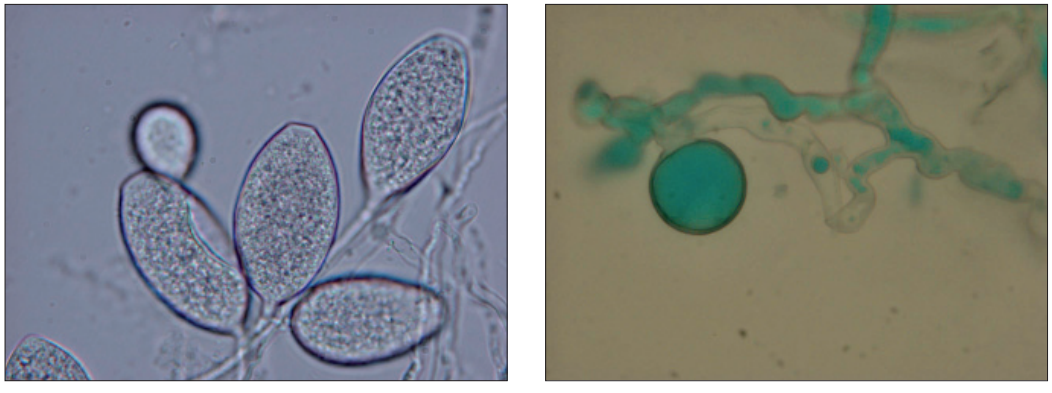

Fig. 4.
Sporangia (a), chlamydospores and coralloid hyphae (b) typical for *Phytophthora ramorum*

Mating type and origin

At first it was believed that the reason why we have not had a same kind of epidemic in Europe that has occurred in North America was that different mating types occurred in Europe (A1) and in North America (A2). However, in 2003 the occurrence of isolates of *P. ramorum* belonging to A1 type was reported from horticultural nurseries in North America and A2 type from Europe (Hansen et al. 2003a, Werres, De Merlier 2003). The AFLP-fingerprinting clustered European and American isolates separately within individual clades according the mating type (Ivors et al. 2004). Also the morphological characters separated the mating types in most cases so that the European isolates were much more homogenous than the North American isolates (Werres, Kamiski 2005). But, the genetic diversity among European isolates was greater than among *P. ramorum* isolates from North America (Brasier 2003, Werres, Zielke 2003, Brasier, Kirk 2004, Ivors et al. 2004). The A1 isolates grew faster had larger chlamydospores and did not produce gametangia with *P. cambivora* (Werres, Kaminski 2005). This suggests that the pathogen was separately introduced into North America and Europe from a third area which remains unknown, but probably localities in Asia.

OTHER *PHYTOPHTHORA* SPP.

A new *Phytophthora* species, described a few years ago, is *P. inundata*, which infects *Salix* in riparian ecosystems (Brasier et al. 2003). It also has other woody hosts such as *Aesculus, Olea* and *Prunus* and might be highly pathogenic after flooding or water logging (Brasier et al. 2003). Extensive studies on oak decline have revealed the presence of new species such as *P. quercina, P. psychrophila, P. europaea, P. uliginosa* and *P. pseudosyringae* (Jung et al. 1999, 2002, 2003). The last *Phytophthora* was also found in necrotic fine roots and in stem lesions of *F. sylvatica* and *A. glutinosa* (Jung et al. 2003). *P. quercina* was the most frequently isolated species from rhizosphere soil near declining oaks in Sweden (Jönsson et al. 2003). There was also a correlation between the presence of the pathogen and the vitality of oak stands (Jönsson et al. 2005). *P. nemorosa* is also a newly described species, which was found during an intensive survey for sudden oak death and *P. ramorum* in California and Oregon (Hansen et al. 2003b). A similar survey in the UK found *P. kernoviae*, which was isolated most often from *F. sylvatica*, also on necrotic lesions of *Q. robur* and *Liriodendron tulipifera* (Brasier et al. 2005).

In Finland a new, homothallic *Phytophthora* sp. from *Rhododendron* was shown to be highly pathogenic to many woody hosts including Norway spruce (Fig. 5).

Fig. 5.
Dying Norway spruce seedlings inoculated with a homothallic, unidentified *Phytophthora* sp. Inoculations were done placing mycelial plugs from agar culture on wounds on stems.

CONCLUSIONS

Studies made over the past decade have shown that many new *Phytophthora* species are associated with diseased trees. Most of them are not native in the area where they are a serious problem, e. g. *P. ramorum*, the cause of sudden oak death, is introduced separately to North America and Europe. Even old, native *Phytophthora*s might create new combinations through sexual recombination or somatic fusion which are more virulent than their parents. Typical for *Phytophthora* are also hybrids, a new combination produced by parents representing two different *Phytophthora* species, as was in the case for the *P. alni*-complex, which has altered riparian ecosystems throughout Europe. The fact that *P. ramorum* is present in a large forest area in Oregon shows that the assumption that *Phytophthora* spp. cannot adapt to weather conditions in Nordic countries is not true. Thus, we must be ready to prevent the spread of these introduced pathogens. The movement of infected plants should be avoided by strict quarantine regulations and control of all suspicious ornamentals and seedlings.

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SIROCOCCUS CONIGENUS: A NEW PATHOGEN IN FINNISH FOREST NURSERIES

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ABSTRACT

Recently, a new canker disease has been found on container-grown Norway spruce seedlings in Finnish forest nurseries. Using traditional isolation techniques and DNA based methods assays were made to identify the most common fungi in the cankers (Lilja et al. 2005). The fungi isolated from cankers were first separated into morphological groups based on colony colour, pattern and growth rate on malt extract agar. 18S rDNA profiling indicated that several Operational Taxonomic Units (OTUs) inhabited the cankers, some (OTU1 and OTU2) of which could be linked to the mycelial cultures which had been isolated from the cankers. Subsequent fungus isolations were also made from damped-off seedlings bearing pycnidia and conidia, resembling those of the conifer shoot pathogen *Sirococcus conigenus,* on the needles (Lilja et al. 2005). Although the cultures linked to OTU1, which was the most common isolate, differed morphologically from each other, the random amplified microsatellite analysis showed that isolates from cankers and from damped-off seedlings were genetically highly similar and represented a single species. Later, when the cultures were kept in natural sunlight, they all produced conidia morphologically similar to *S. conigenus* (Lilja et al. 2005). Recent inoculations showed that *S. conigenus* is pathogenic to 6- and 7-week-old seedlings where it caused desiccation and browning of needles, plus some of the seedlings died. A 60 h dark period at 4 – 5 °C before inoculation did not increase seedling mortality. One reason for this unexpected result might be that the inoculations were made under ambient conditions not conducive to efficient photosynthesis. In a third experiment, in which seedling trays were not covered with plastic after inoculation, the inoculation failed and all seedlings remained green and healthy and the pathogen could not be recovered from the seedlings.

INTRODUCTION

Today more than 61% of the 162.5 million forest tree seedlings produced in our nurseries are container-grown Norway spruce (Anonymous 2004). According to Poteri et al. (2005) some new cultural practices such as short day treatments (black-out) may increase the risk of fungus infections. Recently, a new type of canker disease has occurred on container seedlings in Finland. In past springs some nurseries culled over 10% of the Norway spruce, *Picea abies*, seedlings because of the cankers. The first symptom of the disease appears on individual seedlings where yellowing and wilting of the top occurs. Later needles on the current year shoot turn brown and fall off and lesions appear on the stems. A resin drop may occur on the affected area. Eventually the lesions either become compartmentalized or they girdle the stem and kill the seedling shoot (Fig. 1).

S. conigenus is known as a fungus affecting cones and shoots of several species of conifers in temperate and boreal forests in Europe and North America (Sutton 1980, Smith et al. 2003). In Finland this fungus has earlier been reported as occurring on cones and from forest trees (Kujala

1950, Tian Fu, Uotila 2002). As early as 1950 Kujala found black pycnidia on frost damaged shoots of Norway spruce, and identified the fungus as *S. conigenus*. More recently Tian Fu and Uotila (2002) isolated the pathogen from cones and dead shoots of Norway spruce saplings in the forest.

In nurseries *S. conigenus* causes a seed-borne damping off and tip blight and stem and branch cankers on the current-year growth on older seedlings (Sutherland et al. 1981, Sutherland 1987, Motta et al. 1996, Nef, Perrin 1999). Sutherland (1981) reported that post germination spread of the pathogen occurs from disease loci originating from seed-borne inoculum. Succulent needles of older seedlings are infected by conidia liberated from pycnidia and then the fungus grows into the elongated shoots where lesions and cankers form (Sutherland et al. 1981, Sutherland 1987).

This article is a synopsis of an article published in the Canadian Journal of Forest Research (Lilja et al. 2005). It also gives additional results from new tests done to confirm the pathogenity of *S. conigenus* isolated from cankers on container-grown Norway spruce seedlings. We also did work to determine if low temperature and a dark period before inoculation affect the rate and expression of disease symptoms.

Fig. 1.
Canker on two-year old Norway spruce

MATERIAL AND METHODS

Fungus isolations

Fungus isolations were made from 2-year old, container-grown Norway spruce seedlings which had lesions and cankers on the lower part of current year's stems. Later, isolations were also made from damped-off seedlings which had already developed pycnidia and conidia, resembling those of the conifer shoot pathogen *S. conigenus,* on the needles (Lilja et al. 2005). The isolated fungi were first divided into groups based on colony colour and pattern and growth rate on malt extract agar. In spring, when the cultures were exposed to sunlight on a laboratory window bench, some cultures sporulated, making it possible to also measure the conidia and confirm the identification using morphological characteristics.

18S rDNA profiling of cankers

Fungal 18S rDNA was also directly amplified from cankers using fungus-specific primers to detect the fungi inhabiting the cankers. The same primers were used to amplify the 18S rDNA from the mycelium from the cultures of each of the morphological groups isolated from the cankers and from damped-off seedlings. The amplification products obtained directly from cankers and from the mycelium from cultures were compared using denaturing gradient gel electrophoresis (DGGE) and similar mobility was used as the criterion to link mycelial cultures with directly amplified PCR products (Lilja et al. 2005).

RAMS-analysis

To compare the genetic similarity of isolates (LILJA et al. 2005) random amplified microsatellite (RAMS) analysis was done on single isolates from each morphological group from both cankers and damped-off seedlings.

Pathogenicity tests

In the first experiment, Norway spruce seeds (origin SV 111) were sown in peat (M6, Kekkilä Corp., Finland) in plastic pots (17 cm x 12 cm and 7 cm deep) on May 20, 2003. There were 12 seedlings in each of 20 pots. On July 8, when the seedlings were ca. six weeks old (height ca. 5 cm), they were inoculated with 1 ml of *Sirococcus*–conidia-suspension (10^4 conidia/ml) or pure water (controls) by pipetting a suspension onto the seedling's terminal shoot. Conidia were obtained from cultures of isolate S02 (originating from cankers on Norway spruce seedlings) grown on MA for 6 weeks in natural sunlight on a window bench in the laboratory. Sporulating cultures were then flooded with 20 ml of sterile demineralized water and the spores were gently dislodged using a glass rod. The spore suspension was collected into a separate bottle (100 ml) and the spore concentration was determined using a haemacytometer (Fuchte-Rosenthal). One hundred and twenty seedlings in 10 pots were inoculated and other 120 served as controls. Prior to inoculation half of the seedlings were placed in the dark for 60 h at 4 – 5 °C to predispose them to *Sirococcus* infection (WALL, MAGASI 1976, SUTHERLAND et al. 1981). After inoculation the trays were covered with a plastic sheet to keep the humidity high for 72 h. The seedlings were kept under fluorescent lights with an intensity of 8,000 lx and 8-h photoperiod in the laboratory at 20 - 22 °C. They were watered twice a week. The health of the seedlings was assessed visually every week for 6 weeks.

A second inoculation experiment, using seedlings from the same seedlot as for experiment 1, was made in the autumn of 2003 (experiment 2). The only difference was that the seedlings were 7-weeks old when inoculated. In a third experiment, also done in the autumn of 2003 (experiment 3) container-grown, 7-week old seedlings (Plantek-81, Lännen tehtaat), produced by the Suonenjoki nursery, were used for inoculation. The seedlings were inoculated in trays as before, but were not covered with a plastic sheet after inoculation.

In all of the experiments, to fulfil Koch's postulates, isolations were made 4 weeks after inoculation from 10 inoculated seedlings showing disease symptoms (but not dead) and from 10 non-inoculated, control seedlings.

The significance of differences in the number of diseased and dead seedlings in the different treatments: (i) pretreatment before inoculation (in light and warm or dark and cold), and (ii) inoculation (with *S. conigenus* spore suspension or pure water) were determined by analysing the data using the GLM-procedure in SPSS (ANONYMOUS 2002). The used model included: pretreatment, inoculation, replication and pretreatment x inoculation. F-tests were used to determine the significance of differences between the pre-treatment and inoculation treatment data (ANONYMOUS 2002).

RESULTS

Isolates

The most common isolates from cankers on 2-year old seedlings were separated into three groups based on colony colour, growth rate and pattern on MA, since they did not sporulate. Later when cultures were obtained also from damped-off seedlings, these cultures formed a new morphological group. The groups were: (i) the T-group characterized by an olivaceous green colony with immersed mycelium, (ii) the S-group with the same colony colour, but with a slower growth rate, (iii) the V-group, in which isolates had a whitish colony with immersed mycelium, and (iv) the K-group with the brown immersed colony (Fig. 2). The K-group isolates were from damped-off seedlings.

Later, when the cultures were kept (in the spring) in sunlight on a laboratory window bench most isolates in all morphological groups produced pycnidia and conidia morphologically similar to *Sirococcus conigenus*.

Fig. 2.
Mycelial cultures of *Sirococcus conigenus* in different morphological groups

18S rDNA profiling

DGGE profiling of 18S rDNA directly from cankers on seedlings resulted in profiles composed of one to nine amplification products depending on the primer pairs used. These results indicate that one very common Operational Taxonomic Unit (OTU 1) occurred in cankers, but that no specific fungus occurred at a detectable level in all cankers. In addition, several other amplification products were observed in the DGGE-analyses (other OTUs). The migration of amplification products from mycelial cultures, in different morpholocical groups (S-, T-, V- and K-group) were identical to the most common bands (OTU 1) obtained in direct analyses. It should be noted, that the cultures did not produce pycnidia and conidia at that time.

Confirmation of identification of isolates in different groups

Random amplified microsatellite (RAMS) analysis on single isolates from each morphological group from cankers and damped-off seedlings showed that the RAMS patterns of all isolates were highly similar. The banding patterns of isolates representing groups V and S were identical to that of the isolate representing group K, which had been isolated from damped-off seedlings which had already developed pycnidia and conidia, resembling those of *S. conigenus,* on the needles. The isolate representing the group T only differed in the migration rate of one marker.

CONCLUSION

Although the morphology of isolates in the S-, T- V- and K-group was different, results obtained by DGGE profiling and RAMS analysis indicated that they represent the same species.

Pathogenicity tests

The inoculation with *S. conigenus* spore-suspension was successful and increased the percent of diseased seedlings and the mortality of seedlings in the two first experiments ($p < 0.01$), but the predisposition treatment, in which seedlings were stored before inoculation in dark at 4 - 5 °C for 60 h did not significantly increase the final mortality rate of seedlings ($p > 0.05$) (Tables 1 and 2). However, in the first experiment where the seedlings were kept in dark and cold before inoculation, diseased seedlings were present 2 weeks after inoculation (Table 1). The age of seedlings did not affect disease incidence ($p > 0.05$). In both of the first experiments all diseased seedlings died within 6 weeks.

Tab. 1.
Percent of diseased Norway spruce seedlings and mortality two, four or six weeks after inoculation. The seedlings were six weeks old when inoculated with a conidial suspension of *Sirococcus conigenus* or water (control). Half of the seedlings were kept 60 h in the dark and cold before inoculation. Values are means, plus or minus the standard error of the mean.

Pretreatment	Treatment	Time after inoculation				
		2-week		4-week		6-week
		Diseased	Dead	Diseased	Dead	Dead
Temperature: 20 – 24 °C Light intensity: 8,000 l*x*	Control	0	0	0	1.6 ± 1.6	1.6 ± 1.6
	S. conigenus	0	0	34.9 ± 10.6	13.3 ± 6.7	48.2 ± 8.9
Temperature: 4 – 5 °C Dark: 60 h	Control	0	0	0	0	0
	S. conigenus	14.9 ± 6.10	0	15.0 ± 4.8	39.9 ± 8.7	54.9 ± 5.6
Statistical significance	Pretreatment	0.26	-	0.00	0.00	0.00
	Treatment	0.26	-	0.11	0.00	0.64
	Pretr. x Treat.	0.03	-	0.14	0.00	0.48

Tab. 2.
Percent of diseased Norway spruce seedlings and mortality two, four or six weeks after inoculation. The seedlings seven weeks old when inoculated with conidia suspension of *Sirococcus conigenus* or water (control). Half of the seedlings were kept 60 h in dark and cold before inoculation. Values are means, plus or minus the standard error of the mean.

Pretreatment	Treatment	Time after inoculation				
		2-week		4-week		6-week
		Diseased	Dead	Diseased	Dead	Dead
Temperature: 20 – 24 °C Light intensity: 8,000 l*x*	Control	0	0	1.6 ± 1.6	0	1.6 ± 1.6
	S. conigenus	8.3 ± 6.4	0	9.9 ± 4.8	41.6 ± 9.4	51.6 ± 8.1
Temperature:4 – 5 °C Dark: 60 h	Control	0	0	0	0	1.6 ± 1.6
	S. conigenus	0	0	28.3 ± 7.2	24.9 ± 8.7	53.3 ± 6.7
Statistical significance	Pretreatment	0.22	-	0.79	0.21	0.99
	Treatment	0.22	-	0.00	0.00	0.00
	Pretr. x Treat.	0.22	-	0.06	0.25	0.78

In the third experiment, in which seedling trays were not covered with a plastic sheet after inoculation, the inoculation failed and all seedlings were green and healthy 6 weeks after inoculation.

Material from diseased and healthy looking seedlings were plated onto agar 4 weeks after inoculation. In the first experiment the number of successful isolations of *S. conigenus* was high for inoculated seedlings. In most cases the pathogen was obtained from desiccated seedlings or seedlings having brown needles (Table 3). *S. conigenus* was also detected in the second experiment. However, the percentage of inoculated seedlings, from which the pathogen was re-isolated, was lower than in the first experiment (Table 3). In all experiments healthy, control seedlings were pathogen free as were the inoculated seedlings in the third experiment.

Tab. 3.
Percentage of seedling isolations yielding *Sirococcus conigenus* four weeks after inoculations. Ten seedlings were sampled from each treatment, usually two seedlings from each replication. Half of the seedlings were kept 60 h in dark and cold before inoculation. Values are means, plus or minus the standard error of the mean, n = 10.

Pretreatment	Treatment	Experiment 1	Experiment 2
Temperature: 20 – 24 °C	Control	0	0
Light intensity: 8,000 l*x*	*S. conigenus*	80 ± 12.2	60 ± 60.5
Temperature: 4 – 5 °C	Control	0	0
Dark: 60 h	*S. conigenus*	80 ± 12.2	70 ± 12.2

DISCUSSION

Traditional isolation technique failed to reveal the pathogen responsible for causing the canker disease on Norway spruce. This was because the most commonly isolated fungi did not sporulate during the early phase of the study (Lilja et al. 2005). Consequently, it was only possible to divide the cultures isolated from cankers into different groups based on colony pattern, colour and growth rate. DNA based methods have been successfully applied to detect specified pathogens directly in plant tissues (e. g. Bahnweg et al. 2000, Paavolainen et al. 2001). Another relatively new technique for analysing fungal communities is 18S rDNA profiling, which should, at least in theory, be useful when no specific pathogen is suspected beforehand. This analysis is based on the amplification of partial 18S rDNA by fungus-specific primers (e. g. Kowalchuk et al. 1997, Vainio, Hantula 2000), and separation of the resulting DNA-fragments by denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (Tsuchiya et al. 1994) or restriction analysis (Vaneechoutte et al. 1992). In our study the DGGE profiling of 18S rDNA directly from cankers revealed that several OTUs inhabited the cankers and the most common OTU 1 could be linked to mycelial cultures also isolated from the cankers and from damped-off seedlings. In the RAMS analysis the banding patterns of isolates from each morphological group from cankers and damped-off seedlings were highly similar. On damped-off seedlings pycnidia were already present and the dimensions of the two celled, fusiform conidia were within the limits for *S. conigenus* as were also the conidia produced later on most cultures (Kujala 1950, Sutton 1980, Ellis, Ellis 1985, Tian Fu, Uotila 2002, Smith et al. 2003).

Prior to 2002 *S. conigenus* had not been isolated from nursery seedlings in Finland, but it was known to occur in forests on cones and on dying shoots of conifer trees (Kujala 1950, Tian Fu, Uotila 2002). Results of this study show that *S. conigenus* isolated from cankers on nursery seedlings of Norway spruce was pathogenic to 6- and 7-week old seedlings where it caused desiccation and browning of the needles of spruce and eventually seedling death.

In the first two inoculation experiments, *S. conigenus* was pathogenic although the mortality of Norway spruce seedlings was lower than that of seedlings in tests done with North American spruce species (Sutherland et al. 1981). The mortality of Sitka spruce (*P. sitchensis*) and Engelmann spruce (*P. engelmannii*) was 87 - 98% and 73 - 98%, respectively (Sutherland et al. 1981). However, in our tests the percentage of diseased and dead Norway spruce seedlings was 48 - 55% which is much higher than in inoculations done with *S. conigenus* isolates from cones (Tian Fu, Uotila 2002). In those tests the average infection percentage of Norway spruce and Scots pine seedlings was 14%, whereas inoculation of cones resulted in pycnidia production on all inoculated cones (Tian Fu, Uotila 2002). It has been suggested that the host susceptibility might vary for *S. conigenus* (Peace

1962, O'Brien 1973, Illingworth 1973). According to Peache (1962) the fungus, at that time a new pathogen on nursery seedlings of lodgepole pine (*Pinus contorta*), was a different variety from that on Norway and Sitka spruce. However, a recent study showed that genetic variation among *S. conigenus* isolates from pine and spruce from Europe and eastern and western North America was low (Smith et al. 2003). Only the isolates from hemlock (*Tsuga canadensis*) formed a separate group based both on genetic markers and cultural characteristics (Smith et al. 2003).

Previous researchers have identified some of the conditions which predispose trees to *S. conigenus*. In Austria, fertilization and unbalanced tree nutrition have impact on the expression of *Sirococcus* shoot blight on Norway spruce in the forest (Angleberger et al. 2002, 2003). Needle analysis revealed significant differences in the foliar contents of Mg, Ca, P and Mn between healthy and diseased Norway spruce trees (Angleberger et al. 2003). Based on inoculation experiment results, high humidity at or near 100 % RH is required for at least 1 day after inoculation for infection to be successful (Smith 1973, Wall, Magasi 1976). In our third experiment, where the inoculated seedlings were not enclosed in plastic bags, no infection occurred and attempts to re-isolate the pathogen from these seedlings failed. In the first two experiments, the trays were covered with plastic sheeting for 72 h after inoculation. This obviously kept the humidity high enough, because the number of diseased seedlings was high in both experiments. One unexpected finding was that the number of infected seedlings subjected to continuous illumination and temperature at 20 °C was almost the same as that for seedlings kept in the dark at 4 - 5 °C for 60 h before inoculation. In previous studies, dark period before inoculation has been a good tool to predispose seedlings and confirm the success of infection (Funk 1972, Wall, Magasi 1976, Sutherland et al. 1981). For example, Funk (1972) sprayed hemlocks with a conidial suspension and symptoms appeared only on seedlings that had been subjected to a dark period before inoculation, but not on seedlings that had been kept continuously in light. In our case, one possible reason for the failure to find that darkness did not predispose seedling to infection might be that the inoculation experiment was done using an illumination capacity of only 8,000 lx which is roughly 10 - 15 % of the lighting intensity of normal daylight (Gates 1980).

In our studies we inoculated 6- and 7-week old seedlings. In our nurseries the cankers from which the *S. conigenus* has been isolated have been on 2-year old seedlings. The first symptom of the disease appears on individual seedlings in July. Needles on the current year shoot have already turned brown beginning from the base of needles, and later the fungus has presumably spread into the growing shoots where lesions form. Evidently, the lesions have over time either be compartmentalized, causing small cankers or they have girdled the stem and caused the death of seedling shoots. In 2002, the pathogen was isolated for the first time from damping-off affected seedlings with pycnidia on needles. However, as we cannot be sure that the pathogen in our nurseries is seed-borne, the inoculum source for nursery seedlings might well come from nearby forests.

In conclusion the DNA based methods together with traditional isolation techniques clearly showed that one fungus species was common in the cankers. Later when the cultures in the different morphological groups sporulated and spore measurements were taken and inoculation trials made with spore suspensions we were convinced that *S. conigenus* was the main pathogen responsible for the new canker disease. Also, our inoculation experiments showed that under suitable conditions this fungus is very pathogenic to Norway spruce seedlings.

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IDENTIFICATION OF *ARMILLARIA* SPECIES FROM SOIL BY NESTED-PCR REACTION

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Armillaria sp. is generally distributed white-rot fungus comprising 7 European species. A*rmillaria borealis, A. ectypa, A. gallica* and *A. tabescens* are generally considered as weak parasites whereas *A. mellea, A. ostoyae* and *A. cepistipes* as serious pathogens of the stressed trees. The similarities of their culture features represent a problem for identification. The most common method of identifying *Armillaria* isolates is based on pairing test using the comparison of mycelium isolates with single-basidiospore isolated of known identity. However, this method is time consuming and in the case of diploid samples the results are often difficult to interpret. In recent years the molecular biological methods based on analysis of ITS region of rDNA were established. The non-specific primers ITS1 and ITS4 based on the conserved regions of nuclear small rDNA and nuclear large rDNA are used for its amplification. Therefore, they cannot be used for specific amplification of ITS region of *Armillaria* from natural samples without previous isolation and cultivation of *Armillaria* mycelium which is time consuming.

In this study, we describe a new set of specific primers AR1 and AR2 suitable for direct identification of European *Armillaria* sp. (except *A. ectypa*) from soil based on the conserved sequences within the ITS region of *Armillaria* species. DNA was extracted by commercial isolation kit which proved to be rapid and easy when compared with published classical isolation protocols. For amplification nested PCR reaction with external non-specific primers ITS1 and ITS4 and internal specific primers AR1 and AR2 was used. Consequently, individual species were distinguished by restriction analysis of their amplicons by restriction endonucleases *Hinf*I and *Mbo*I. However, species *A. mellea* and *A. tabescens* could be discriminated on the basis of different lengths of amplicons.

Up today 20 soil samples of forest soil collected in Czech Republic were successfully analysed. The identification of *Armillaria* species in the soil samples was in agreement with the fact that these species had previously been found in the respective areas. Presently the method is further tested by Mendel's University of Agriculture and Forestry in Brno.

DETECTION OF FUNGI IN FINE CONIFER SEEDLING ROOTS IN FOREST NURSERIES: MORPHOTYPING, ISOLATION AND DIRECT SEQUENCING

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ABSTRACT

Fungi colonizing healthy root tips of *Pinus sylvestris* and *Picea abies* grown in forest nurseries were assessed by morphotyping, isolation and direct sequencing. The overall community consisted of 33 morphotypes. Mycelial isolation from 8,535 root tips representing 12 morphotypes yielded 616 isolates which after sequencing were identified as 46 taxa. Direct ITS rDNA sequencing of 130 root tips representing 33 morphotypes identified 60 fungal taxa. Only 14% of taxa were detected by both isolation and direct sequencing methods. Taxa often detected by direct sequencing were *Amphinema byssoides*, *Phialophora finlandia*, *Rhizopogon rubescens*, *Suillus luteus*, and *Thelephora terrestris*. On the other hand, *P. finlandia* and *Phialocephala fortinii* were most often isolated as mycelia. Our results demonstrate the power of direct sequencing as an identification method compared with fungal isolation and morphotyping. However, the latter remains a fast, cheap and reliable method for a preliminary assessment of the presence or absence of mycorrhizal colonization.

INTRODUCTION

Fungal colonization of root systems is an important factor in determining seedling vigour and, consequently, their quality (Smith, Read 1997). A number of mycorrhizal fungi have been shown to have a positive impact on seedling health and productivity in forest nurseries (Jumpponen 2001, Sampagni et al. 1985, Sinclair et al. 1982, Stenström et al. 1997). Furthermore, following the transfer and outplanting of the seedlings into the field, mycorrhizal fungi promote survival, establishment and growth of young trees in newly established forest plantations (Kropp, Langlois 1990, Le Tacon et al. 1994, Perry et al. 1987, Stenström et al. 1990). The main mechanisms for this are thought to be enhanced uptake of water and nutrients (Smith, Read 1997), lengthened root life (Wilcox 1996), and protection against environmental stress factors such as drought, pathogens and heavy metal pollution (Chakravarty, Unestam 1985, Colpaert, Vanassche 1992, Morin et al. 1999, Ortega et al. 2004, Van Tichelen et al. 2001).

Previous studies on root-associated fungal communities in forest nurseries have mainly been done by morphotyping (morphological and anatomical identification) of mycorrhizal root tips (Grogan et al. 1994, Ursic et al. 1997), fungal isolation (Kope et al. 1996, Lilja et al. 1992) or a combination of both these methods (Danielson et al. 1984, Ursic, Peterson 1997). More recently, it has been demonstrated that direct sequencing of fungal DNA from root tips could be a powerful tool for identification of fungi (Egger 1995, Horton, Bruns 2001). The method has proved to be sensitive for the detection of potentially all root-inhabiting fungi, in particular the taxa that are usually overlooked both by morphotyping and isolation, e. g. latent pathogens or slow-growing endophytes (Kernaghan et al. 2003). The main aim of the present work was to identify fungi colonizing roots of healthy *Pinus sylvestris* and *Picea abies* seedlings in forest nurseries, and to compare the efficacy of different identification methods: morphotyping, pure culture isolation and direct DNA sequencing.

MATERIALS AND METHODS

Pinus sylvestris and *Picea abies* seedlings were collected from six forest nurseries situated within a radius of 150 km in the south-western and central part of Lithuania. Samples were collected in April 2001 and 2002. After collecting, the root systems were excised from the stems, individually packed into plastic bags, transported to the laboratory and kept at 4 °C for a maximum period of four weeks.

Mycorrhizal tips were identified by the presence of a mantle, external hyphae or rhizomorphs, the absence of root hairs, a slightly swollen apex and, in pine, dichotomous branching of the fine roots. Macroscopic features were examined using a Carl Zeiss Stemi 2000-C dissection microscope (Oberkochen, Germany). In the absence of macroscopic mycorrhizal features, sections were made of root tips using a razor blade to verify the presence of a Hartig net. Root squashes were used to examine the mantle, hyphae and rhizomorphs microscopically. These features were examined using a Carl Zeiss Axioplan microscope (Oberkochen, Germany) at 1,000x magnification. The morphotypes were identified as far as possible by comparison with published descriptions (Agerer 1986 - 1988, Agerer et al. 1996 - 1998). Following the examination, up to five mycorrhizal root tips of each morphotype were taken from each root system and placed separately in 1.5 ml centrifuge tubes, labelled and stored at -40 °C for direct DNA sequencing.

The isolation of fungal cultures was attempted from 8,535 individual mycorrhizal root tips, and included multiple representations of 12 different morphotypes. Prior to isolation, root tips were placed in 10 - 20 mm net bags (mesh size 0.2 x 0.2 mm), sterilized in 33% hydrogen peroxide for 15 - 60 s, and then rinsed three times in sterile deionized water (Danielson 1984, Sieber 2002). Tips were plated onto modified Melin Norkrans medium (Marx 1969) and incubated at room temperature in the dark. Dishes were checked daily and emerging cultures were transferred onto fresh agar medium. Fungal mycelia were examined under a microscope, and were grouped accordingly to morphological characteristics. For identification, one to five representative cultures from each morphological group were ITS rDNA sequenced. Extraction of DNA, amplification and sequencing followed the method described by Rosling et al. (2003).

From 33 different morphotypes, 1 - 22 individual root tips were selected for direct sequencing. More replicates were taken from more common morphotypes, to encompass, when available, both tree species. A total of 130 root tips was subjected to direct sequencing of the internal transcribed spacer (ITS) of the fungal ribosomal DNA (rDNA). Databases at both GenBank (Altschul et al. 1997) and at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala were used to determine the identity of sequences. The criteria used for deciding on the taxon or genus for a given strain was its intra- and interspecific ITS sequence similarity to those present in the databases.

RESULTS

In both pine and spruce, 45.5 - 100% of studied plants were colonized by mycorrhizal fungi. The level of mycorrhizal colonization varied considerably between individual plants, and significant differences were observed between plants from different nurseries. A total of 33 distinct morphotypes was observed among the total of 30,166 root tips from 660 plants, studied during the present work, 14 of which were unique for pine, 7 for spruce, and 12 (36.4%) were found on both tree species. The most common morphotypes on pine were: “Yellow brown”, “Thelephora”, “Piceirhiza bicolorata”, and “Suillus luteus”, observed on 12.7 - 94.5% of plants and up to 63.8% of root tips examined. In root systems of spruce the dominant morphotype was “Amphinema” (on 28.5 – 91.8% of plants, 10.2 – 80.5% root tips), followed by “Piceirhiza bicolorata” and “Thelephora”.

Direct sequencing of 130 root tips with 33 distinct morphotypes revealed the presence of 60 fungal taxa, and pure culture isolation from 8,535 root tips with 12 morphotypes yielded 616 isolates representing 46 taxa. The other 7,919 tips (92.8%) remained sterile, probably due to surface sterilization. When pooled, direct sequencing and isolation detected a total of 93 fungal taxa, 74 of which (79.6%) were identified at least to a genus level. The overlap between the two methods was low. Only 13 (13.9%) of taxa were both sequenced and isolated, 47 (50.5%) were detected exclusively by sequencing, and 33 (35.5%) exclusively by isolation. Within the set of morphotypes that was both sequenced and subjected to isolation, a total of 63 taxa were detected but only 8 of these (12.7%) were detected using both of the methods.

The fungi, most commonly detected by direct sequencing were the basidiomycetes *Suillus luteus*, *Thelephora terrestris*, *Rhizopogon rubescens*, *Amphinema byssoides*, *Inocybe* sp. NS114, and ascomycetes from the genera *Wilcoxina* and *Tuber*, and *Phialophora finlandia*. The fungi, most frequently isolated, included the ascomycetes *P. finlandia*, *Phialocephala fortinii*, and representatives from the genera *Hymenoscyphus*, *Leptodontidium* and *Oidiodendron*. Moreover, almost all of the fungi from the three latter genera were not detected during direct sequencing from root tips, but detected exclusively following pure culture isolations. Nevertheless, the efficiency of isolation was much lower than that of direct sequencing. Thus, during the sequencing a maximum of 4 fungal taxa was detected in just a single root tip, and on average the method revealed the presence of 1.23 - 1.61 distinct fungi per one root. By contrast, the detection of a similar number of taxa by isolation would require processing of 150 – 250 root tips; the success of isolation, expressed as the average number of cultures isolated from a single tip, was 0.03 - 0.09. The difference between those proportions, when compared by chi-squared test, was highly statistically significant ($p < 0.0001$).

Single, distinct morphotypes commonly hosted several fungal taxa. In fact, there were only 4 morphotypes (12.1% of all observed), where just one taxon of fungus was detected, while the remaining 29 hosted 2 - 41 different taxa each. On average, from each single morphotype, direct sequencing, as well as sequencing or morphological identification of isolates revealed 6.5 fungal taxa. Some less defined mycorrhizal morphotypes were associated with a high diversity of taxa. These were "Yellow brown" (41 spp.), "Amphinema" (27 spp.), "Thelephora" (19 spp.), "Suillus" (15 spp.), "Yellow" (14 spp.) and "Suillus luteus" (13 spp.).

On the other hand, individual fungal taxa were also commonly detected in different mycorrhizal morphotypes. Thus, among the total 93 taxa in the present study, 46 (49.5%) were found in different morphotypes, inhabiting 2 - 19 morphotypes each. On average, an individual taxon there was likely to be found in 2.4 different morphotypes. Fungi, detected in the highest diversity of morphotypes were *Phialophora finlandia* (in a total of 19 morphotypes: sequenced from 14 and isolated from 10), *Phialocephala fortinii* (8 : 1 and 8), *Thelephora terrestris* (7 : 7 and 0), *Wilcoxina* sp.706 (7 : 7 and 1), *Rhizopogon rubescens* (6 : 6 and 1) and *Hymenoscyphus* sp. 701 (6 : 3 and 4).

Despite the high diversity found within the morphotypes and the relatively wide range of morphotypes in which individual fungal taxa were detected, both direct sequencing and, to a lesser extent isolation, confirmed our morphotyping. There were 11 morphotypes among the total of 33, which were deemed to represent a certain fungal genus. In fact, the corresponding mycorrhizal taxa were sequenced or isolated from 10 of them. Thus, *Amphinema byssoides* and *Suillus luteus* were both sequenced and isolated from the respective "Amphinema" and "Suillus luteus" morphotypes. From the "Cenococcum" morphotype we detected *Cenococcum geophilum* by sequencing; from "Hebeloma", - *Hebeloma* sp. NS21; from "Laccaria", - *Laccaria proxima*; from "Rhizopogon", - *Rhizopogon rubescens*; from "Suillus", - *Suillus bovinus* and *S. granulatus*; from "Thelephora", - *Thelephora terrestris*; from "Tuber" - *Tuber* sp. 734 (also isolated) and sp. NS206A and *Hymenoscyphus* sp. 678 was isolated from the "Piceirhiza bicolorata" morphotype, which is known to host *Hymenoscyphus* spp. (Vrålstad et al. 2000). However, in one case (9.1%)

our morphotyping apparently misidentified the fungal genus, since *Rhizopogon rubescens* was sequenced from the morphotype "Tricholoma".

On the other hand, despite the fact that in 10 taxonomically defined morphotypes the corresponding taxa were present, the proportion of root tips from which these taxa were sequenced was not always high (33 - 100%). For the "Amphinema" morphotype the "right" fungus was sequenced from 3 root tips out of 7; in "Cenococcum" from 1 root tip out of 1; in "Hebeloma" from 1 out of 1; in "Laccaria" from 2 out of 4; in "Rhizopogon" from 1 out of 1; in "Suillus" from 3 out of 9; in "Thelephora" from 5 out of 15; in "Suillus luteus" from 11 out of 13, and in "Tuber" from 5 out of 14. However, despite this mismatch, other mycorrhizal taxa were sequenced from "mismorphotyped" root tips, showing that all these root tips were indeed mycorrhizal.

A total of 68 taxa of fungi was found in root systems of pine, and 52 taxa in spruce. Among those, 41 were unique for pine, 25 unique for spruce, and only 27 (28.7%) were found on both tree species, indicating that mycorrhizal roots of both tree species in most cases were colonized by different fungal taxa. Besides the differences in fungal community structure, both tree species exhibited profound differences in richness of taxa, as fungal diversity in root systems of pine was significantly higher than that in spruce. In pine, the most commonly sequenced fungi were the basidiomycetes *Suillus luteus*, *Thelephora terrestris*, *Rhizopogon rubescens*, and the ascomycete *Phialophora finlandia*, while in spruce the most commonly sequenced taxa were the basidiomycetes *T. terrestris*, *Amphinema byssoides*, *Inocybe* sp. NS114, and the ascomycetes from the genera *Wilcoxina* and *Tuber*. The most frequently isolated fungi were to a large extent common for both tree species, and included the ascomycetes *P. finlandia*, *Phialocephala fortinii*, and the representatives from the genera *Hymenoscyphus*, *Leptodontidium* and *Oiodendron*.

DISCUSSION

In the past, most of the community studies of mycorrhizal fungi have been based exclusively on morphotyping (Fransson et al. 2000, Grogan et al. 1994, Ursic et al. 1997). However, it has already shown (Kåren, Nylund 1997, Wurzburger et al. 2001), that morphotyping on its own is not sufficient and that mismatching of mycorrhizal morphotypes with ITS types occurs. Accordingly, it was found that an individual fungal taxon may form mycorrhizal roots with different morphologies and that mycorrhizal roots with similar morphologies may be formed by different taxa.

The present study demonstrates that morphotyping, despite being a reliable tool in determining some fungal genera, provides only very limited information on fungal diversity in mycorrhizal root tips. Distinct morphotypes were very frequently found to host surprisingly many taxa, while a particular taxon was commonly encountered in several different morphotypes. Our results indicated that the presence of several mycorrhizal basidiomycetes within a single morphotype is not uncommon, as e. g. *Paxillus involutus*, *Rhizopogon rubescens*, *Rhizopogon* sp. NS164, *Suillus bovinus* and *S. granulatus* were sequenced or/and isolated from the "Suillus" morphotype, while *Amphinema byssoides*, *Laccaria proxima*, *R. rubescens*, *Thelephora terrestris* and *Tomentella ellisii*, - from the "Thelephora" morphotype. On the other hand, *Cenococcum geophilum* and *Hebeloma* sp. NS21 were detected by direct sequencing, and *Suillus luteus* sequenced and isolated from corresponding morphotypes only, implying that their mycorrhizal structures in the fine roots might be more consistent. However, it has also been reported that *C. geophilum* may concurrently colonize roots with other fungal taxa (Kåren, Nylund 1997, Zak, Marx 1964). In most cases, the fungi inhabiting root tips together with the other taxa were the ascomycetes *Phialophora finlandia* and *Phialocephala fortinii*, present in 19 (57.6%) and 8 (24.2%) of morphotypes, respectively. Occasionally, our work revealed the presence of fungal pathogens of conifer seedling roots, such as *Nectria radicicola* (teleomorph of *Cylindrocarpon de-*

structans) or *Fusarium oxysporum*. The present study thus shows that functionally different fungal taxa co-exist within mycorrhizal root tips.

A prerequisite for discovering high diversity is the combination of different methods and sampling strategies. In particular, the diversity of detected fungi increased due to combining direct sequencing with isolation, as different fungi were often detected by each of the methods. The direct sequencing, for example, more commonly yielded mycorrhizal basidiomycetes such as *Laccaria* spp., *Rhizopogon* spp., *Suillus* spp., *Tomentella* spp, *Thelephora terrestris*, many of which were seldom or never isolated into pure culture. Isolation, on the other hand, more commonly detected ascomycetes, such as *Phialophora finlandia*, *Phialocephala fortinii* or *Oidiodendron* spp. In a related Canadian study (KERNAGHAN et al. 2003) the comparison of species data obtained both by direct sequencing and isolation from conifer seedling roots also showed different pictures of fungal species composition and abundance. Thus, despite lower efficiency compared with direct sequencing, pure culture isolation proved to be a valuable complementary tool for analysis of fungal communities colonizing conifer seedling roots.

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ALDER DECLINE IN POLAND

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ABSTRACT

In Poland, the problem of an ever increasing decline and dieback of alders has been continuing since 2000. At some sites tree dying has occurring en masse, while elsewhere the disease is developing slowly. Abnormal mortality among alders has already been noted in many of Poland's forest districts, particularly those within the Lublin Regional Directorate of the State Forests (RDSF). Research undertaken in 2002 revealed that *Phytophthora alni* was the main cause of this decline in many forest districts. The severe drought of 2002, with its negative consequences for forest ecosystems, e. g. low soil water and a decrease in the water table, dramatically increased tree mortality. Last year, in Lublin RDSF the area afflicted by alder decline exceeded 2,460 ha, a high proportion of the trees already being dead. In other regional directorates the situation was similar with Katowice RDSF reporting 3,017 m^3 and Wrocław RDSF 3,267.2 m^3 of dead trees. These losses have been causing foresters much economic concern. Taking into account simultaneous health problems with ash, they have faced major problems when it comes to choosing the right composition of forest tree species for the most humid sites.

Key words: alder decline symptoms, *Phytophthora alni* isolations, health status of stands

INTRODUCTION

Alders in Poland are an important component of forest stands as well as riparian ecosystems. The appearance of the new disease phytophthorosis among European alders is thus of great concern. This disease was first noted in 1993 in the United Kingdom (Brasier et al. 1995, Gibbs et al. 1994) and in the following year from Germany (Hartmann 1995, Jung, Blaschke 2004) and then from Austria (Cech 1997, 1999). During the last IUFRO conference devoted to *Phytophthora*, held in Freising (Germany) in 2004, the several examples of severe alder decline were cited. While the British and French cases of dieback among alders have been noted mainly along rivers, those in Germany have also involved nursery seedlings and alders growing on former agricultural land (Schumacher et al. 2001). Furthermore, the disease has not remained confined to these countries over the last decade, but has spread to include all of Europe from Italy to Sweden (Orlikowski et al. 2003). The causal agent has been described as *Phytophthora alni* (Gibbs et al. 2003, Brasier et al. 2004), a fungus causing rotting of roots and the root collars, stem-bases or the entire trunk (Oszako, Orlikowski 2004). In Poland in the last 8 years black alder has been particularly affected by dieback and death (Oszako et al. 2003). This new disease is becoming increasingly severe and it is of great concern among Polish foresters (Oszako 2005). The aim of this paper is to identify the range of alder decline in Poland, the factors involved in disease development and propose a solution to the problem.

MATERIALS AND METHODS

Research was made on alder decline from 2000 to 2005 and involved five areas: (i) an analysis of information concerning alder stands sent in by foresters to the Department of Forest Phytopathology of the Forest Research Institute, especially in the form of the so-called "disease indication sheets"; (ii) data obtained from certain regional directorates of the State Forests as regards alder death; (iii) analyses of samples of plant material, soil and water taken from nurseries and forest stands or sent in to the Department of Forest Phytopathology; (iv) identification of fungi or algae-like organisms to generic or species levels using traditional methods and (v) analysis (of similarities and differences) of the descriptions of the disease available in the European literature.

Plant material

Samples of diseased bark, roots, parts of stems and trunks of alder were taken from both nurseries and streamside (mainly tributaries of the Vistula) trees and from forest stands (mainly in the RDSFs of Warsaw and Lublin). The study trees were 5 to 90 years old. Isolations were made from fragments of bark and wood at the point where damaged and healthy tissues meet. For seedlings and young trees these were roots and trunk bases, while in older trees they were oval or irregularly-shaped, grey-brown or black patches. The samples were placed in sterile plastic bags and brought to the laboratory where within 10 to 20 hours after sampling pieces of diseased tissue (following surface sterilization) were plated onto potato-glucose (PDA) medium, as well as being placed in apples for isolation of *Phytophthora* spp. Segments of diseased tissue (about 0.5 cm long) from each tree were plated out in at least six Petri dishes with PDA. After 2 to 3 days incubation the fungi growing from the segments were transferred onto PDA slants in test tubes.

Soil analysis

Analysis of soils for *Phytophthora* zoospores was done using rhododendron leaf traps. These were placed in cuvettes each containing 0.5 kg of soil collected from the vicinity of trees showing signs of dieback, and watered subsequently. After several days (once brown patches had appeared on the leaf pieces), fragments of diseased tissue were plated onto PDA medium. After 24 to 48 hours incubation, the colonies growing up around diseased fragments of tissue were transferred into test tubes with PDA. The resulting cultures were identified to genus and species levels.

Analysis of water

Isolation of *Phytophthora* spp. from water was done with the aid of traps in the form of the apical parts of the alder and rhododendron stems. These were placed in the water and, after 3 to 5 days incubation the number of patches and then the amount of diseased tissue were recorded. Following flame disinfection of the surface, the samples were transferred to PDA medium; the remaining procedure was the same as for isolation of *Phytophthora* spp. from soil.

RESULTS AND DISCUSSION

The occurrence of alder and disease symptoms

Black alder is a component of mixed broadleaf forests, especially those on marshy or wetland sites, plus it is a major species growing along riverbanks and lakes shores and other bodies of water. Black alder is also grown in parks and gardens and even as hedges. Consequently, it is plentiful enough for possible outbreaks of *P. alni*, which might spread across the entire country, i. e. correlating to the host plant range. The pathogen most likely enters the host through roots (Werres 1998). The precise place of infection and course immediately thereafter are unknown. Most likely from the infection site

the pathogen spreads to the root collar and then up the trunk. Species of the genus *Phytophthora* probably destroy cambium tissues. Infection is thus accompanied by a cessation of incremental growth in plants and by splitting of the tissues the pathogen spreads into the trunk. The destruction of the vascular tissues results in the tree suffering from water stress which becomes especially evident during hot weather. A combination of high temperature and a lack of water may lead to rapid dieback and death of trees. Similarly, disease development may be slower when the growing season is favourable in the availability of water or when conditions are not conducive for the pathogen. All *Phytophthora* species require water for zoospore development. Diseased trees may be identified easily since they have smaller leaves and are chlorotic.

These symptoms, though characteristic for phytophthorosis, may also be caused by other root pathogens, e. g. the honey fungus (OSZAKO, ORLIKOWSKI 2004). Dark discoloration appears on trunks, while affected bark is soft and watery, and a watery discharge may be common. The underlying tissue is often red-brown.

The red-brown discoloration always spreads from base of the trunk upwards, the lesion assuming a lenticulate or lingulate shape clearly discernable from the healthy, pale tissue. Infected trees often bear a heavy seed crop, only to die shortly thereafter. However, it is possible for trees to survive and even regain their health. The development of disease symptoms may be enhanced by prolonged drought. Some state of health, inventory data of trees along rivers indicate rapid and unavoidable mortality among infected trees, e. g. in the southern UK, northeast France, south western and eastern Germany and north eastern Poland, while trees such as those from Upper Austria relate to markedly improved tree health. Very little is known on the influence of the vitality of the host-trees on the development of the disease, but such knowledge would help to explain, for example, appearance of the disease following water-table fluctuations caused by extended drought or flooding. In marshy ecosystems in The Netherlands (Die Wieden), no visible signs of damage were noted among trees even though the pathogenic strain of *P. alni* was present.

When the first symptoms of phytophthorosis were noted in England in 1993 (GIBBS et al. 1994), all the diseased trees were characterized as having abnormally small, chlorotic leaves and discharges on the trunks. In Poland, the latter phenomenon has not always been observed, e. g. in Spała Forest District, trees with damaged roots did not have a cankerous discharge on trunks, just bark splitting. Only after removal of bark was necrosis evident on living tissues, particularly at the bases of dying stems. In its advanced stage the disease also causes necrosis of the phloem. *Phytophthora alni* has been isolated from such tissues. The death of crown stems was thus a consequence of malfunction of vascular tissues within the tree trunks. As in the UK, the dead bark of infected boughs and trunks was only barely visible as a surface concavity. Only after cutting was the boundary of the active necrosis seen as "tongues of flame". The development of the infection in such instances was rapid and encompassed the entire girth of the trunk in one growing season during which the crown branches still retained their leaves. If infection included only part of the trunk the area below the infected tips formed secondary crowns from outgrowths along the trunk. Such behaviour provides trees with a means of survival even though this phenomenon was seldom seen. Healthy, dying and dead trees were noted growing adjacent to one another. It was quite common for the skeletons of dead trees to stand out "like a sore thumb" amongst otherwise healthy trees, or vice versa. A similar situation is common along many of Poland's riparian ecosystems (OSZAKO, ORLIKOWSKI 2005).

For 10 to 20 year-old trees, crown symptoms can be rather difficult to distinguish from a distance. Only with careful scrutiny of the trunk does it become possible to see splits in the bark and tissue necrosis extending 10 to 20 cm above the cracks, visible externally as detached bark. External disease symptoms on nursery seedlings are first visible from a distance by their chlorotic leaves which eventually become brown, and fall off or else remain on seedlings. Diseased alder

seedlings, from which the soil has been removed, have cankerous necroses at the trunk base, i. e. a dark, necrotic deformation of the tissue. It is from such sites that *P. alni* is most often isolated. Usually, diseased roots of alder seedlings are dark and when cut they reveal the infection courts. Since the external appearance of such trees differs little from healthy trees, especially where some soil moisture is maintained in the nursery, they may be overlooked as being healthy and outplanted in the forest or on river banks. Only in the new conditions of the wet habitat (alder forest or riparian ecosystems) and water saturated soil water the disease resumes development. Under such conditions zoospores are produced that can infect new root and root collar tissue or nearby mature trees. The disease may then spread further downstream including many kilometres from the original source.

The health of alder stands in Poland

The area of alder stands with damaged trees has increased since 2000 from 250 to 3,039 ha. The damage has increased markedly in certain years, notably 2002 (Figure 1).

Over the last 4 years areas of Poland within 7 SF regional directorates have experienced a decline of alder stands, i. e. in the RDSFs in Białystok, Lublin, Łódź, Olsztyn, Radom, Warsaw and Zielona Góra. Most damage has occurred in the Forest Districts of Lublin RDSF. In 2004, alders suffering decline extended over 2,460 ha of that District (Figure 2). Evidence of the losses is represented by the amounts of deadwood being removed. Depending on the regional directorate, these were 536 to 3,267 m^3 in 2004 which illustrates the seriousness of the situation.

More severe instances of alder decline and dieback were to be noted in the Puławy, Tomaszów, Rudnik, Świdnik, Strzelce, Chełm, Zwierzyniec, Radzyń Podlaski, Biłgoraj, Lubartów and Biała Podlaska Forest Districts (Table 1).

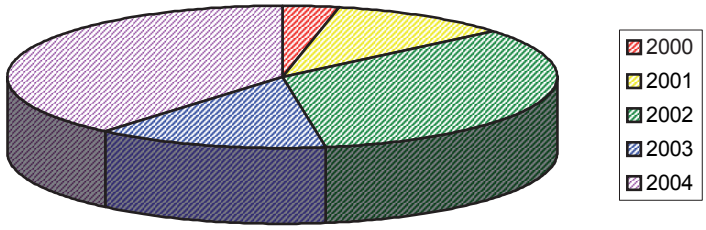

Fig. 1.
Damage to alder stands from 2000 to 2004

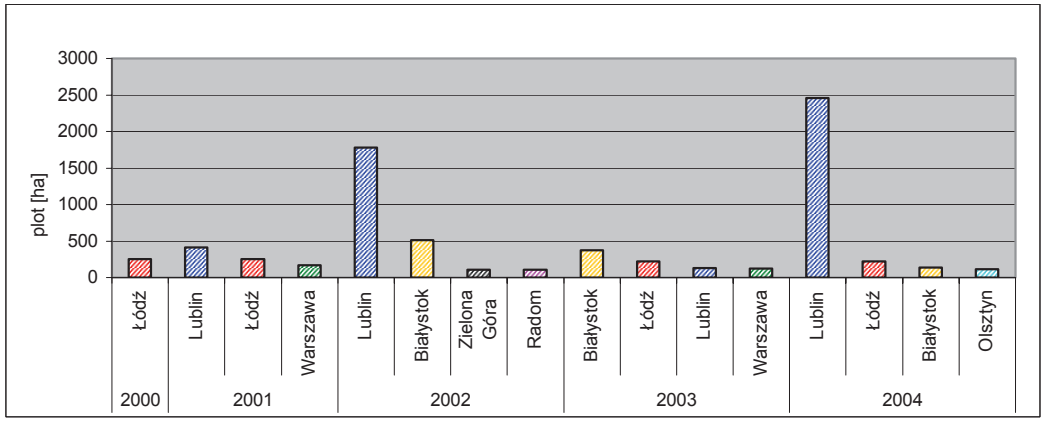

Fig. 2.
Damage to alder stands from 2000 to 2004 in different RDSFs

Tab. 1.
Dieback area (ha) of alder stands within the Lublin Regional Directorate of the State Forests

Forest district	Degree of stand damage		
	limited	moderate	severe
Biała Podlaska	128	1.3	0.79
Biłgoraj	14.3	8.4	1.65
Chełm	133	1	3.454
Kraśnik	2.65	0	0
Lubartów	13.1	2.1	1.18
Międzyrzec	19.6	22.6	0
Parczew	37.7	8.35	0
Puławy	101	31.2	**26.36**
Radzyń Podlaski	57.4	2.97	1.7
Rozwadów	31.3	9.66	0
Rudnik	168	34.9	4.95
Sarnaki	64.9	7.01	0
Sobibór	**235**	26.9	0
Strzelce	10.7	7.78	3.72
Świdnik	27.2	30.5	4.2
Tomaszów	1.14	2.98	7.18
Włodawa	42.7	0	0
Zwierzyniec	54.3	**42.2**	1.77

Decline was especially prevalent in alder stands in age classes IVa, IIIb and IVb (Table 2). However, the largest affected areas occurred in the 55 to 66 age-class (Table 2). In many countries the disease has caused death of individual trees and entire stands. Particularly threatened are alders growing along, or adjacent to stream banks and lake shores (Gibbs et al. 2003).

Tab. 2.
Area of to alder stand damage by tree age class in the Lublin RDSF

Age class	Area of damage	Age class	Area of damage
Ia	51.44	IVa	205.43
Ib	34.95	IVb	148.07
IIa	63.18	Va	78.96
IIb	80.54	Vb	78.77
IIIa	95.62	VIa	16.33
III	177.25	VIb	13.86

Isolation of *Phytophthora alni* in Poland and sources of infection

To date, most positive isolations have originated from samples from north eastern Poland, though symptoms of the disease and excessive dying are evident countrywide. The most isolation attempts from tree bark and nursery soils, plantations and stands with alder have been from forest districts within the Lublin and Warsaw RDSFs. However, young (11-year old) alder plantations established in the Miękinia Forest District following the 1997 floods are also diseased, showing characteristic phytophthorosis, and yielding *P. alni* from cankered, trunk bark (Oszako, Orlikowski unpublished). Usually isolates of *P. alni* subsp. *alni* were obtained, however, alder leaf traps (in water) revealed the presence of other *Phytophthora* spp., e. g. *P. citricola* and *P. cactorum*

(ORLIKOWSKI, unpublished). Frequently *P. alni* is obtained from river water used to irrigate nursery seedlings. When outplanted these are transfer *P. alni* to forest or riparian ecosystems. Such a situation was confirmed by the isolating of *P. alni* from alder seedlings (Table 3).

From 1,011 isolation attempts made from diseased alders analysed 17 genera and species of fungi were isolated (Table 3). The largest groups (of 220 and 190 instances respectively) consisted of fungi from the genera *Phytophthora* and *Fusarium*. *Phytophthora alni* was most often isolated from young plants (up to 10 years old), less often from older trees (up to 30 years old) never from mature trees (of over 60 years old). The sources of inoculum are soil and saplings growing in forest nurseries, or ornamental plants transferred to plantations containing alder saplings infected via contact with infected soil or water. In turn, infected riverside trees and forest trees are becoming a reservoir for the disease, from which the pathogen may be transferred via water or wind, on vehicle tyres, and so forth. The disease is known to be transmitted by gastropod molluscs and insects (inter alia wasps), which are attracted by the lesions on the bark of diseased trees (T. JUNG, personal communication).

Possibilities for limiting the spread of the disease and the need for research

The work on the occurrence of *P. alni* in forest nurseries shows that alder saplings are a primary source of infection capable of transferring the pathogen to new sites. Diseased trees are often planted to stabilize riverbanks and result in a source of inoculum which is spread by water. For example, JUNG et al. (2000) observed that the disease was spread downstream. This suggests that infections of the root collar or bare roots of riparian trees take place via lenticelles or adventitious roots. But, in Germany *P. alni* from roots of in nursery infected seedlings was thought to be the major source of inoculum for forest stands (JUNG et al. 2000). This shows the need for the rapid development and implementation of phytosanitary regulations for plants exported to disease-free countries. Nurseries producing samplings free of *P. alni* pathogens will need to be part of a certification system. As such it will be necessary to devise detection procedures using molecular tools for detecting *P. alni* in soil, water and plant tissues. Until then preventative methods need to be used, e. g. a ban on the use of river water for irrigating plants as is now done in some forest districts. As well, disease surveys of nurseries during the growing season would help, especially if used in conjunction with appropriate disinfection of soils. Fungicides presently recommended for plant protection in forest nurseries include Folpan 80 WG, Previcur 607 and SL Spinaker 607 SL (systemic agents), and Biochikol 020 PC (a biological agent with fungicidal properties and a stimulator of resistance on the basis of chitosan). Fungicides recommended for ornamental nurseries (apart from Biochikol 020 PC) are Biosept 33 SL (grapefruit extract), Saprol, Alliette 80 WP or Mildex 711, 8 WG.

Ongoing work to determine the effective renewal of riverside planted trees may also be helpful. Preliminary information on the level of health achieved by trees planted in this way in Germany is quite encouraging. Finally, one should not forget about selection for resistance on the basis of trees not showing disease symptoms. Trials seeking to increase the immunity of host plants have not so far yielded encouraging results, suggesting that in nature genes for resistance may be rare. A key issue to better understanding the infection process is to determine the role played by factors stimulating its development, e. g. environmental conditions and their influence on the host-plant's capacity to counteract pathogens. Also, more information is needed on the genetic diversity and stability of the strains of *P. alni* as now found in Europe, plus continued monitoring of the state of health of trees on established permanent observation plots, to add in better understanding disease development and modelling and forecasting.

CONCLUSIONS

1. Fungus isolations made from diseased seedlings and older alder trees point yield *P. alni* subsp. *alni*. In Germany, France and the UK, this subspecies of the pathogen is responsible for the widespread decline of alder trees.
2. Seventeen genera and species of fungi were obtained from diseased alders, i. e. in order of frequency of occurrence: *Phytophthora alni*, *Trichoderma* spp., *Mucor* spp., *Penicillium* spp., *Fusarium solani*, *Botrytis cinerea*, *Alternaria alternata*, *F. culmorum*, *Rhizoctonia solani*, *F. avenaceum*, *Pythium ultimum*, *Pythium* sp., *Phytophthora* sp., *F. oxysporum, F. equiseti*, *Chaetomium* spp., *Cryptosporiopsis* spp. The largest group consisted of species of *Phytophthora* and *Fusarium*. Of these *P. alni* is the greatest risk to alders.
3. The isolation of *P. alni* from nursery soils shows that the pathogen may be transferred on seedlings from nurseries to both reforestation and afforestation sites. There is a need to check nursery soils for *P. alni* and prevent the spread of the pathogen on seedlings. In Poland sources of *P. alni* are:
   - seedlings and infested soil in forest nurseries, or ornamental plants,
   - infested soil in plantations into which diseased alder saplings have been planted,
   - infected riverside trees and forest trees whose young stems or leaves may fall directly into water or onto the soil, and from there be moved for example by water and wind,
   - seeds of alder infected via contact with infected soil or water,
   - snails, slugs and insects.
4. Based on information obtained from neighbouring countries, particularly Germany, the next few years will likely see further spread of the disease. This presents the problem as to which tree species can be used on the wettest alder or alder-ash habitats.

Genus / species	I		II		III		IV		V		VI		VII		VIII		IX		X		XI		XII		XIII		XIV		XV	
	10 seed-lings 5 months old		11 seed-lings 6 months old		17 seed-lings 1.5 years old		14 saplings 3 years old		9 trees 5-6 years old		5 trees 6-8 years old		6 trees 8 years old		6 trees 9 years old		9 trees 8-10 years old		24 trees 10 years old		4 trees 10-25 years old		4 trees c. 30 years old		3 trees up to 60 years old		67 years old		2 trees 170 years old	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Alternaria alternata	2	3	2	5	6	14		5	4	7					1	3			3	7	4	11	2	5	3	4				
Botrytis cinerea	2	4	1	3	5	11					3	5	2	5	5	8	3	7	1	2	3	7	1	4	2	5			2	10
***Chaetomium* sp.**									2	3									2	3										
***Cryptosporiopsis* sp.**																					2	5								
Fusarium avenaceum																	6	11	7	14	2	3			1	1				
Fusarium culmorum	-	-	4	9	1	2	7	18			2	4			5	10			6	14										
Fusarium equiseti																	2	3	2	5			2	5						
Fusarium oxysporum																			1	4							1	5	2	5
Fusarium solani	1	3	1	2	3	5	4	8	4	7	1	2	3	5					13	30			2	5	3	7	1	3		
***Mucor* spp.**	3	5	5	8	6	9	10	22	3	8	5	9	2	3	7	16	5	7	14	28	4	6	3	6	3	5	1	2	2	6
***Penicillium* spp.**	4	7	1	4	6	12	8	17	2	7	3	6					2	4	5	9	3	5			3	7			1	3
Phytophthora alni	6	14	6	17	4	16	5	19	5	14	3	9	4	15	5	18	6	22	10	44	1	9	2	8						
***Phytophthora* sp.**					2	7	2	8																						
***Pythium* sp.**					4	9					2	3							3	5										
Pythium ultimum	3	7	2	4	3	7							3	7																
Rhizoctonia solani	5	23	5	9	2	3																								
***Trichoderma* spp.**	4	11	1	2	4	7	7	19	5	10	2	5	6	14	7	20	5	16	11	25	3	11	3	7	3	6	1	4		

A = number of plants from which fungi were isolated. B = number of isolates obtained.

Tab. 3.
Isolation results from seedlings, saplings and trees in different forest sub-districts across Poland

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BIOTIC DAMAGING AGENTS IN FOREST NURSERIES IN THE CZECH REPUBLIC

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A review of the major fungal and animal pests causing damage to forest nurseries in Czechia is presented. In particular agents or their groups, the recent occurrence, damage symptoms, significance or other notes are given. The most important fungal pathogens are as follows: the genera *Pythium*, *Phytophthora, Fusarium*, *Cylindrocarpon*, *Verticillium* or *Pestalotia* causing damping off and root rot of seedlings; *Botrytis cinerea* infecting all growth stages from seeds to plantings; *Phytophthora cactorum* damaging particularly beech seedlings; of needle-casts mainly *Lophodermium pinastri* and *Lophodermium seditiosum* on Scots pine, or *Mycosphaerella pini* on Austrian pine and some other pine species; of mildews *Microsphaera alphitoides*, *Phyllactinia guttata* or *Sawadea bicornis*, of rusts *Cronartium ribicola, Coleosporium tussilaginis* s. l., *Melampsora pinitorqua, Pucciniastrum epilobii* and *Gymnosporangium sabinae*. Of animal pests the following examples of important agents can be given: *Oligonychus ununguis* as the most significant representative of mites on conifers; aphids of the family Pemphigidae on roots of conifers; weevils of the genus *Otiorhynchus*, particularly *O. sulcatus*, feeding in larval stages on roots; larvae of *Melolontha* spp. on roots; adults of *Hylastes cunicularius* on roots and collars of conifers; the cecidomyid *Contarinia fagi* damaging to beech; some songbirds and rodents can cause damage in nurseries as well.

GREMMENIELLA ABIETINA ON NORWAY SPRUCE SEEDLINGS IN A FINNISH FOREST NURSERY: PRELIMINARY RESULTS

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ABSTRACT

Damage caused by *Gremmeniella abietina* is common on Scots pine in Finnish forest nurseries. Production of Norway spruce seedlings has increased in recent years and the proportion is nowadays larger than that of Scots pine. Some top-dying has been found on spruce seedlings. The aim of our experiments was to investigate whether or not Norway spruce seedlings are susceptible to *G. abietina*, and to determine the symptoms and when possible susceptibility is greatest during production of 1- or 2-year old seedlings.

Seedlings were inoculated with conidia of both A(LTT)- and B(STT)-type of *G. abietina* at different times during the summer. Symptoms caused by inoculation and natural infection on spruce seedlings were checked and compared to that observed on pine seedlings. After inoculation, 40 - 80 % of the seedlings became diseased.

The symptoms observed on Norway spruce seedlings were very similar to those observed on pine seedlings: the needles turned brown during the spring following inoculation, starting at the needle base. In Norway spruce the diseased needles often first occurred in the middle part of the shoot whereas on pine they were located at the top of the shoot. The pycnidia developed about two years after inoculation (on pine one year after inoculation). On spruce the lower part of the shoot often remained alive. The susceptible period for first and second year seedlings was different, approximately in the same way earlier reported for pine seedlings. Natural infection in 2002 caused more disease on pine seedlings than on spruce seedlings.

Our experiments show that Norway spruce seedlings can also be susceptible to *G. abietina* under Finnish nursery conditions.

INTRODUCTION

Gremmeniella abietina (LAGERB.) (imperfect stage *Brunchorstia pinea* (KARST.) HOHN.) causes disease on conifers, especially on pines. *Gremmeniella* commonly damages pine seedlings in nurseries (KURKELA 1967, JANCARIK, UROSEVIC 1973, SKILLING 1969) and the symptoms are usually distinct. On Norway spruce the fungus was described by LAGERBERG (1913). Since then there have been some reports and studies of the disease on spruce in Europe, e. g. ROLL-HANSEN, ROLL-HANSEN 1973, BARKLUND, UNESTAM 1988, SOLHEIM 1986, KAITERA et al. 2000.

In Finland the production of Norway spruce seedlings has increased during the past few decades and it is now larger than that of pine. Some top-dying of spruce seedlings has been found, and the cause is not always evident.

For *Gremmeniella* infection, the susceptible phase of first-year pine seedlings during the growing season appears to be later than that of second-year pine seedlings (PETÄISTÖ 1999, 2005, PETÄISTÖ, KURKELA 1993). The aim of these studies is to (i) investigate the ability of *Gremmeniella* to cause disease on young first- and second-year Norway spruce seedlings, (ii) determine the susceptible phases of the seedlings to the disease, and (iii) describe the symptoms.

MATERIALS AND METHODS

1 Experiment 1: 2002/2003. Inoculation during various seedling growth phases during the first growing season

Seeds were sown on 22 April 2002, in Plantek-81F containers. Each tray contained 9 rows of seedlings with 9 seedlings/row. The seedlings were grown in a nursery production greenhouse in Central Finland and moved outside on 16 July 2002.

The inoculation times were 25 June, 20 August and 11 September, and there were three trays for each inoculation time. In each tray four rows were inoculated, one row was left as a barrier, and the last four rows acted as controls. The seedlings were inoculated with conidia of *G. abietina A*-type isolates (isolates from Antti Uotila), 2.5 x 10^6 conidia/ml, 200 µl/seedling, by pipetting the suspension onto the top of the main shoot. The surface of the seedlings was kept moist for two days after inoculation by spraying with water. The seedlings were overwintered outdoors and were examined in the spring of 2003.

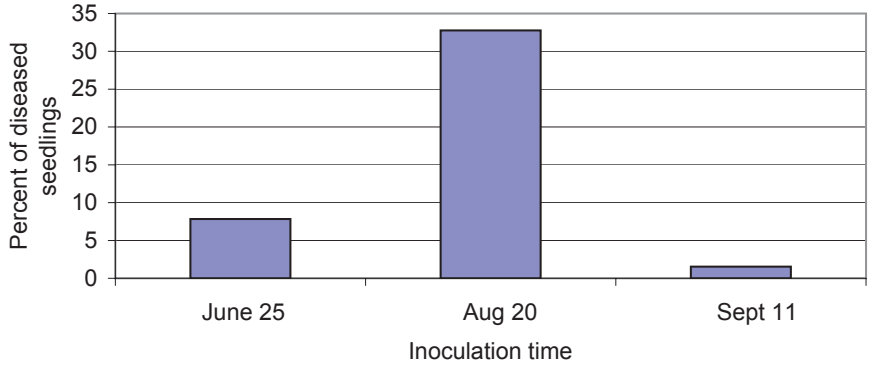

Fig. 1.
Experiment 1: Disease occurrence in spring on 1-year old Norway spruce seedlings after inoculation in June, August and September. Values are the percent of diseased seedlings for inoculated and non-inoculated (Control) seedlings

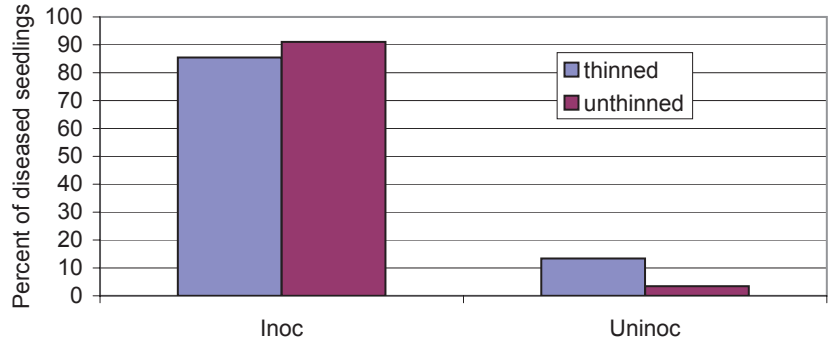

Fig. 2.
Experiment 2: Disease occurrence for inoculated and non-inoculated (control treatment), thinned and unthinned, 1-year old seedlings

2. Experiment 2: 2002/2003. Inoculation of thinned and unthinned seedlings

The seedlings were from the same seed lot as in Experiment 1. The seedling trays were moved outside on 16 July 2002. The shoot of every second seedling in rows 1 - 5 in each tray was cut off. The trays were located in two rows with the thinned halves of the trays being closed towards each other. Trays with untreated seedlings were placed around the experimental trays.

Inoculation was done in the same way as in Experiment 1. The inoculation date was 18 July 2002, and the inoculation was done for seedlings in four trays, with other four uninoculated trays serving as the control treatment. The seedlings were overwintered outdoors and were examined in spring 2003 and in summer 2004 for evaluating disease severity.

The air temperature and relative humidity were measured continually with HMP 133Y sensors (Vaisala, Helsinki, Finland) on four thinned and four unthinned halves of the trays. The measurements were made about three centimetres up from the surface of the growing medium.

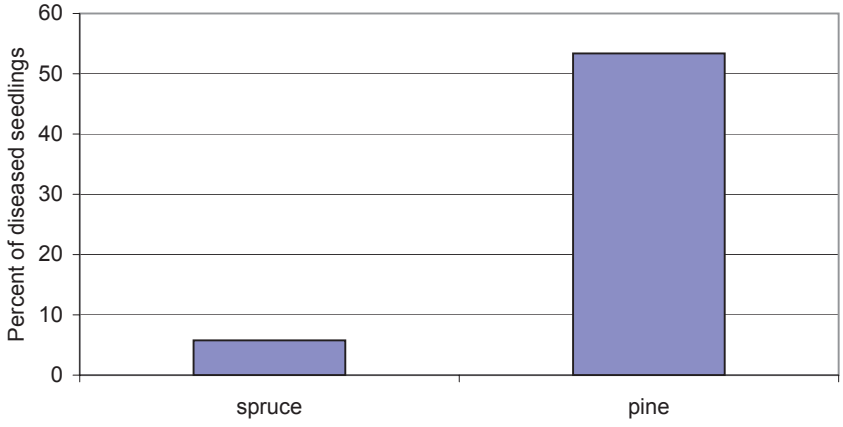

Fig. 3.
Experiment 3: Disease occurrence after natural infection of first-year Norway spruce and Scots pine seedlings

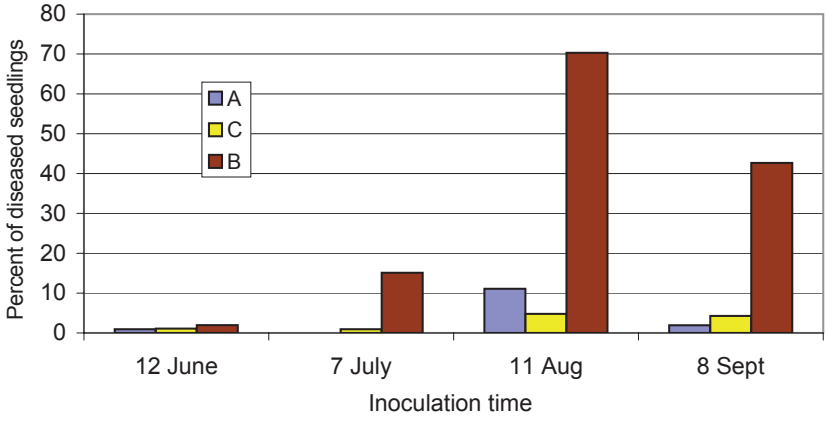

Fig. 4.
Experiment 4: Disease occurrence in spring after inoculation in June, July, August and September with the A- and B-type pathogen (C = non-inoculated). Above, 1-year old seedlings and below 2-year old seedlings

3. Experiment 3: 2002/2003. Natural infection

Three trays of seedlings (same seed lot as in Experiments 1 and 2) were moved outdoors on 24 June 2002, and placed near diseased *Pinus cembra* pines (about 20 years old) (PETÄISTÖ et al. 2005), and the symptoms were checked in the spring of 2003.

4. Experiment 4: 2003/2004. Effect of age and growth phase of the seedlings

Experiment 4 involved second-year container-grown, Norway spruce seedlings (from the same seed lot as in Experiments 1 and 2) and the first-year seedlings (sown in the spring of 2003 in a greenhouse and moved outdoors in early July). At the beginning of September 2003, the seedlings were moved into an unheated greenhouse to prevent frost damage, and were moved back outdoors at the end of October. The inoculation dates were 12 June, 9 July, 11 August and 8 September 2003. At each inoculation date there were four trays of seedlings of each age.

The seedlings in the first three rows (3 x 9 = 27 seedlings) of the trays were inoculated with A-type conidia, and the seedlings of the seventh to ninth rows were inoculated with B-type conidia. The fungus isolates were supplied by Antti Uotila and had been stored in liquid nitrogen and in a deep freezer in cryoprotectant. The seedlings in rows 4 - 6 were not inoculated.

During the winter of 2003 - 2004 the seedlings were kept outdoors. The seedlings were examined on 10 May 2004.

Fig. 5.
Diseased needles on the shoots of Norway spruce

Fig. 6.
Diseased needles shed from seedling shoots

RESULTS AND DISCUSSION

In Experiment 1 in the spring of 2003, disease occurrence was the highest on seedlings inoculated on 20 August 2002 (first-year seedlings) (Fig. 1).

In Experiment 2, inoculation affected the percentage of diseased seedlings, but thinning had no clear effect on disease occurrence (Fig. 2). The temperature was about one degree (°C) higher and the relative humidity (%) about 4 (%) lower among the thinned seedlings.

In Experiment 3, the percentage of diseased seedlings, examined in the spring of 2003 was clearly lower on spruce (5.8%) than on pine (53.4%) (Fig. 3).

In Experiment 4, disease occurrence was high in seedlings inoculated with B-type conidia. In the case of the first-year seedlings, inoculation with the B-type pathogen in August resulted in more disease than did inoculation at the other dates. In the case of the second- year seedlings, inoculation with the B-type pathogen in July resulted in more disease than did the inoculations done in June, in September and in August. The results were generally the same for the inoculations done with the A-type pathogen (Fig. 4).

In Experiments 1 - 4 the symptoms in the spring following inoculation resulted in: the base of the needles quickly turning brown and the entire needle was soon killed (Fig. 5). The dead needles were soon shed during the summer (Fig. 6). Most of the lower part of the main shoot remained alive, and the lateral shoots continued to grow (Fig. 7).

In these experiments pycnidia were first detected about two years after the inoculation. The pycnidia occurred on the shoot or very often on the scar from which the needle had been shed (Fig. 8). Pycnidia in the earlier experiments with pine seedlings often appeared in the spring following inoculation (e. g. PETÄISTÖ et al. 2005).

The results showed that the symptoms on Norway spruce needles were very similar to those on pine needles, i. e. in the spring after infection the base of the needles turned brown. However, for the Norway spruce seedlings diseased needles were also very often located in the middle part of the shoot as opposed to being on the top of pine shoots. However BARKLUND and UNESTAM (1988) reported in an experiment with 2-year old spruce seedlings that the disease occurred more frequently on the base of the shoot than on the upper parts.

Fig. 7.
The lower part of diseased seedlings, shoots alive

Fig. 8.
Pycnidia on Norway spruce seedlings about two years after inoculation

Diseased needles on spruce seedlings were soon shed during the summer, while in the case of the pine seedlings, infected during their first year, the diseased needles remained on the shoots for a long time. On spruce the lower part of the shoot often remained alive, while in pine the whole shoot frequently died.

The susceptible phases for the first- and the second-year seedlings were different, and about the same as reported earlier (PETÄISTÖ 1999, 2005, PETÄISTÖ, LAINE 1999, PETÄISTÖ, KURKELA 1993, PETÄISTÖ, JUNTUNEN 2000) for pine seedlings with the main susceptible phase of the second-year seedlings being earlier than that for first-year seedlings. These results are different from those reported from Britain by AITKEN (1993) who found that the both 2- and 3- year old seedlings were more susceptible in August than in June. AITKEN (1993) suggested that the reason was the additional 'lamma' growth, e. g. due to warm weather in late summer.

In contrast to the results of TERHO and UOTILA (1995), the B type pathogen caused more disease than the A type in Experiment 4 (2003 - 2004). The germination of conidia in the suspension used was high for both types and the spore numbers in both suspensions was the same. In experiments (1 and 2, 2002 - 2003), where only A-type conidia were used for inoculation, disease occurrence was however high (40 - 60%). The difference in the capacity of the A- and B-type isolates of the pathogen to cause disease on Norway spruce seedlings requires further studies.

Natural infection in 2002 caused more disease on pine than on spruce seedlings. Scots pine is generally known to be more susceptible than the Norway spruce to *Gremmeniella*.

The occurrence of the differences in susceptibility between conifers is also affected by the growth rhythm, bud development and how the various growth phases coincide with the spore dispersal. In summary, it is clear that there are risks of *G. abietina* damage on Norway spruce seedlings produced in Finland.

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DETERMINING FUNGICIDE EFFICACY AGAINST SNOW BLIGHT *(PHACIDIUM INFESTANS)* BY USING A SCOTS PINE SHOOT BUNCH TECHNIQUE

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ABSTRACT

In Finland snow blight (caused by the fungus *Phacidium infestans*) is a common disease on Scots pine (*Pinus sylvestris*) seedlings which must be controlled by fungicides. The disease mainly occurs in North and Central Finland where snow cover remains throughout winter.

In the autumns of 2003 and 2004 a mixture of cyprodinil and propiconazole (sold as the commercial product Basso®) and a mixture of prochloraz and propiconazole (Stereo® EC) were tested for their efficacy against the disease by applying the fungicides to bunches of pine shoots. Propiconazole (Tilt® 250 EC) was the trial reference product as it is approved for use in Finnish forest nurseries. All products significantly reduced *P. infestans* infections. The efficacy of the products was similar, except in the 2004 trial when a mixture of cyprodinil and propiconazol significantly lowered infection.

INTRODUCTION

In recent years 54 - 57 million Scots pine (*Pinus sylvestris* L.) seedlings have been produced in Finland (Anonymous 2004a). Practically all these are one-year old, container grown seedlings in which the seeds are sown in March - April in plastic (film) covered houses. The seedlings are grown in the houses until being transferred outdoors for hardening off before the end of July. The seedlings are shipped the next spring either after cold storage in packages or after over wintering outdoors under snow cover.

Scots pine seedlings suffer from two major nursery diseases in Finland, *Scleroderris* canker caused by *Gremmeniella abietina* (Lagerb.) Morelet and snow blight caused by *Phacidium infestans* P. Karst. Both of these diseases are common on pine trees surrounding nurseries and so the diseases on nursery seedlings need to be controlled with fungicides (Lilja 1986, Lilja et al. 1997). Snow blight is restricted to areas with permanent snow cover and it occurs mainly in Central and North Finland. The history of controlling snow blight in nurseries is almost as long as is the history of intensive growing of pine seedlings (Björkman 1948, Jamalainen 1961).

Control of snow blight is challenging as fungicides should be applied just before the permanent snow cover occurs which means in Central Finland the middle of November. Studies on the biology of *P. infestans* (Björkman 1948, Kurkela 1993) showed that apothecia develop and ascospore liberation starts under favourable conditions as early as September and will continue until snow comes.

The fungus penetrates healthy needles under snow cover and hyphae can grow at temperatures as low as -3 ºC. Hyphal growth takes place under snow and can spread up to 30 cm from the infection point. Thus, under high densities seedlings on benches and in containers can suffer considerable losses.

Since the 1950ies several chemicals have been developed to control snow blight on pine seedlings. New active ingredients have replaced all the very early ones, but there is still a continuous need to evaluate and choose those fungicides that are both efficient and safe enough to use. This is especially true in northern regions where the environment is conducive for the disease and so fungicide sprayings need to be applied several times and late in the autumn when cool temperatures and often high rainfall may increase washing off of the protective fungicides and cause harmful leakages into the environment (JUNTUNEN 2002).

The goals of the work described here were to: (i) describe a pine shoot bunch method for evaluating the effect of different fungicides against snow blight caused by *P. infestans* and (ii) give the results for two trials made to test and compare the efficacy of three fungicides against *P. infestans*.

MATERIALS AND METHODS

The fungicide trials were done in the autumns of 2003 and 2004 at the Finnish Forest Research Institute Suonenjoki Research Unit (62º 39', N 27º 03' E). The pine shoot method which was used to evaluate the efficacy of the fungicides was a modification of the one given by SAKARI LILJA (unpublished) and was based on earlier work by BJÖRKMAN (1948). Otherwise, the general trial design and lay-out followed EPPO standards (ANONYMOUS 2004b).

For the trials pine twigs bearing apothecia of *P. infestans*, i. e. the inoculum, were collected during a dry spell in mid-September and stored in plastic bags at +5 ºC until used. The test shoots were collected at the end of October (again when the weather was dry) in a local forest with no heavy occurrence of snow blight. The 30 - 40 cm long shoots had at least two whorls of growth bearing healthy, green needles and were stored in black plastic bags at +5 ºC until used.

Tab. 1.
The fungicide treatments applied to pine shoot bunches in the efficacy trials against *Phacidium infestans* in 2003 and 2004

Year	Treatment no.	Fungicide	Active ingredient	Concentration (l/ha)
2003	1	Control/water		
	2	Tilt® 250 EC	propiconazole 250 g/l	0,5
	3	Stereo® EC	prochloraz 400 g/l propiconazole 90 g/l	1,6
	4	Basso®	cyprodinil 250 g/l propiconazole 62,5 g/l	1,25
2004	1	Control/water		
	2	Tilt® 250 EC	propiconazole 250 g/l	0,5
	3	Basso®	cyprodinil 250 g/l propiconazole 62,5 g/l	1,25
	4	Stereo® EC	prochloraz 400 g/l propiconazole 90 g/l	1,6

In both years, four fungicide treatments were applied (Table 1) of which Tilt® 250 EC (active ingredient propinconazole) is approved for use in Finnish forest nurseries against snow blight. Consequently, it served as the efficacy reference for the new products being tested, i. e. Basso® (active ingredients cyprodinil and propiconazole) and Stereo® EC (active ingredients prochloraz and propiconazole).

The fungicides were applied on November 18, 2003, and November 17 in 2004. During the treatment in 2003 the air temperature was 1 ºC in the plastic-sheet covered greenhouse and 15 ºC in 2004 in the glass-covered greenhouse. For treatment, five healthy shoots were bound together to form a bunch which was soaked for 1 - 2 sec in a solution of the test fungicide. Altogether 40 randomly-selected, bunches, 10 bunches/treatment, were soaked starting with the control (water) treatment. After treatment, the bunches were left to dry overnight on the floor of a plastic-covered greenhouse. Next day, one inoculum twig was placed into each bunch and after the bunches were tightly rebound they were placed at random in a 5 x 8 arrangement on the ground in a forest. To prevent cross-contamination among bunches, i. e. via growth of the pathogen under the snow, a 50 cm space was left between each bunch. The bunches remained on the ground over winter.

The percentage of *P. infestans* infected needles in each test shoot was assessed next spring after snow melt, i. e. on April 20 in 2004 (at the time total temperature sum = 27 day degrees) and on April 25 in 2005 (7 d. d.).

The data were subjected to analysis using a SPSS statistical package (version 13.0) to determine the effect of the treatments on the percent of infected needles. The data were transformed to the arcsin and then subjected to a Univariate Analysis of Variance and then the significance of mean differences was determined using the Student-Newman-Keuls test.

RESULTS

In both years over 50% of the control needles became infected although there were some differences in the amount of disease between the two years (Fig. 1). In 2003 there were clearly more *P. infestans* infections than in 2004 in all treatments except the Basso® treatment. In both years all the fungicides significantly reduced disease on the needles as compared to the corresponding control (water-treated) bunches (Fig. 1, Table 2).

In both years Basso® and Stereo® were the most effective fungicides and the reference product Tilt® was slightly less effective. In the treatments done in the autumn of 2003, Basso® had the lowest infection percentage, but in the autumn 2004 trial Stereo®-treated shoots had the lowest disease occurrence, however, the differences did not significantly differ from the other fungicides (Table 2).

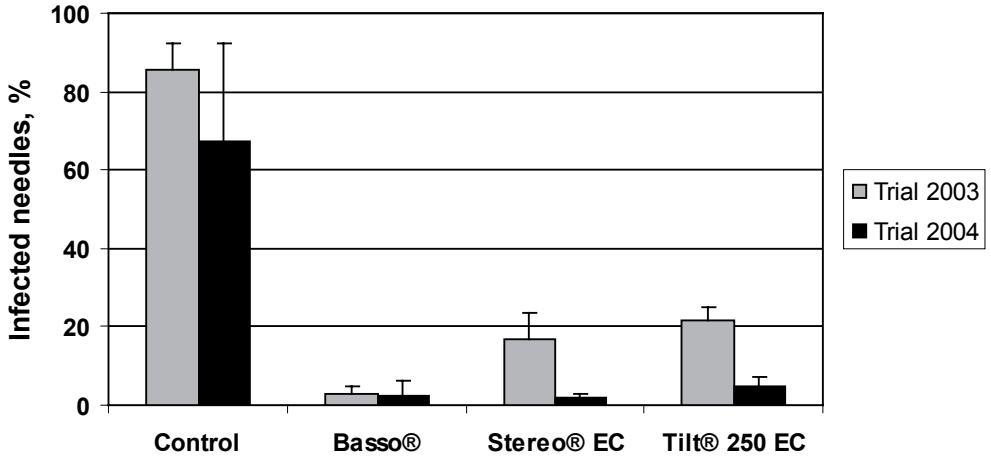

Fig. 1.
Percentage of *Phacidium infestans*-infected needles in the different fungicide treatments done in 2003 and 2004. Columns show the means of 10 bunches of pine shoots and the bars on top of the columns are the standard error of the mean (sem).

Tab. 2.
The effect of different fungicide treatments on the percentage of *Phacidium infestans*-infected needles in the pine shoot bunches in the 2003 and 2004 trials

Treatment	Diseased needles (%) in a shoot bunch Mean ± sem	
	Trial 2003	Trial 2004
Control	85.5 ± 11.49 a	67.2 ± 25.27 a
Basso® 1,25 l/ha	2.8 ± 3.29 b	2.6 ± 3.66 b
Stereo® EC 1,6 l/ha	17.0 ± 11.74 c	1.7 ± 1.34 b
Tilt® 250 EC 0,5 l/ha	21.4 ± 10.91 c	5.0 ± 2.16 b

Note: The means of diseased needles (%) followed by different letters differ from each other according to Univariate ANOVA followed by Student-Newman-Keuls-test, $n = 10$, $P < 0.05$.

DISCUSSION

The pine shoot bunch method was a useful and easy way to determine the efficacy of the different fungicides against snow blight. Probably the most critical component of the technique is the inoculum twigs as the number of apothecia and the amount of ascospores may vary among twigs depending on the collection site and year, or both.

The difference in the infection percentages for the control treatments between the two years in which the trails were done may indicate differences in the amount of inoculum. Perhaps in the autumn of 2004 the inoculum twigs had fewer apothecia on the needles or a significant proportion

of ascospores may have been liberated before the twigs were collected. It is known that ascospore production and timing of release, which is dependent on rainfall, may vary considerably between years (KURKELA 1993).

It is also possible that conditions were more favourable in 2003 - 2004 than in 2004 - 2005 for the infection of the needles and hyphal growth under the snow. According to the weather data collected at the trial site between November 15 and December 15, 2003, the average air temperature was -1,8 ºC and the average temperature on the ground surface -2,2 ºC while the corresponding temperatures were -4,3 ºC and -4,9 ºC, respectively, in 2004. Maybe primary infection was more successful in 2003 due to the milder late autumn weather and hyphal growth was also greater in the spring of 2004 due to the 10 - 15 cm deeper snow cover.

The two test fungicides represented new products which are composed of two different active ingredients. In the trials these formulas gave better control against *P. infestans* than did Tilt® 250 EC which has propiconazole as the single active ingredient (250 g/l). Propiconazole is a second but a minor component in the two other products. If the amount of propiconazole as an active ingredient is compared for the test fungicides, the Stereo® EC treatment contained propiconazole equivalent to 144 g/ha and Tilt® 250 EC and Basso® treatments each contained 125 g/ha and 78 g/ha of propiconazole, respectively.

The fungicide Basso® seemed to be equally effective in both years while the efficiency of Stereo® and Tilt® varied between the two years. Both of these products controlled proportionally more snow blight in the 2004 trial than in the 2003 trial. When the materials were applied in 2003 the temperature was significantly lower (plastic sheet-covered greenhouse house, temperature 1 ºC) than in the autumn of 2004 when the greenhouse temperature was 15 ºC and disease control was also better. In operational sprayings one source of variable results is due to rain falls occurring in outdoor areas too soon after spraying that may wash off fungicide and cause also leakages into the environment. In the present trials such leakage was not a problem as the treated bunches were left to dry over night in a shelter before they were transferred outdoors. Temperature during the treatment time may explain some of the variations between the years as generally it is recommended that pesticides be applied at temperatures above 5 ºC.

To control snow blight, nurseries can also use integrated methods and not chemicals alone (ROLL-HANSEN 1989). Removing the infected lower branches from the young pine trees growing in the neighbourhood of a nursery is usually an efficient way to reduce inoculum. Such pruning should be done just after snow melt and before infected needles have fallen off.

ACKNOWLEDGEMENTS

Mr. Sakari Lilja is acknowledged for suggesting the use of the shoot bunch technique (method) and Mr. Osmo Korhonen for assisting in the trials.

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INCIDENCE OF *CIBORIA BATSCHIANA* ON *QUERCUS ROBUR* AND *Q. PETRAEA* ACORNS: EFFECT OF COLLECTION YEAR AND OAK STANDS, COLLECTION METHOD, AND ANNUAL RAINFALL

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The fungus *Ciboria batschiana* causes a serious disease of stored *Quercus robur* and *Q. petraea* acorns. Acorns acquire the fungus prior to collection and subsequently during storage the pathogen intensifies on infected acorns and spreads to healthy acorns.

To learn more about the epidemiology of *C. batschiana* and possible means of preventing initial infection, acorns of *Q. robur* and *Q. petraea* were collected from various oak stands from 2000 to 2003, and from both the forest floor and mesh nets spread over the floor. As well, rainfall data throughout these years were collected to determine a possible link between rainfall before and during fall collection of acorns and initial infection rate of acorns by *C. batschiana*.

The results showed that (i) *Ciboria batschiana* occurs on acorns from across the Czech Republic, (ii) infection level of acorns differs by year and by stand, (iii) higher incidence of *Ciboria* on acorns of *Quercus petraea* than on *Q. robur* acorns, (iv) *Ciboria* occurs on acorns collected from both forest floor and from nets and (v) infection is higher in years with higher precipitation in August and especially in September.

ACKNOWLEDGEMENT

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GREMMENIELLA INFECTION ON PINE SEEDLINGS PLANTED AFTER FELLING OF SEVERELY *GREMMENIELLA* INFECTED FOREST

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During 1999 and 2001 the most severe *Gremmeniella* epidemic ever appeared in Sweden. Big forest areas needed to be clearcut in advance followed by replanting. In this investigation we wanted to find out to what extent newly planted seedlings became infected and also if remaining twigs and branches support new infections. Seedlings were planted on clearcut areas felled in 2001 in the most affected areas of Sweden. They were planted in 2002, 2003 and 2004 and infection was controlled the year after planting.

Seedling planted in 2002, the year after felling, were infected between 50 to 90 % the following year showing that it is unsuitable to replant already the year after felling due to sever *Gremmeniella* infections. The infection decreased for seedling planted two and three years after felling but at this time there was a big variation between different areas. The infection was not influenced so much if twigs and branches were left on the clearcut areas. Seedling planted in the adjacent diseased forest became much more infected than seedlings planted on the clearcut areas. The different result will be discussed.

HISTORY OF IUFRO WORKING PARTY 7.03.04 (DISEASES AND INSECTS IN FOREST NURSERIES) SINCE ITS INCEPTION IN 1990 TO PRESENT (2005)

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ABSTRACT

This paper summarizes the history of IUFRO WP 7.03-04 (Diseases and insects in forest nurseries) since its formation in 1990 until the present (2005). Since inception of the WP members have met every two or three years for a total of six times. As the organizer and first leader of the WP I have given the reader some of my thoughts and reasons for my comments when I wrote the proposal for forming the WP. For example, I believe that our WP is unique in that the membership consists of both pathologists and entomologists. As well, I have given my opinions on how well I believe that we have done in completing our original goals, e. g. one place where we have excelled is that we have published the proceedings of all of our meetings. These are listed here. A summary table lists our meeting dates and localities, WP leaders/meeting hosts, attendance and paper presentation numbers, and a general summary of each meeting's theme.

INTRODUCTION

I suppose that it is appropriate that I write this short history of our working party (WP) since I am responsible for both forming the WP and also for hosting the first meeting at Victoria, British Columbia, Canada, in 1990. At that time, and even today, I consider myself to have been very fortunate to have done my Ph.D. research on nursery diseases and then to have worked in this area for almost 33 years. I would say a third of a century, but that sounds a lot longer! I doubt if there are few others who have been so lucky. I use the word 'work', but I never considered one day of my career as being work. It was always so interesting that if my employer would have only known I would have done it without compensation! But, that's another story and what we want to dwell on here is the short history of this working party. In the late 1980s an entomology colleague of mine returned from an IUFRO WP meeting and told me all about the wonderful experience of participating in a small meeting (by most standards) where everyone spoke the same scientific language. So, after some thinking I said to myself, 'why are there so many IUFRO working parties in any number of insignificant areas (sic) and nothing dealing with such an important area as nursery diseases and insects'? So, after making enquires at IUFRO Headquarters I was asked to prepare a 'Proposal for Formation of an IUFRO Working Party on Nursery Diseases and Insects' dated July 24, 1987. ' Believe it or not, after 23 years I still have a copy of the two-page proposal. The first paragraph of the proposal is a short introduction telling about the ever increasing production of forest nursery seedlings worldwide, how diseases and insects adversely affect seedling production and the fact that no existing IUFRO WP covered the subject area for the proposed WP. The proposal then went on to cover six topics which IUFRO needed to consider before giving the go ahead on forming the WP. What follows is a summary of the contents of each point and then where appropriate I have commented on certain topics that were uppermost in my thoughts at the time - and even today. Again, where fitting I have said a bit about our successes and shortcomings in each of the areas.

THE PURPOSE OF THE WORKING PARTY

Here the proposal emphasized that the WP would promote, worldwide an interest in and support for research and management of diseases and insects in forest nurseries. As well, the WP would encourage cooperation in research and dissemination of information, e. g. via publication of the proceedings of each meeting, and finally that we would hold meetings every two or three years where we would cover these topics and tour local nurseries.

At the time I wanted the WP to be a worldwide forum where those of us who were working with nursery pests would have a common group where we could communicate with one another. I especially wanted a group where those members of the WP in developing countries got to know others in the field and also in giving them the opportunity to meet others outside their homeland. The word 'worldwide' was of particular significance. Here we have had only moderate success as the majority of our WP meetings have been held in either North America or Europe (Table 1). The 2003 meeting, while held in India, was somewhat disappointing as turnout from other countries was particularly low with outsiders coming only from Finland, the Czech Republic and Canada. Undoubtedly, world unrest at the time and an outbreak of SARS (a viral respiratory illness) were largely responsible for the lack of participation from other countries, but we need to do more to hold successful meetings outside North America and Europe. Too, we need to do more to encourage active participation by WP members from outside our traditional areas of strength. One area where we have had good success is in publication of the WP proceedings with the 1990, 1993, 1996 and 1999 meetings resulting in publication of paper versions and the 2003 meeting proceedings being published electronically. We are indebted to the folks at The Finnish Forest Research Institute, and above all Dr. Arja Lilja, who came to our rescue and made sure that the 2003 meeting proceedings got published. As I write Zdenka Procházková is busy preparing the proceedings of our 2005 meeting for publication.

THE TOPICS TO BE INCLUDED AND EXCLUDED

In the proposal I stated that the working WP would be unique in that both entomologists and pathologists would be welcome as members, particularly if they were dealing with fungus, bacterial and nematodes as pathogens or insect pests affecting broadleaf and conifer seedlings of bareroot and container-grown seedlings. Weed problems would not fall under the jurisdiction of the WP.

To date, (see overall themes given in Table 1) we have managed to maintain a good balance of participation by both entomologists and pathologists in WP meetings. At our meetings both the pathology and the entomology presentations have covered a wide range of pathogens and diseases and insect pests on numerous seedling species around the world and under various types of nursery cultural practices.

JUSTIFICATION FOR FORMATION OF THE WP

Here the proposal reiterated the importance of nursery diseases and insects and also the fact that no other IUFRO WP covered this topic. Too, it emphasized that management of nursery pests differs from other types of forest-related pests even when specific pests occur in both nurseries and the forest. As well, it was stated that while another IUFRO WP dealt with nursery operations it was not directly relevant for nursery pathologists and entomologists.

Such justifications are as relevant today as they were in 1987 when I wrote the proposal.

EXPRESSION OF INTEREST BY OTHER SCIENTISTS TO SUPPORT THIS WP

Here I solicited and then attached letters of support from scientists in Australia, India, Mexico, New Zealand, Sweden and the USA. The last letter was from Dr. Harry Powers, an outstanding American pathologist, who was extremely active in IUFRO. As well, I stated that the proposed leader was Canadian.

Obviously this interest in supporting the WP has continued throughout the years with many of our colleagues from several countries assuming the role as leader of the WP or hosts for our meetings, or both (Table 1).

Tab. 1.
Summary of IUFRO working party S7.03-04 (Diseases and insects in forest nurseries) meetings from inception in 1990 to 2005

Year and meeting place	Leader or local host	Number of attendees	Number of papers presented	Overall themes
August 23 - 30, Victoria, B.C., Canada	Jack Sutherland	49	43	Diseases and insects around the world; invited papers including reviews and new techniques
October 3 - 10, 1993, Dijon, France	Robert Perrin	63	35	Diseases and insects, especially in European nurseries; nematodes; biological control; IPM
May 19 - 24, 1996, Gainesville, Florida, USA	Robert James and Frank Barnard	31	25	Diseases and insects; alternatives to methyl bromide soil fumigation
July 25 - 28, 1999, Suonenjoki, Finland	Steve Fraedrich and Arja Lilja	39	25	Holistic pest management; pest surveys
April 6 - 8, 2003, Peechi, Kerala, India	Zdenka Procházková and C. Mohanan	25	13	Nursery diseases and insects, especially in India
September 12 - 14, 2005, Uherské Hradiště, Czech Republic	Zdenka Procházková	30	29	*Gremeniella* canker; quarantine diseases; powdery mildews; nematodes

THE OPPORTUNITIES FOR A WORKSHOP

I stated ’It is highly likely that the WP could schedule a workshop within 2 years and certainly within 3 years of its formation’.

I proposed that the first meeting be held at Victoria, British Columbia, Canada were there are excellent facilities for holding the meeting and visiting a variety of nearby forest nurseries. Too, it was stated that participants could on their way to the meeting, or afterward, visit nurseries, universities and research facilities elsewhere in British Columbia or Canada, or in the nearby northwest USA.

Fortunately we were able to meet our commitment and the first meeting of our WP was held in Victoria in August, 1990 (Table 1). Since then we have met each 2 to 3 years, i. e. in Dijon, France, Gainesville, USA, Suonenjoki, Finland, Kerala, India and in 2005 in Uherské Hradiště, Czech Republic (Table 1). We look forward to our next meeting in Hawaii, USA, with Michelle Cram as WP leader and Bob James as local host.

A STATEMENT OF WILLINGNESS AND QUALIFICATIONS FOR A WP LEADER AND CO-LEADER

The proposal concluded with the listing of my credentials for being the first leader of the WP and those of Dr. Bruce Brown of Australia for being the deputy leader, i. e. not only our professional qualifications, but also our willingness as shown both by our soliciting support for the WP and in preparing the proposal for IUFRO headquarters. Unfortunately, Bruce retired soon afterward and he was no longer active in the WP.

I will end with what I feel are some relevant comments. Firstly, I think that all of us can be justifiably proud of this WP and what we have accomplished. Nothing is perfect, but we have done well over the years. We can be pleased with our record in helping one another be better at what we do whether that is in research, nursery pest management, as nursery managers or a wide array of other activities related to the goals of the WP. We have an outstanding reputation for hosting excellent meetings and for publishing our results. The immediate future looks bright for our next meeting in Hawaii and as I write the proceedings of the exceptional meeting held in the Czech Republic in 2005 are being prepared for publication. No one can predict the future, but we must be vigilant if we want the WP to continue and be successful.

Finally, I will say that if you have only obtained technical information from being a member of IUFRO WP S7.03-04 then I feel sorry for you. You see, our real accomplishments have been in meeting and becoming friends with colleagues from all over the world, in understanding new and different cultures, and beliefs and ways of living. For those of you who have attended all or only a few of our meetings how can you forget the thrill of going salmon fishing near Victoria, in being shown the wonderful countryside and eating the delicious food in France, in floating on an inflatable raft down that long, cold stream in Florida, in sitting in a smoke sauna in Finland, in absorbing the wonderful culture in Kerala, or in seeing the folk festival and visiting a wine cellar in Moravia. Our WP is as much about these things as it is about anything else.

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Available only in electronic form at the following URL (as of February, 2006): http://www.metla.fi/julkaisut/workingpapers/2005/mwp011-en.htm

ERADICATION OF AN OUTBREAK OF CHESTNUT BLIGHT (CAUSED BY *CRYPHONECTRIA PARASITICA* (MURRILL) BARR.) IN FOREST NURSERY AT KLADÍKOV

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Protection against the introduction of organisms harmful to plants or plant products into the Czech Republic from other Member States of the European Union and from third countries, against their spread within the territory of the Czech Republic and against the introduction of these harmful organisms into the territory of other Member States of the European Union and third countries is one of the main task of State Phytosanitary Administration (SPA).

Czech Republic was acclaimed as a protected zone for *Cryphonectria parazitica* (MURILL) BARR. In the forest nursery Kladíkov in 2004 year there were found symptoms of chestnut blight and *Cryphonectria parasitica* was determined in samples. Chestnut blight was detected on *Castanea sativa* trees about 25 – 30 years old.

Based on these results there were acclaimed extraordinary phytosanitary measures by SPA for eradication an interception of chestnut blight spreading. There were destroyed 896 seedlings of *Castanea sativa* and over 1 million seedlings of *Quercus* genus. Also all full-grown trees *Castanea sativa* were got down and burned.

After these measures many inspections were done. Chestnut blight is not recorded in this range in year 2005.

FUNGI ASSOCIATED WITH ROOT DECAY OF CONIFER SEEDLINGS IN FOREST NURSERIES AND AFFORESTED SITES

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ABSTRACT

Fungi colonizing decayed fine roots of *Pinus sylvestris* and *Picea abies* seedlings were assessed by pure culture isolations and direct sequencing of affected tissue in three types of terrain: bareroot forest nurseries, afforested clear-cuts and abandoned farmland. The isolation from 1,500 roots of 480 seedlings (240 of each tree species) yielded 1,110 isolates which, based on morphological characters and ITS rDNA sequencing of the mycelia, were found to represent 87 distinct taxa. Direct ITS rDNA sequencing from decayed sections of 140 roots (70 of each tree species) yielded 160 sequences representing 58 taxa. In total, 131 taxa were found, 92 of which (70.2%) were identified at least to a genus level. Fungi most commonly isolated were the pathogens *Fusarium oxysporum* (25.6%) and *Nectria radicicola* (14.9%). Conversely, direct sequencing most frequently revealed presence of the endophyte *Phialocephala fortinii* (33.1%) and Unidentified sp. NS234A2 (10.0%). There were no significant differences between species richness in different types of terrain, but it had marked effect on fungal community structure. Our results demonstrate that different fungi are associated with the root decay of conifer seedlings in different types of terrain.

INTRODUCTION

Seedlings, infected with root-decay fungi, might have reduced survival rates following outplanting even when aboveground disease symptoms in the nursery are absent. For example, the transfer of *Rhizoctonia*-inoculated, sound-looking plants from the nursery to the field resulted in 25% and 70% mortality of pine and spruce seedlings after the first growing season, and increased to 39% and 93% after the second season (Lilja, Rikala 2000). Therefore, the assessment of root health condition at early stages is of practical importance. Moreover, the success of a plantation might also depend on the presence of root pathogens in afforested areas, as transferred seedlings are likely more susceptible to infection due to recent replanting stress.

To date, such studies are scarce, and previously fungal communities in decayed roots of conifer seedlings were mainly assessed by fungal isolations into pure culture (Lilja et al. 1992, Kope et al. 1996). However, despite the large number of isolated fungi, it was noted that this method could be biased towards fast growing species and provide only a portion of total fungal community inhabiting diseased roots. More recently, it has been demonstrated that PCR based molecular methods can be a powerful tool for identification of fungi (Donaldson et al. 1995, Hamelin et al. 1996, Hantula et al. 2002). For example, the direct sequencing of fungal DNA from roots has proved to be a sensitive method for the detection of potentially all root-inhabiting fungi, in particular species that are usually overlooked by isolation, e. g. latent pathogens, slow-growing endophytes and non-culturable species (Kernaghan et al. 2003). The main aim of the present work was to determine species composition and relative abundance of fungi colonizing roots of decayed *P. sylvestris* and *P. abies* seedlings in three types of terrain: bareroot forest nurseries, afforested clear-cuts and abandoned farmland. In order to achieve this, pure culture isolations were combined with direct sequencing of fungal DNA from decayed root tissue.

MATERIALS AND METHODS

Diseased *Pinus sylvestris* and *Picea abies* seedlings were collected in July 2003 from three bareroot forest nurseries, three replanted clear-cuts and one afforested farmland in Lithuania. All four plantations were established during the spring of the same year. Aboveground symptoms of all sampled seedlings were needle discoloration and poor growth. Following excavation, they all showed root dieback and decay. Excavated root systems were excised from the stems, individually packed into plastic bags and transported to the laboratory, where they were washed in running tap water. From each root system, three to five core roots with decay symptoms were randomly selected, and from each selected root, a single segment about 5 mm in length was cut at the zone of advancing decay. Three to four of those were immediately used for isolation of fungi into pure culture. In addition, from 10 randomly selected plants from each site, one segment per root system was designated for future direct sequencing. Then, it was placed in 1.5 ml centrifugation tube, labelled and stored at -40 °C.

The isolation of fungal cultures was attempted from 1,500 core roots derived from 240 pine and 240 spruce seedlings. Prior to isolation, the segments cut from the zone of advanced decay were surface sterilized for 1 minute in 33% hydrogen peroxide and rinsed three times in autoclaved, deionized water. Then, they were placed on agar medium in 5 cm Petri dishes (single segment per dish) and incubated at room temperature in the dark. The dishes were checked daily and all outgrowing mycelia were immediately subcultured.

For initial isolation from the nursery plants we used three types of agar medium (one type per each root from a single plant), i. e. 2% water agar, vegetable juice agar (BARKLUND, UNESTAM 1988) and Hagem agar (STENLID 1985). Moreover, for additional root samples we also used an apple as an intermediate nutrient source (HANSEN et al. 1979). In the latter case, a surface-sterilized root segment was initially placed inside the apple. Following colonization, a piece of rotting apple tissue from the margin zone was removed and placed onto agar. All isolations from replanted clear-cuts and afforested farmland, as well as all subsequent subculturings of all obtained strains were done exclusively on Hagem agar.

Isolated cultures were grouped into mycelial morphotypes accordingly to morphological characteristics of the mycelium. For identification, one to ten representative cultures from each morphotype were ITS rDNA sequenced. The initial aim was to sequence at least three isolates per group, and one or two strains were sequenced only in cases when no more was available. Larger groups were sequenced more extensively. Extraction of DNA, amplification and sequencing followed the method described by ROSLING et al. (2003). Representatives from 15 sporulating cultures that had not been taxonomically defined by sequencing were sent for identification to the Central Bureau of Fungal Cultures (CBS) in Utrecht, the Netherlands.

One hundred and forty segments of core roots representing 70 pine and 70 spruce seedlings were selected for sequencing of the internal transcribed spacer (ITS) of the fungal ribosomal DNA (rDNA). Extraction of DNA, amplification and sequencing followed the method described by ROSLING et al. (2003). Databases at both GenBank (ALTSCHUL et al. 1997) and at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala were used to determine the identity of sequences. The criteria used for deciding on the taxon or genus for a given strain was its intra- and interspecific ITS sequence similarity to those present in the databases.

Species richness and structure of fungal communities were analysed in relation to isolation media, type of terrain and tree species. Fungal community structures were compared by calculating qualitative (S_S) Sorenson similarity indices (MAGURRAN 1988). The occurrence of given fungus in respective datasets was compared by chi-squared tests, which were calculated from actual numbers of observations (presence/absence data) (FOWLER et al. 2001).

RESULTS

Out of 1,500 roots used for isolation, 1,110 (74.0%) produced fungi, and the remaining 390 (26.0%) were either colonized by bacteria or remained sterile. As in all cases a single isolate per root was obtained, this part of work yielded a total 1,110 of distinct cultures, which, following mycelial morphotyping, morphological and molecular identification, were found to represent 87 taxa. Of those, 77 (88.5%) were identified at least to genus. Fungi, most frequently isolated were ascomycetes and deuteromycetes *Fusarium oxysporum*, *Nectria radicicola*, *Nectria* sp. 702, *Trichoderma harzianum*, *Phialocephala fortinii*, *Penicillium spinulosum*, *T. viride* and *Zalerion varium*.

The exclusive use of three different types of agar for isolations from nursery material did not result in increased diversity of obtained strains, as compared with the material from clear-cuts and farmland where only Hagem agar was used. It was supported by the analysis of species richness, as here the differences between the media on number of outgrowing taxa were in all cases statistically insignificant (chi-squared tests; $p = 0.10 - 0.81$), and in all cases it was demonstrated that the similar isolation effort would yield rather similar numbers of fungal taxa from each of three types of agar media (data not shown). The isolations using apple produced five taxa, all of which were isolated using Hagem agar.

Despite rather similar richness of taxa, in nursery plants there were certain differences in community structure isolated on different agar media, since the Sorensen similarity indices between the three sets were moderate ($S_S = 0.51 - 0.55$). This could be an indication of different species favoured by different media, but also it could be the result of large natural diversity. To check this, also clear-cut and farmland isolations (all done exclusively on Hagem agar) were divided into three random datasets each, and analysed in the similar manner. The results showed that the Sorensen similarity indices within each of them, despite the single medium, were only slightly higher, - $S_S = 0.51 - 0.67$ for clear-cuts, and $S_S = 0.63 - 0.70$ for farmland. This indicated that observed differences between agar media in nursery material are more likely to originate from high natural diversity than from the agar used. Moreover, accumulation curve of isolated taxa did not reach asymptotic value (data not shown) indicating both high diversity and the potential that further fungal taxa would have been discovered with increased effort of sampling and isolation.

Amplification of fungal ITS rDNA from 140 root segments was successful for 123 (87.9%), producing one to four distinct amplicons in each of the PCR products. Direct sequencing of all amplicons resulted in 160 sequences representing 58 fungal taxa. Fungi, most commonly detected by direct sequencing were the ascomycetes *Phialocephala fortinii*, Unidentified sp. NS234A2, *Leptosphaeria* sp. 1169, *Nectria radicicola*, *Nectria* sp. 702, *Xenochalara juniperi*, *Fusarium oxysporum* and *Zalerion varium*. When pooled, direct sequencing and isolation detected a total of 131 fungal taxa, 92 of which (70.2 %) were identified at least to a genus level.

For both pine and spruce, the highest absolute numbers of taxa were found in forest nurseries (56 and 54), followed by afforested clear-cuts (36 and 40), and abandoned farmland (24 and 16). Observed differences, however, have arisen due to different sampling efforts on each of terrains, since, accordingly to species accumulation curves, similar sampling effort (100 roots) would have revealed much closer levels of fungal diversity on all terrain types for both tree species, i. e. 21 - 24 for pine and 16 - 24 for spruce (data not shown). Consequently, in all intraspecific comparisons, the differences in species richness among the sites were statistically insignificant (chi-squared test; $p = 0.16 - 0.61$).

However, despite rather even species richness, there were notable differences in fungal community structure between the terrains, indicating that different species cause root decay of conifer seedlings in forest nurseries, afforested clear-cuts and abandoned farmland. Thus, in every intraspecific comparison the Sorensen similarity indices were low, varying between 0.22 - 0.39 for

pine, and 0.09 - 0.26 for spruce. On the other hand, it must be remembered that to some extent such low indices have arisen due to high overall diversity and presence/absence of occasional taxa, while the dominant fungi were of more common occurrence in different types of terrains. Fungal diversity in decayed roots of both tree species was similar, and a total of 87 taxa of fungi were found in pine, and 93 taxa in spruce. Among these, 39 were unique for pine, 44 unique for spruce, and only 48 (36.6%) were found on both tree species, indicating that decayed roots of both tree species in most cases were colonized by different fungi.

DISCUSSION

Our results show that the most common fungi isolated from decayed roots in nurseries were also commonly isolated from diseased conifer roots in some other forest nurseries in Europe (Unestam et al. 1989, Ericson et al. 1991, Lilja et al. 1992, Kacprzak 1997). This indicates that similar means for control of root diseases could be applicable. Yet, in contrast to the above studies, we seldom isolated *Rhizoctonia* spp., and *Pythium* spp. was not isolated at all. The *Pythiaceae* and *Rhizoctonia*-like fungi were reported to be very important pathogens causing damping-off and root-rot of conifer seedlings (Lilja 1994). High moisture content in the growth substrate favours the growth and infection by these pathogens (Vaartaja, Morgan 1961, Hendrix, Campbell 1973, Venn et al. 1986). However, the substrate of our study sites was characterized by dry sandy loam podzol, what likely contributed to low incidence of these pathogenic fungi.

Our results demonstrated that different species colonize decayed roots of conifer seedlings in forest nurseries, afforested clear-cuts and abandoned farmland. This indicates that indigenous soil fungi of clear-cuts and agricultural land colonized roots of outplanted seedlings already during several weeks. The most common decay fungi isolated in forest nurseries were the representatives of *Fusarium* spp., in clear-cuts *Nectria* spp., and in abandoned farmland species of *Penicillium* and *Trichoderma*.

On all sites, except for pine from the clear-cuts, the most frequent species detected by direct sequencing of decayed tissue was *Phialocephala fortinii*, but it was rarely isolated using agar media. In a previous study it was the most commonly isolated from healthy mycorrhizal conifer seedling roots (from 29% of over 8,000 root tips), and was only seldom recorded by direct sequencing (from 0.8% of 150 root tips) (Menkis et al. 2005). Therefore, the possibility cannot be excluded that in healthy root systems the fungus does persist latently as an endophyte, and is commonly isolated from the mycorrhizal root tips, since on agar it grows faster than mycorrhizal species. Decayed roots are already colonized by the pathogens, which, on agar media, exhibit faster growth than *P. fortinii*, thus the presence of the fungus is explicitly manifested by direct sequencing from decayed tissue. The exact role of the species in root dieback is unknown, but earlier we hypothesized about the possible shift from endophytic to pathogenic behaviour along with the changes in health and resistance of a tree (Menkis et al. 2004).

Occasionally, our work revealed the presence of mycorrhizal fungi, such as *Tuber rapaeodorum* or *Wilcoxina mikolae*. The present study thus showed that functionally different fungi, e. g. mycorrhizal, endophytic, saprophytic, pathogenic, are present in decayed core roots of conifer seedlings. In the present study about 30% of the taxa had remained unidentified. This is not surprising, knowing that only about 5% of the fungi have been described so far (Hawksworth 1991, 2001). In other studies of fungal communities from soil and woody substrates the proportion of unidentified taxa was similar (Kernaghan et al. 2003, Landeweert et al. 2003, Rosling et al. 2003, Tedersoo et al. 2003, Lygis et al. 2004a, b, Vasiliauskas et al. 2004, 2005), showing that the sequence coverage in our databases is still limited, and that huge future potential does exist in exploring natural fungal communities.

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DAMAGE IN NORWEGIAN CHRISTMAS TREE PLANTATIONS CAUSED BY FUNGI AND NEMATODES POSSIBLY INTRODUCED ON NURSERY STOCK

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ABSTRACT

For several fungi causing disease on Christmas trees there has been a close association between those organisms detected in nurseries and disease outbreaks in production fields in Norway. In 2004, severe *Phytophthora* root rot was observed in a Christmas tree plantation of Nordmann fir (*Abies nordmanniana*) a few months after outplanting, that must have been caused by infected transplants. *Rhizosphaera kalkhoffii* has caused serious needle cast on different species of fir and spruce (*Picea* spp.) and has often been found on transplants produced both in Norway and on imported material. *Thysanophora penicillioides* has often been associated with needle cast on Christmas trees in Norway, and it has commonly been detected on nursery stock. *Phomopsis* sp. kills needles and shoots and girdles the stem of small trees in production fields. The fungus has been detected several times in nurseries, where it may be a serious problem on young seedlings. *Sclerophoma* sp. has killed shoots on Norway spruce (*Picea abies*) and Nordmann fir in Christmas tree plantations and has also been found on nursery stock and fir seeds.

In 2002, nematodes (*Pratylenchus* spp.) were found in the rhizozone of poorly-growing Nordmann fir Christmas trees a year after planting. No harmful nematodes were found in the soil between the plants. This indicated that the nematodes were introduced by the seedlings. During investigations in the nursery where the plants originated from different nematodes were found throughout the production chain.

INTRODUCTION

Traditionally, Norway spruce (*Picea abies*) has been the dominant Christmas tree in Norway, but different species of fir (*Abies* spp.) have taken increased market shares over the last two decades. Approximately 1 million Christmas trees are planted yearly, and 70% are fir. Nordmann fir (*A. nordmanniana*) is the dominant Christmas tree species, but sub alpine fir (*A. lasiocarpa*) is common in areas where the climate is too harsh for Nordmann fir. Other firs, like Korean (*A. koreana*), Turkish (*A. borlumeriana*) and Noble (*A. procera*) are only used to a limited extent for Christmas trees, and the latter is mainly grown for bough production.

Compared to forest production, there is hardly any tolerance for damaged foliage on Christmas trees. Many airborne fungi are associated with diseased foliage and shoots on Norwegian Christmas trees; *Botrytis cinerea, Chrysomyxa abietis, Cytospora* spp., *Kabatina* spp., *Nectria* sp., *Pestalotiopsis* spp., *Phaeocryptopus nudus, Phomopsis* spp., *Pucciniastrum epilobii, Rhizosphaera kalkhoffii, Sclerophoma* spp., *Thekopsora areolata* and *Thysanophora penicillioides* (Talgø, Stensvand 2003a, 2005). Soilborne diseases caused by different *Phytophthora* and *Armillaria* species are also troublesome, but have so far only been found in a few fields (Talgø, Stensvand 2005). In addition, nematodes seem to be the cause of poorly developed root systems in several fields (Talgø, Stensvand 2003a).

Except for *Pestalotiopsis* spp. and *T. penicillioides,* all the fungi mentioned above are reported on Christmas trees elsewhere in Europe (PERNY et al. 2002) or from USA (CHASTAGNER et al. 1997). Many of the genera (*Botrytis, Cytospora, Kabatina, Nectria, Pestalotiopsis, Phomopsis* and *Phytophthora*) are also reported from forest nurseries in Europe (NEF, PERRIN 1999) or from woody ornamental and tree nurseries in USA (JONES, BENSON 2001). Furthermore *R. kalkhoffii*, *Sclerophoma* sp., and *T. penicillioides* have been found on nursery stock in Norway (TALGØ, unpublished data). Several soilborne fungi and oomycetes (such as species of *Pythium*, *Phytophthora*, *Fusarium*, *Cylindrocarpon* and *Rhizoctonia*) can cause damping off during seed propagation in nurseries. Airborne fungi like *B. cinerea*, and species of *Alternaria*, *Phoma* and *Phomopsis* can also cause symptoms resembling damping off. Seedborne fungi like *Sirococcus* sp. can also affect seedlings. Disease causing organisms in nurseries may cause severe problems when transferred by seedlings to production fields, especially *Phytophthora* spp.

In Nordic tree nurseries 17 genera of plant parasitic nematodes have been reported (MAGNUSSON 1981). Parasitic nematodes damage the root system on plants, resulting in stunted growth, discoloration, low vigour and mortality. Restrictions in the use of nematicides will most certainly result in a large increase in nematode damage in many countries. No nematicides are available in Norway. Nematodes have the potential to cause extensive damages. From USA it is reported that damages from nematodes cause farms and nurseries millions of dollars in annual crop losses (DUNN 2001).

Sufficient control of diseases is much easier to accomplish in nurseries than after the plants are spread over large areas in production fields. Over a period of five years we have surveyed Norwegian nurseries and Christmas tree fields for presence of diseases caused by true fungi, oomycetes and nematodes, and here we present a preliminary and brief overview of this work.

Some of the work was previously presented internationally (TALGØ, STENSVAND 2004), and as fact sheets for growers (TALGØ, STENSVAND 2003b, 2003c, 2003d, 2003e, 2003f).

MATERIALS AND METHODS

Airborne pathogens (*Rhizosphaera, Thysanophora, Phomopsis* and *Sclerophoma*)

Between 2000 and 2005 several hundred fir and some spruce samples were investigated for presence of airborne fungi. The standard procedure was to incubate needles and shoots with disease symptoms in saturated air (moist chambers) at room temperature and examine them for fungal growth using a dissecting microscope. Sample size varied from a few needles to whole 4 - 5-year old trees or branches from older trees. Depending on the condition of the samples, the length of the incubation period varied. Normally 2 - 5 days gave satisfactory fungal growth and sporulation, but samples were sometimes incubated longer. When needed for further identification, isolations were carried out from incubated material. Occasionally isolations were carried out prior to incubation from the area between diseased and healthy tissue.

Loose, green needles were collected from a tree (none from the ground) with heavy needle cast (Fig. 1), and 250 randomly chosen needles were incubated with stomata facing upwards (different from subalpine fir, Nordmann fir have stomata only on the underside of the needles). For *T. penicillioides* the number of stomata per needle covered with conidiophores were estimated on a scale from 0 to 5 (0 = none, 1 = < 1%, 2 = 1 - 10%, 3 = 11 - 25%, 4 = 26 - 50% and 5 = > 50%).

Soilborne pathogens (*Phytophthora* and nematodes)

Samples were collected in the spring of 2004 in a severely damaged Nordmann fir Christmas tree field in Rogaland county on the southwestern coast of Norway. The symptoms resembled those caused by *Phytophthora* spp. The planting had been established the previous year. Symptoms included poorly developed roots and discoloration under the bark from the stem basis downwards. The foliage had different stages of drought symptoms; pale green, yellow or brown. Isolations were carried out from the area between healthy and diseased tissue both from roots and stem base. ITS amplification was carried out on the isolated culture, and the DNA sequence was determined.

In 2002, newly established Nordmann fir Christmas trees in a field on the southwestern coast of Norway did not grow as well as expected. The trees did not necessarily die, but they suffered poor growth. Root development was very poor or none at all. The plants were analysed for possible presence of *Phytophthora* spp., but no such fungi were detected. A styrofoam tray that the plants had been transported in from the nursery to the grower, was examined for fungi and nematodes. Further samples for nematode analysis were collected from the nursery by the extension service. A total of 19 samples from growth medium, styrofoam trays, roots and soil in different seedbeds were analysed. Nematodes were extracted from the styrofoam trays using the Baermann-funnel technique (HOOPER 1986) and the Seinhorst elutriator (SEINHORST 1988). The styrofoam trays were soaked in water prior to extraction.

RESULTS AND DISCUSSION

Airborne pathogens (*Rhizosphaera, Thysanophora, Phomopsis* and *Sclerophoma*)

R. kalkhoffii and *T. penicillioides* are both associated with needle cast on different fir and spruce species used as Christmas trees in Norway. They have both frequently been traced on nursery stock on both plants produced in Norway and imported plants. *R. kalkhoffii* has been found on discoloured needles and *T. penicillioides* both on discoloured and green needles that drop during winter. Both fungi are associated with the stomata. *R. kalkhoffii* is a coelomycete that has pycnidia growing in rows along the stomata. *T. penicillioides* is a hyphomycete with relatively long conidiophores (resembling *Penicillium* spp., hence the species name *T. penicillioides*). The conidiophores of *T. penicillioides* are also located along the stomata rows.

Figure 1 illustrates how dramatic needle cast can be. Of the 250 incubated needles from the tree in Figure 1, 198 (79%) had *T. penicillioides* growing from the stomata. Out of the 198 needles with

Fig. 1.
Severe needle cast on Nordmann fir (*Abies nordmanniana*). Rogaland county, October 2005. Photo: V. Talgø

T. penicillioides, 117 needles (59%) had more than 50% of the stomata per needle covered with conidiophores (scored 5 on a 1 - 5 scale). Figure 2 illustrates what the score of 5 very often looked like.

Besides *T. penicillioides* on the 250 needles from the tree in Figure 1, 54 needles (22%) had pycnidia of *Sclerophoma* sp. (located both on upper and lower side of the needles) and 27 needles (11%) had pycnidia from *R. kalkhoffii* in the stomata rows. Only rarely was more than one fungus found on the same needle, and if so, they grew in separate locations on the needle.

The tree adjacent to the tree with needle cast in Figure 1 also started to loose needles on the side facing the damaged tree, indicating that needle cast may spread in the field. In other locations we have observed trees with needle cast standing next to perfectly healthy trees, indicating that there may be individual differences in resistance. Damage from *T. penicillioides* and *R. kalkhoffii* may be due to impeded gas exchange, because the stomata are more or less blocked by fungal growth.

R. kalkhoffii has been reported on a wide variety of conifer hosts from around the world, and it is common in Europe (Maanen, Gourbičre 1997). From Canada, *R. kalkhoffii* is reported on *Abies*, *Pinus*, and *Pseudotsuga* (Funk 1985). In the Pacific northwest it causes significant disease on *Picea* (Chastagner et al. 1997). According to Kendrick (1961), *T. penicillioides* was only observed on needles after they had dropped to the forest floor, and thus the fungus was considered as being a saprophyte. Also Van Maanen and Gourbière (1997) described the fungus on decaying conifer needles (litter samples) in a European study. *T. penicillioides* is also described as a saprophyte by Ellis and Ellis (1997). So far the only literature cited that describes *T. penicillioides* in living needles is from a needle entophyte survey in Finland on Norway spruce. In that investigation, *T. penicillioides* was isolated occasionally from green needles collected from trees (Moller, Hallaksela 1998). Shedding of green needles during the winter is also reported on Nordmann fir in Denmark, where it is most prominent from December to February, but some winters are said to be worse than others. One theory from Denmark suggested that a sudden drop in temperature before the plants were fully hardened in the fall resulted in needle cast (Lyhr 1994).

Phomopsis spp. have been found on a wide range of host plants in Norway, both on conifers and broad leaved trees (Talgø, Stensvand 2003b). This fungus is primarily a problem in nurseries, but is also frequently found in Christmas tree fields on buds that have failed to open in the spring, and on damaged needles and shoots from both spruce and fir. On the southwest coast of Norway, thousands of relatively newly transplanted subalpine fir and spruce were girdled by *Phomopsis*. The pathogen had obviously followed the nursery stock. *Phomopsis* canker is reported on Christmas trees from USA (Chastagner et al. 1997) and Austria (Perny et al. 2002).

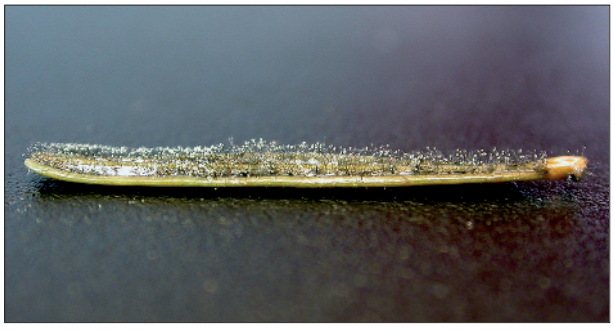

Fig. 2.
Thysanophora penicillioides on a Nordmann fir needle from the tree in Fig. 1 incubated for 11 days. Photo: V. Talgø

Sclerophoma spp. are described as weak pathogens that infect plant tissue wounded by other fungi, frost, insects, mites, or other damaging agents. *Sclerophoma* is often traced on dead needles and shoots from both fir and spruce in Norwegian Christmas tree fields. It is associated with canker wounds and girdled shoots, indicating that the fungus is capable of infecting non-lignified shoots and young needles. This is also reported from Austria (Perny et al. 2002). Often *Sclerophoma* is the only pathogen traced on dead shoots, but on samples from a severely dam-

aged Nordmann fir field (Fig. 3) the shoots were girdled at the base by *Phomopsis* sp. and covered with pycnidia of *Sclerophoma* from the girdled area to the tip of the branches. This particular field was surrounded by a 40-year old spruce forest (*Picea abies*) where we detected *Phomopsis* sp. on dead shoots. Further investigations are necessary to find whether or not it is the same *Phomopsis* sp. attacking Nordmann fir and Norway spruce. *Sclerophoma* has been found on nursery stock in Norway and also on fir seeds, and there is thus a possibility of transferring the disease from nurseries to production fields.

Soilborne pathogens (*Phytophthora* and nematodes)

A *Phytophthora* sp. was isolated from Nordmann fir. Both morphological characteristics and ITS rDNA sequencing of the isolate showed closest similarity to *P. inundata.* The field where the diseased Nordmann fir trees grew was well drained, but the annual precipitation is high (> 2,000 mm) at the southwest coast of Norway where the field was located, and thus the conditions were favourable for *Phytophthora* spp. Approximately 70% of the plants were dead or dying within few months after planting. The massive infestation in a relatively newly established field, and the fact that the field had previously only been used for grass production over decades, strongly indicated that the disease had followed the imported transplants.

In Michigan in USA *P. inundata* has been found on fir (Catal et al. 2005). *P. inundata* has also been isolated from several other host plants in different countries: from roots of horse chestnut (*Aesculus hippocastanum*) and *Salix matsudana* in the UK, from river water in France, from alder (*Alnus* sp.) debris in a pond in Denmark, from olive roots (*Olea* sp.) in Spain and from roots of *Vitis* sp. in South America (Brasier et al. 2003).

We have also found *P. cambivora* on 15-year old noble fir and *P. megasperma* on 7-year old subalpine fir in Norway (Talgø et al. 2005). The two latter pathogens may as well have followed the transplants, but that is not possible to verify so many years after planting. *Phytophthora* spp. (*P. citricola* and *P. citrophthora*) are well known to cause disease on Lawson Falsecypress/Port-Orford-cedar (*Chamaecyparis lawsoniana*) in bough plantations, public parks and private gardens in Norway (Talgø, Stensvand 2003c), but has never been reported from forest stands.

A *Pratylenchus* sp. was extracted from soil debris in the styrofoam tray that had been used for transportation of plants from the nursery to the grower. A *Pratylenchus* sp. was also found in roots that had penetrated the styrofoam walls. The amount of soil and roots in the styrofoam tray was very limited, thus the number of nematodes traced were only 3 and 2, respectively. Also the fungus *Cylindrocarpon* sp. was isolated from the roots. Damage from nematodes may enhance the disease development caused by this otherwise weak fungal parasite. It was the extraction of nematodes from this particular styrofoam tray that led to the investigation in the nursery. Plant

Fig. 3.
Damaged shoots on a Nordmann fir Christmas tree. The shoots were girdled by a *Phomopsis* sp., and the dead and dying shoots were full of pycnidia from a *Sclerophoma* sp. Rogaland county, October 2005. Photo: V. Talgø

parasitic nematodes feed on plant tissue either from outside the host (ectoparasitic) or within host tissue (endoparasite). The feeding is done by means of a slender, hollow feeding spear (stylet). During the feeding process the plant cells are injured by enzymes produced by the nematodes (DUNN 2001). Secondary invaders like *Cylindrocarpon* can thereby enter the cells. The *Cylindrocarpon* sp. detected in the styrofoam tray prior to the investigation in the nursery, may have added to the disease problem in the field, but no further investigations were carried out.

Nematodes (*Pratylenchus penetrans, P. crenatus, P. fallax*, *Paratylenchus* sp., *Trichodorus* sp. and spiral nematodes) were found throughout the production chain in the nursery, from the seedbeds to fully-grown transplants (Table 1). In general, *P. crenatus* and *P. fallax* are considered less damaging than *P. penetrans*, but depending on plant species and cultivars, the response may differ. *Trichodorus* spp. and *Paratylenchus* spp. are also considered quite damaging on various hosts. Figures in Table 1 indicate that the nematode population had been building up over the years in the seedbeds, reaching a total of 1,016 nematodes in a 250-gram sample taken from the 12-year old seedbed. In the seedbed from 2000, there were 120 *Pratylenchus* spp. per 250-gram soil. The seedlings used for the Christmas tree field where the poor growth was observed, originated from this particular seedbed. The frequent occurrence of root lesion nematodes (*Pratylenchus* spp.) indicated that the nematodes might have contributed to the crop failure. Connections between the occurrence of *P. penetrans* and damaged nursery seedlings are reported on conifer species in different countries; *Pinus* sp. in Germany (WEISCHER 1956), *Cryptonectria japonica* in Japan (MAMIYA 1969) and *C. lawsoniana* in Belgium (MAESENEER 1964).

Tab. 1.
Number of different nematodes found in 19 samples collected in a nursery in 2002; from seedbeds, growth medium, styrofoam trays and the rhizozone of seedlings from different plant species (including some roots). Sampling took place in October and sample size was 250 gram soil ± roots (except for the styrofoam trays).
P. = *Pratylenchus*, *pen.* = *penetrans*, *cren.* = *crenatus, fall.* = *fallax, Par.* = *Paratylenchus* sp., and *Tric.* = *Trichodorus* sp.

Investigated material	*P. pen.*	*P. cren.*	*P. fall.*	*P.* sp.	*Par.*	*Tric.*	Spiral nem.
Seedbed prepared for 2003	0	101	0	149	0	0	20
Seedbed from 2002	19	9	5	5	0	3	0
Seedbed from 2000[1]	52	51	0	17	13	23	0
12-year old seedbed covered with cloth[2]	91	182	91	91	523	38	0
Old seedbed covered with cloth[3]	143	107	72	107	343	0	0
Peat soil for containers	0	0	0	0	0	0	0
1/1 winter injured Nordmann fir	0	0	0	10	0	0	0
2/1 Nordmann fir	0	0	0	50	0	10	0
2/1 subalpine fir	33	0	8	8	0	0	0
1/1 subalpine fir	0	0	0	25	0	0	0
2/1 dead plant	0	0	0	33	0	0	0
1/1 subalpine fir, bad condition	0	0	0	15	0	0	0
Transplanted seedlings, fall 2002	0	0	0	10	0	0	0
Transplanted seedlings, fall 2002	0	0	0	5	0	0	0
Transplanted seedlings, fall 2002	0	0	0	5	0	0	0
Nordmann fir in containers	0	0	0	15	0	5	0
Subalpine fir in containers	0	0	0	0	0	0	0
Styrofoam tray, steamed	0	0	0	0	0	0	0
Styrofoam tray, not steamed	0	0	0	0	0	0	0

[1] The poorly developing plants in the Christmas tree field described under soil borne pathogens in the article, originated from this seedbed.
[2] The samples were taken under the cloth/Mypex (no containers with plants were placed on top at the time of sampling).
[3] The samples were taken under the cloth/Mypex (roots from container seedlings placed on top of the seedbed had penetrated the cloth).

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Seed borne fungi on *Abies* spp.

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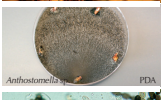
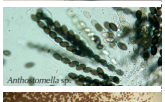
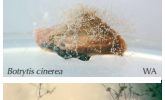
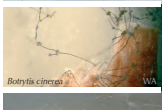
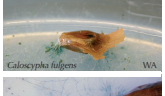
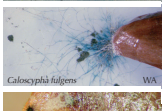










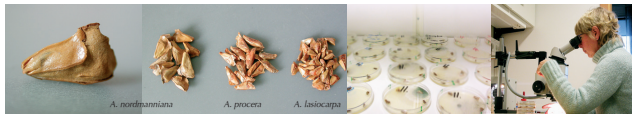


Introduction

In Norway, nordmann (*Abies nordmanniana*) and subalpine fir (*A. lasiocarpa*) are the most common Christmas tree species, and noble fir (*A. procera*) the dominant species for bough production. In spring 2005, a survey was undertaken to examine for presence of seed borne fungi on these fir species.

Material and methods

- Twelve seed samples; five nordmann fir, four sub alpine fir and three noble fir
- Seeds produced in Austria (nordmann fir), Canada (subalpine fir), Georgia (nordmann fir), Norway (noble and subalpine fir) and Russia (nordmann fir)
- 100 seeds per sample pre-treated in 1 % NaOCl, plated on potato dextrose agar (PDA) and incubated for 5 to 7 days at 20 ± 2 ºC
- 100 seeds per sample pre-treated in H_2O_2, plated on water agar (WA) and incubated for 15 to 19 days at 15 ± 2 ºC
- Alternating 12 h NUV-light and 12 h darkness



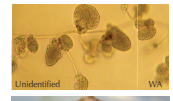
Results and discussion

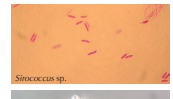
The results are presented in the table and illustrated by photos (alphabetic order). *Sirococcus* and *Fusarium* species are well known to cause damping off in nurseries, but are not reported to cause problems in production fields. Species of *Alternaria, Phoma, Botrytis, Cylindrocarpon,* and *Trichothecium* can also cause damages on seedlings. Seeds infected with *Caloscypha* will not germinate. *Anthostomella* is reported on cone scales of pine (*Pinus* spp.), but is not described as pathogenic. *Aureobasidium* is described as a saprophytic or weakly parasitic cosmopolitan fungal genus. *Aureobasidium* sp. is commonly found in Norway on buds of subalpine fir which fail to open in spring. On WA the majority of the seeds contaminated with *Aureobasidium* were accompanied by conidiomata of *Sclerophoma* (see photo of *Aureobasidium* on WA). PDA is a rich medium where *Aureobasidium* grows very rapidly and hence probably covers up the *Sclerophoma*. *Sclerophoma* is associated with dying of current year shoots on both fir and spruce (*Picea* spp.). *Acremonium, Acromoniella, Aspergillus, Cephalosporium* (no photo), *Cladosporium, Dictyopolyschema, Epicoccum, Mucor, Penicillium, Rhizopus,* and *Trichoderma* have at varying degrees been present on incubated Christmas tree samples from both spruce and fir, but are not considered to be primary causes of disease. *Chaetomium* has not been observed on diseased Christmas tree samples. All the identification work from the test is not completed. A so far unidentified fungus (photos on top right) was present on a number of samples, but particularly on subalpine fir seeds produced in Norway (57 % of the seeds from one location).

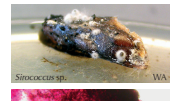
Fungi isolated from fir seeds (*Abies nordmanniana, A. lasiocarpa,* and *A. procera*) produced in five different countries. The table shows percent contamination in samples of 200 seeds (average of 100 on WA and 100 on PDA).

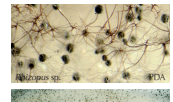
Fungal genera	Nordmann fir (*Abies nordmanniana*)					Subalpine fir (*Abies lasiocarpa*)				Noble fir (*Abies procera*)		
	Austria[1]	Georgia[2]	Georgia[3]	Georgia[4]	Russsia[5]	Canada[6]	Canada[7]	Norway[8]	Norway[9]	Norway[10]	Norway[11]	Norway[12]
Acremonium					0.5							
Acromoniella	1								0.5			
Alternaria	4	0.5	0.5	0.5	0.5				0.5	2.5		0.5
Anthostomella									0.5			
Aspergillus	0.5		1		2.5	1	0.5	3	1	0.5	5	14
Aureobasidium	4	1	0.5		3			5	18	30	1	22
Botrytis	0.5		0.5							12.5		1
Caloscypha						0.5	0.5					
Cephalosporium	2.5		0.5	0.5			0.5			1		
Chaetomium	0.5											
Cladosporium	1	0.5			2			1.5		1.5		5.5
Cylindrocarpon			1									
Dictyopolyschema	3	9.5	23.5	5	3.5		2.5	2.5	21			0.5
Epicoccum	1.5				1						0.5	0.5
Fusarium	0.5	1	0.5							1	1	
Mucor		1	4	1.5	0.5							0.5
Penicillium	20.5	32	46	36	2.5	18	1	2	23.5	10.5	21	14.5
Phoma	0.5		0.5		0.5				0.5	1		
*Rhizopus**		++	++		+	+					+	+
Sclerophoma					1			0.5		22		1
Sirococcus												31
Trichoderma	3	33.5	34	59	3	36.5	58.5					
Trichothecium	19	27.5	43	3.5		8.5	20			1		
Unidentified		3.5	1	3.5	31.5	11.5	9	4	57		7.5	26.5

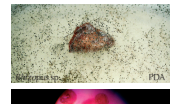
[1-12] Origin of seed samples: [1]Stefan, 2004 [2]Tlugi, Ambrolauri, 2004 (via Denmark) [3]Nedzvi, Borjomi, 2004 (via Denmark) [4]Tlugi, Ambrolauri, 2001 [5]Arbyz, Zelenczuk, 2003 [6]Sicamos, British Columbia, 2002 [7]Chawhels Mt., British Columbia, 2004 [8]Biri, 1998 [9]Biri, 2002 [10]Kaupanger, 2002 [11]Sogne, 1998 [12]Stange, 1998
*Rhizopus is so fast growing that it was not possible to tell which seed it originated from (five seeds per Petri dish) and is therefore marked with: ++ = a lot , + = some

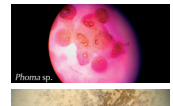


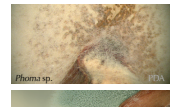


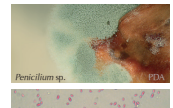


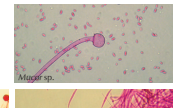


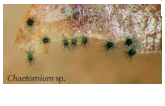


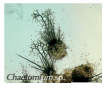


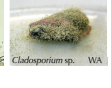


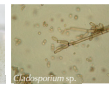


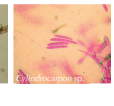


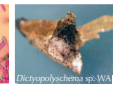


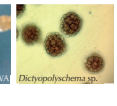


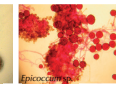


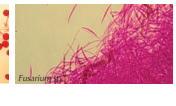












SEED BORNE FUNGI ON FIR

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ABSTRACT

In Norway, Nordmann (*Abies nordmanniana*) and subalpine fir (*A. lasiocarpa*) are the dominant Christmas tree species and noble fir (*A. procera*) the dominant species for bough production. In the spring of 2005 a survey was undertaken to determine the presence of fungi on seeds of these three plant species. Twelve seed samples were tested; five from Nordmann fir, four from subalpine fir and three from noble fir. The test included seeds produced in Austria (Nordmann fir), Canada (subalpine fir), Georgia (Nordmann fir), Norway (noble and subalpine fir) and Russia (Nordmann fir). The testing was done in a certified seed laboratory in Norway. One hundred seeds per sample were pre-treated in 1% NaOCl and plated on potato dextrose agar (PDA), and 100 seeds per sample were pre-treated in H_2O_2 and plated on water agar (WA). The PDA Petri dishes were incubated for 5 to 7 days at 20 $\pm$ 2 ºC and the WA Petri dishes for 15 to 19 days at 15 $\pm$ 2 ºC, both under alternating 12 h NUV-light and 12 h darkness. To date not all of the fungi have been identified, but fungi from several genera have been found: *Alternaria*, *Anthostomella*, *Aspergillus, Aureobasidium, Botrytis, Caloscypha, Cephalosporium, Chaetomium, Cladosporium, Dictyopolyschema, Epicoccum, Fusarium, Mucor, Penicillium, Phoma, Rhizopus, Trichoderma,* and *Trichothecium. Caloscypha* was only found on one subalpine fir seedlot from Canada and *Anthostomella* on one subalpine fir seed from Norway. All the other fungi were more commonly found in all the samples. *Fusarium* species are well known to cause damping off in nurseries, but are not reported to cause problems in production fields. Species of *Alternaria, Phoma, Botrytis* and *Trichothecium* can damage seedlings. *Anthostomella conorum* is reported on cone scales of pine (*Pinus* spp.), but is not described as pathogenic. *Aureobasidium* is described as a saprophytic or weakly parasitic cosmopolitan fungal genus. *Aureobasidium* sp. is commonly found in Norway on buds of subalpine fir which fail to open in spring, and it will therefore be included in a pathogenicity test.

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PROGRAMME

Sunday, September 11, 2005

12:00 - 18:00	EXCURSION (Buchlov, Buchlovice)	
20:00	ICEBREAKER (meet in lobby of Hotel Slunce)	

Monday, September 12, 2005

8:00 - 9:00	REGISTRATION	
9:00 - 9:10	**Karel Vančura**	Official opening
9:10 - 9:25	**Jack Sutherland**	IUFRO WP 7.03.04 Diseases and insects in forest nurseries: history
Session 1		
9:25 - 10:10	**Robert James**	Evolution of our knowledge of *Fusarium* diseases in forest nurseries
10:10 - 10:40	COFFEE BREAK	
Session 2		
10:40 - 11:00	**Raija-Liisa Petäistö**	*Gremmeniella abietina* on Norway spruce in Finnish forest nurseries
11:00 - 11:20	**Isabella Børja**	Damage on Norway spruce seedlings associated with *Gremmeniella* and *Phomopsis*
11:20 - 11:40	**Marja Poteri**	Control of *Gremmeniella abietina* and *Phacidium infestans* on Scots pine in Finnish forest nurseries
11:40 - 12:00	**Elna Stenström**	*Gremmeniella* infections on pine seedlings planted after felling severely *Gremmeniella* infected forest stands
12:00 - 13:30	LUNCH	
Session 3		
13:30 - 13:50	**M. W. Baksha**	Major forest nursery pests and diseases of Bangladesh
13:50 - 14:10	**Vítězslava Pešková**	Biotic damaging agents in forest nurseries in Czechia
14:10 - 14:30	**Andrej Kunca**	Occurrence of pest agents in Slovak forest nurseries from 1996 to 2004

14:30 - 14:50	**Elna Stenström**	Biological control of *Botrytis cinerea* in *Pinus sylvestris* seedlings in Swedish forest nurseries
14:50 - 15:10	COFFEE BREAK	
Session 4		
15:10 - 15:30	**Libor Jankovský**	The risk of introduction of quarantine pest important for forest nurseries in the Czech Republic
15:30 - 15:50	**Miroslava Bednářová**	*Dothistroma* needle blight in Czech Republic
15:50 - 16:10	**Jan Šamánek**	Eradication of the outbreak of chestnut blight (caused by *Cryphonectria parasitica* (MURRILL) BARR) in a forest nursery in Kladikov
16:10 - 16:40	COFFEE BREAK	
16:40 - 17:00	**Arja Lilja**	*Sirococcus conigenus* a new pathogen in Finnish forest nurseries
17:00 - 17:20	**Rimvydas Vasiliauskas**	Fungal communities in diseased conifer seedling roots in forest nurseries and afforested sites
17:20 - 17:40	**Arja Lilja**	*Phytophthora* spp. a new threat to forest seedlings
17:40 - 18:00	**Tomasz Oszako**	Forest nurseries as a source of *Phytophthora alni* – a cause of the recent alder decline in Poland
18:00 - 19:00	**POSTER SESSION**	Meeting room

Tuesday, September 13, 2005

7:30 - 13:00	EXCURSION	FGMRI RS Uherské Hradiště Nursery Kladíkov
13:00 - 14:30	LUNCH	Hotel Slunce
Session 5		
14:30 - 14:50	**Venche Talgø**	Damage in Norwegian Christmas tree plantations caused by fungi and nematodes possibly introduced by nursery stock
14:50 - 15:10	**Michelle Cram**	Biology and management of *Longidorus americanum* in a southern USA nursery
15:10 - 15:30	**Miloslava Kavková**	Oak powdery mildew (*Microsphaera alphitoides*): biology, epidemics and potential control in Europe

15:30 - 15:50	**Menkis Audrius**	Detection of fungi in fine conifer seedling roots in forest nurseries: morphotyping, isolation and direct sequencing
15:50 - 16:20	COFFEE BREAK	
16:20 - 16:40	**Miloslava Kavková**	Effect of inoculation of oak plantlets with *Paxillus invulutus* (BATCH.) and FR. and *Laccaria bicolor*
16:40 - 17:00	**Abdalla M. Abdelmonem**	Major diseases of date palm and their control
17:00-17:20	**Jan Lochman**	Identification of *Armillaria* species from soil by nested-PCR reaction
17:20 - 17:40	**Zdenka Procházková**	Incidence of *Ciboria batschiana* on *Quercus robur* and *Q. petraea* acorns: effect of collection year and oak stands, collection method, and annual rainfall
17:40 - 18:00	**Zdenka Procházková**	**Business meeting**

CLOSING OF THE WORKSHOP

19:30	OFFICIAL DINNER	We will walk as a group to wine cellar – meet in hotel lobby at 19:00

Wednesday, September 14, 2005

7:30	Departure from Uherské Hradiště	Meet in hotel lobby at 7:15
10:00 - 12:00	EXCURSION - Budišov nursery	
12:30 - 14:00	LUNCH (in the town of Třebíč)	
14:15 - 16:00	Sightseeing - visit of Jewish town in Třebíč, Basilica, town square	
16:00	Departure for Prague	
18:00 - 18:30	Arrival in Prague	