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ABSTRACTS

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LIST OF PARTICIPANTS

Arvidsson, Bernt	Svenska Skogsplantor AB Box 33, S-551 12 Jönköping Sweden FAX: +46-36-19 07 40 E-mail: bernt.arvidsson@skogsplantor.se
Baksha, Md Wahed	Forest Protection Division Bangladesh Forest Research Institute P.O. Box 273, Chittagong 4000 Bangladesh FAX: 880-31-681585; 681584; 681566 <u>E-mail: bfri@spnetctg.com</u>
Barras, Stan	USDA Forest Service Vegetation Protection & Mgmt. Research (1CEN) P.O. Box 96090, Washington, DC 20090-6090 USA FAX: +1-202-205-6207 <u>E-mail</u> : <u>sbarras/wo@fs.fed.us</u>
Bolumole Ademola Fola	International Society of Tropical Forester, Nigeria :No.1, Fola Bolumole Street, Off Abiola Way, Ring Road, Ibadan Nigeria FAX +234 2413385/ 2312167/ 8109051 <u>E-mail: gcmisl@ibadan.skannet.com</u> cefrad@errands.skannet.com
Cafourek, Josef	Forest Company Jaromêrtice, Forest Nursery Budisov 67503 Budisov Czech Republic FAX: 00420-618-875233
Dwinell, L. D.	USDA Forest Service, Southern Research Station 320 Green St., Athens, GA 30602-2044 USA FAX: 706/559-4287 E-mail: <u>ddwinell/srs_athens@fs.fed.us</u>
Elbakali, Mohamed Abdessamad	Dept. Biologia Vegetal Faculty of Biology, University of Barcelona, Av. Diagonal 645, 08028 Barcelona Spain FAX: 34-34112842 <u>E-mail: elbakali@porthos.bio.ub.es</u>
El-Settawy Ahmed A. A.	Forestry and Wood Tech. Dept. Fac. of Agric. Alexandria Univ. El-Shatby, Alexandria Egypt <u>E-mail : settawy@yahoo.com</u>

Fraedrich, Stephen W.	USDA Forest Service 320 Green Street, Athens, GA 30602, USA FAX: 706/559-4287 <u>E-mail</u> : sfraedrich_athens,srs@fed.us
Glavas, Milan	The Faculty of Forestry, Zagreb Svetosimunska 25 Croatia FAX: 385/1/218616 <u>E-mail: milan.glavas@zg.tel.hr</u>
Gulin, Leif	SCA FOREST AND TIMBER,Bogrundet nursery Box 90, 860 30 Sörberge Sweden FAX: +46-0-60 578131 <u>E-mail: leif.gulin@fat.sca.se</u>
Jarkko Hantula	Finnish Forest Research Institute Vantaa Research Centre P.O. Box 18 (Jokiniemenkuja 1) FIN-01301 VANTAA Finland FAX+358-9-8570 5569 <u>E-mail: jarkko.hantula@metla.fi</u>
Hernandez, George	USDA Forest Service, 1720 Peachtreerd, NW Atlanta GA 30367 USA FAX: 404 347 2776 <u>E-mail: ghernand/r8@fs.fed.us</u>
Holopainen, Jarmo	Dept. Ecology and Environmental Science University of Kuopio P.O.Box 1627, FIN-70211 KUOPIO http://www.uku.fi/~holopain/ FAX: +358-17-163 230 <u>E-mail: jarmo.holopainen@uku.fi</u>
James, Robert L.	USDA Forest Service, 3815 Schreiber Way Coeur d'Alene, ID. 83815-8363 USA FAX: 208-765-7307 <u>E-mail: rjames/rl_ipnf@fs.fed.us</u>
Juntunen, Marja-Liisa	Finnish Forest Research Instutute Suonenjoki Research Station Juntintie 40, FIN-77600 SUONENJOKI Finland FAX: +358-17-513 068 <u>E-mail: marja-liisa.juntunen@metla.fi</u>
Justin, Diji Allen	Faculty of Agricultural Science University of Ibadan Nigeria

Kurkela, Timo	Finnish Forest Research Institute Vantaa Research Centre P.O. Box 18 (Jokiniemenkuja 1) FIN-01301 VANTAA Finland FAX+358-9-8570 5569 <u>E-mail: timo.kurkela@metla.fi</u>
Laatikainen, Tarja	Dept. Ecology and Environmental Science University of Kuopio P.O.Box 1627, FIN-70211 KUOPIO Finland FAX: +358 17 163 229 <u>E-mail: tarja.laatikainem@uku.fi</u> <u>tlaatika@messi.uku.fi</u>
Landis, Tom D.	USDA Forest Service, Cooperative Programs PO Box 3623; 333 First Avenue Portland, OR 97208-3623 USA <u>E-mail: nurseries@aol.com</u>
Lilja, Arja	Finnish Forest Research Institute Vantaa Research Centre P.O. Box 18 (Jokiniemenkuja 1) FIN-01301 VANTAA Finland FAX: +358-9-8570 2575 <u>E-mail: arja.lilja@metla.fi</u>
Ma, Chuo	No.12-1-301, An yuan, An Hui Bei li, Chao Yang District, Beijing 100101 China <u>E-mail: machuo@public.bta.net.cn</u>
Magnano di San Lio Gaetano	Dep. Agrochimica e Agrobiologia Univ. of Reggio Calabria Piazza San Francesco, 2 89061 Gallina-Reggio Calabria Italy <u>E-mail: gmagnano@csiins.unirc.it</u>
Mohanan, C.	Division of Pathology Kerala Forest Research Institute Peechi-680 653, Kerala India FAX: 0487-782249 <u>E-mail: libkfri@md2.vsnl.net.in</u>
Majuri, Marja-Leena	Finnish Forest Research Institute Vantaa Research Centre P.O. Box 18 (Jokiniemenkuja 1) FIN-01301 VANTAA Finland FAX: +358-9-8570 5569 <u>E-mail: marja-leena.majuri@metla.fi</u>

Manninen, Anne-Marja	Dept. Ecology and Environmental Science University of Kuopio P.O.Box 1627, FIN-70211 KUOPIO Finland FAX: +358-17-163230 <u>E-mail</u> : <u>anne-marja.manninen@uku.fi</u>
Niemi Karoliina	Dept. Ecology and Environmental Science University of Kuopio P.O.Box 1627, FIN-70211 KUOPIO Finland FAX: +358-17-163230 <u>E-mail: karoliina.niemi@uku.fi</u>
Ogura, Nobuo	Laboratory of Nematology Forestry and Forest Products Research Institute P.O.Box 16, Tsukuba Norin Kenkyu Danchi Ibaraki 305-8687 Japan FAX: 81(Japan)-298-73-1543 <u>E-mail: nogura@ffpri.affrc.go.jp</u>
Paavolainen, Laura	Finnish Forest Research Institute Vantaa Research Centre P.O. Box 18 (Jokiniemenkuja 1) FIN-01301 VANTAA Finland FAX: +358-9-8570 5569 <u>E-mail: laura.paavolainen@metla.fi</u>
Perrin, Robert	INRA-Flore Pathogene 17 rue Sully, BV 1540, 21034 Dijon Cedex France FAX: 33(0) 80 69 32 26/30 57 <u>E-mail: perrin@dijon.inra.fr</u>
Petäistö Raija-Liisa	Finnish Forest Research Instutute Suonenjoki Research Station Juntintie 40, FIN-77600 SUONENJOKI Finland FAX: +358-17-513 068 <u>E-mail: raija-liisa.petaisto@metla.fi</u>
Poteri, Marja	Finnish Forest Research Instutute Suonenjoki Research Station Juntintie 40, FIN-77600 SUONENJOKI Finland FAX: +358-17-513 068 <u>E-mail</u> : <u>marja.poteri@metla.fi</u>
Prochazkova, Zdenka	Forestry and Game Research Institute Research Station Uherske Hradiste 68604 Kunovice Czech Republic <u>E-mail</u> : <u>vulhmvs@brn.pvtnet.cz</u>

Rikala, Risto	Finnish Forest Research Instutute Suonenjoki Research Station Juntintie 40, FIN-77600 SUONENJOKI Finland FAX: +358-17-513 068 <u>E-mail</u> : <u>risto.rikala@metla.fi</u>
Salerno, Maria Isabel	Centro de Investigaciones de suelos CISAUA, Calle 3 no. 584, 1900 La Plata Argentina <u>E-mail</u> : <u>permerlo@isis.unlp.edu.ar</u>
Segun, Fakolujo	Faculty of Agricultural Science University of Ibadan Nigeria
Smolander, Heikki	Finnish Forest Research Instutute Suonenjoki Research Station Juntintie 40, FIN-77600 SUONENJOKI Finland FAX: +358-17-513 068 <u>E-mail: heikki.smolander@metla.fi</u>
Stenström, Elna	Dept. of forest mycology and pathology Swedish University of Agricultural Sciences (SLU) Box 7026, S-750 07 Uppsala Sweden FAX:+46 (0)18-30 92 45 <u>E-mail: elna.stenstrom@mykopat.slu.se</u>
Sutherland, Jack	Applied Forest Science 4417 Bennett Rd.,Victoria, B.C. V9C 3Y3 Canada FAX: +250-598-1959 <u>E-mail: jsuther@islandnet.com</u>
Suto, Yasuo	5-11-46, Agenogi, Matsue-shi, Shimane 690-0015 Japan <u>E-mail: CZK12557@nifty.ne.jp</u>
Tervo, Leo	Finnish Forest Research Instutute Suonenjoki Research Station Juntintie 40,FIN-77600 SUONENJOKI Finland FAX: +358-17-513 068 <u>E-mail: leo.tervo@metla.fi</u>

Themann, Karin	Biologische Bundesanstalt fur Pflanzenschutz imGartenbau Messeweg 11/12, D-38104 Braunschweig Germany FAX: 0531/299-3009 <u>E-mail: k.themann@bba.de</u>
Vuorinen, Martti	Finnish Forest Research Instutute Suonenjoki Research Station Juntintie 40, FIN-77600 SUONENJOKI Finland FAX: +358-17-513 068 <u>E-mail: martti.vuorinen@metla.fi</u>

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HOLISTIC NURSERY PATHOLOGY: A NEW EMPHASIS ON SEEDLING HEALTH

Tom D. Landis¹

In holistic medicine, the emphasis is on treating patients as whole persons. Some of the basic tenets include the recognition of how environment contributes to disease and a stronger emphasis on prevention. Holistic principles should be applied to forest nurseries because, all too often, nursery pest specialists focus on their own interests and fail to look at the entire seedling. In this article, I propose taking a new perspective on nursery pest problems and giving as much attention to the host as to the pest. In addition, we need to acknowledge the critical importance of environment in nursery pest problems and work towards encouraging seedling health rather than merely treating disease.

¹ National Nursery Specialist, USDA Forest Service, Cooperative Programs, P.O. Box 3623; 333 SW First Avenue, Portland, OR 97208-3623, USA

R.L. James¹, R. Perez², R.K. Dumroese³ and D.L. Wenny³

Using *in vitro* techniques, we determined pathogenicity and virulence of 179 isolates of *Fusarium oxysporum* on young Douglas-fir seedlings. Our isolates were from soil or from healthy or diseased conifer seedling roots at two bareroot nurseries in the Inland Northwest of the United States. Isolates from diseased seedling roots were more virulent than those from healthy seedling roots. All isolates from diseased seedling roots were were virulent. Virulence of isolates was different between nurseries; soil-derived isolates were more virulent at one nursery, while the other nursery had more virulent root-derived isolates. At one nursery, soil-derived isolates were more virulent than those found on conifer roots. About half of the soil isolates were highly or moderately virulent on Douglas-fir germinants. We concluded that both virulent and non-pathogenic *F. oxysporum* isolates are common in forest nursery soils. Further, conifer seedling root infection is common but most isolates from healthy seedlings are either non-pathogenic or exhibit low virulence.

¹ Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection, Coeur d'Alene, ID, USA.

² Biological Technician, Universidad Autonoma de Nuevo Leon, Linares, Nuevo Leon, Mexico.

³ Research Associate and Professor, respectively, University of Idaho Research Nursery, Moscow, ID, USA.

L.D. Dwinell¹ and S.W. Fraedrich¹

The pitch canker fungus, *Fusarium circinatum* (= *F. subglutinans* f. sp. *pini*), is capable of infecting various vegetative and reproductive stages of many pine species. Researchers with the USDA Forest Service found in 1979 that the pitch canker fungus causes mortality of female flowers and mature strobili, and infects and destroys gametophyte tissues of seeds of several pine species indigenous to the southeastern United States. More recent research indicates that seed contamination by the pitch canker fungus is largely restricted to the outer seed coat of otherwise healthy, viable seeds in many seed lots, and that actual infection and destruction of endosperm and embryos may be much rarer events. The incidence of external contamination of Monterey pine seeds varied by geographic location in California. External contamination of pine seeds can be reduced or eliminated by appropriate seed treatments. Current research suggests that 5% and 30% hydrogen peroxide, Benlate^{\Box} and Arasan^{\Box}, alone or in combination may be useful seed treatments for controlling F. circinatum on the surface of seeds. Many questions currently remain unanswered regarding the epidemiology of seed and seedling diseases of pine. The mechanism by which the pitch canker fungus enters pine cones to infest seeds in not understood. Furthermore, there is little empirical data linking seed contamination by F. circinatum with seedling cankers that occur in nursery beds and outplanting sites. Preliminary data suggests that the major result of seed contamination by the pitch canker fungus is pre- and post-emergence damping-off. Pine seed health is a major area of our current research.

¹ USDA Forest Service, Southern Research Station, 320 Green St., Athens, GA 30602

Gladys Lori¹, Maria Isabel Salerno² and Silvia Wolcan¹

A survey of Fusarium species was carried out at two different forest nurseries in Argentina where diseased Pinus radiata seedlings are seldom observed. For this purpose, Fusarium taxa and population levels were determined in the nursery field soils alone and mixed with the organic matter amendments (Casuarina sp. or Pinus sp. litter, respectively) regularly incorporated into the soil before sowing. Pinus radiata seedlings with necrotic root symptoms were taken in order to isolate and identify the Fusarium species present in diseased seedlings. In parallel, Fusarium species present in the rhizosphere soil as well as those present in the treated and untreated nursery field soils where the seedlings were grown were also determined. F. oxysporum, F. solani and F. equiseti were the most frequently recovered species in the field soils at both nurseries. There was a greater diversity in Fusarium taxa and population levels in both nursery soils after the organic matter amendments. In addition to the species mentioned above, F. acuminatum, F. moniliforme, F. graminearum and F. pallidoroseum (=F. semitectum) were also recovered. F. oxysporum, and F. solani were the two species isolated from symptomatic seedlings grown at both nursery soils. These two taxa plus F. equiseti, F. moniliforme and F. acuminatum were isolated from diseased seedlings when grown in the treated soil in one of the nurseries. On the other hand, there were no differences in Fusarium taxa in the rhizosphere soil at either nursery. The three species present in the rhizosphere soil were F. oxysporum, F. solani and F. equiseti. In conclusion, field nursery field soils in Argentina have few Fusarium taxa. Cultural practices encourage the increase in the diversity of Fusarium species as well as in population levels that may be potential pathogens to Pinus radiata seedlings that might increase disease incidence.

¹ CIC-Facultad de Ciencias Agrarias y Forestales UNLP 60 y 119 (1900) La Plata, Buenos Aires, Argentina

² CISAUA-Facultad de Ciencias Agrarias y Forestales UNLP 60 y 119 (1900) La Plata, Buenos Aires, Argentina.

Stephen W. Fraedrich¹ and L. David Dwinell¹

The forest-products industry and nonindustrial private landowners in the southern United States depend on forest-tree nurseries for the production of high quality seedlings. Nursery managers have long used methyl bromide fumigation to provide broad spectrum control of weeds, disease-causing organisms, nematodes, and insects. Due to the phaseout of methyl bromide by 2005, it is imperative to determine the pest problems that will affect nursery production in the future, and to develop appropriate control strategies. Since 1995 we have been assessing pest problems and evaluating various fumigants including metam sodium, basamid, chloropicrin and methyl bromide for the control of pests and the production of seedlings. More recently we have turned our attention to assessing the potential benefits of other chemical (e.g., herbicides, seed treatments) and nonchemical (e.g., solarization, cover crops) pest control practices. Differences in seedling production frequently have not been observed between fumigation treatments and nonfumigated controls in our studies. Weeds, particularly nutsedge (Cyperus spp.), have been the most evident and severe problem. Although herbicides are available for most weeds found in southern pine nurseries, their use to control nutsedge and other difficult-to-control weeds have not been fully developed. We have not observed major losses in our studies that could be definitively attributed to diseases or nematodes. Information is presently lacking on the rate at which populations of disease-causing organisms and nematodes rebound in nurseries following fumigation. Although finding acceptable alternatives to methyl bromide is essential, developing the means to adequately predict pest problems is equally important. In the future, additional emphasis needs to be given to development of more comprehensive integrated pest management programs rather than just the evaluation of broad spectrum fumigants.

¹USDA Forest Service, Southern Research Station, Athens, GA

BIOFUMIGATION IN FOREST NURSERIES AN ALTERNATIVE TO CHEMICAL FUMIGATION?

B. Lebihan¹, R. Perrin¹ and P. Camporata¹

Organic matter, composts, manure usually emit volatile substances such as ammonia and carbon dioxide during the process of fermentation. Such gases revealed to be highly toxic for most of soilborne pathogens and nematodes.

Vegetables crop under greenhouses in the south part of Spain never suffer from soilborne diseases as a result of biofumigation occurrence.

Preliminary results carried out in controlled conditions showed that 48 and 96 hours exposure to volatile substances released from urban composts reduce greatly damping-off caused by *Rhizoctonia solani* to pine seedlings.

Biofumigation associated with solarisation can be the most promising mean of the integrated control of disease prevention program

¹Station de Recherches sur la Flore Pathogène du Sol INRA BV 1540 17 rue Sully 21034 Dijon cedex France

OUTCOME OF FIVE YEARS SOLARISATION IN FRENCH FOREST NURSERIES

B. Lebihan¹, R. Perrin¹ and P. Camporata¹

Attempts were made to use solar heating for controlling soilborne pathogens in trees forest nurseries for the last five years. Solar heating has been applied at spring and at summer times in different places and also in marginally suitable climate. Our results demonstrated that solarization can be very effective against the main fungal pathogens occurring in French forest nurseries but the effectiveness depends greatly on two kind of factors:

- Uncontrolled factors related to environment
- Controlled factors such as type of plastic films, irrigation

Our results showed that a minimum level of the temperature/duration combination is required to expect some efficiency and such a minimum is more easily reached in summer than in spring within the environmental conditions of temperate ecosystems. No more than one or two weeks solar heating is convenient in summer in the south part of France whereas up to two months can be necessary in marginal climate or at spring.

Soil solarisation appears as the most promising alternative to chemical fumigation.

¹Station de Recherches sur la Flore Pathogène du Sol INRA BV 1540 17 rue Sully 21034 Dijon cedex France

DISEASES, INSECTS AND THE USE OF PESTICIDES IN FINNISH FOREST NURSERIES

Marja-Liisa Juntunen

In 1996 a questionnaire on seedling production, growing systems and disease and insects problems was send to forest nurseries in Finland. Twenty-eight nurseries answered the questionnaire. Of these 20 were large nurseries (annual production 4-10 million seedlings) owned by commercial companies while eight were smaller (annual production 0.1-1.5 million seedlings), family-owned nurseries. This survey covered about 60 % of the forest tree seedling production in Finland.

According to the nurseries, stem lesions of birch and root dieback of spruce were the most common diseases in nurseries during the 1996 growing period. These diseases probably caused the largest losses in seedling production from 1990-1995. There were only two nurseries without stem lesions of birch and six without root dieback of spruce. Scleroderris canker caused the worst damage to Scots pine seedlings destined for planting in the spring of 1996. Other diseases were not so common, although they occasionally cause heavy losses at individual nurseries.

Among insects, aphids were the most abundant in nurseries during the 1996 growing year. Half of the nurseries used insecticides to prevent aphid damage. The second most abundant insect was *Lygus* bug. From 1990-95 there were only five nurseries, which did not use insecticides against aphids and *Lygus* bugs.

The nurseries, which replied to the questionnaire, used on average 10.9 kg pesticides (as active ingredients calculated) per million bareroot seedlings and 2.7 kg per million container seedlings. This means in total 640 kilograms of pesticides were used which represents less than 0,1 per cent of all the pesticides used in Finland in 1996. Half of the pesticides used in container seedling production were fungicides, one third insecticides and the rest herbicides.

Most of fungicides and insecticides were used in the production of pine. Chlorothalonil (Bravo 500) was the most commonly-used fungicide. It is registered for control of Scleroderris canker, Lophodermium needle cast and snow blights. Most insecticides were applied to pine and spruce seedlings in the nursery before shipping with permethrin formulations (most used Gori 920) being the most prevalent insecticide used against pine weevil.

¹ Finnish Forest Research Institute, Suonenjoki Research Station, Juntintie 40, FIN-77600 Suonenjoki, Finland

Laura Paavolainen¹, Timo Kurkela¹ and Jarkko Hantula¹

We isolated ascospores and conidia of fungi associated with leaf spots common in birch leaves. The fungi were identified as Pyrenopeziza betulicola and Cylindrosporium spp. by analyzing morphological characteristics with photomicroscopy. Random amplified microsatellite fingerprints were used to show a connection between the teleomorphic P. betulicola and anamorphic Cylindrosporium spp. In pathogenicity tests the birch leaves developed leaf spots when inoculated by P. betulicola mycelia. Based on these results, we concluded that the causative agent of the leaf spot disease in Finland is P. betulicola and its anamorph belongs to Cylindrosporium form genus. To study genetic variation, we collected population samples of this fungus around Finland. We found that the morphologically uniform P. betulicola is composed of two genetically different types A and B. In P. betulicola type A two alleles were found in the ITS region and nine alleles in the 18S rDNA region. In this fungus the level of geographic differentiation between all locations was moderate but statistically significant, indicating that the spore dispersal of P. betulicola occurs mainly on a limited scale. In addition, we studied genetic variation in situ by using a specific marker and denaturing gradient gel electrophoresis. Within one leaf genetically distinct individuals were detected in different spots, indicating that multiple colonization by P. betulicola is a common occurrence in birch leaves. However, the formation mechanism of separate spots was different from that of leaf edges.

¹ Finnish Forest Research Institute, Vantaa Research Centre, P.O. Box 18, FIN-01301 Vantaa, Finland

PROPOSAL TO PREPARE AN IUFRO WORLD SERIES ENTITLED 'FOREST NURSERY DISEASES AND THEIR MANAGEMENT: A WORLDWIDE PERSPECTIVE'

Jack R. Sutherland¹ and Zdenka Prochazkova²

We have obtained permission from IUFRO to write and edit the contributions of others for this proposed publication. The purpose of our oral presentation and subsequent discussions is to solicit the endorsement of this Working Party (S7.03-04) for this project and to recruit working party members to write specific sections. Our present idea is that rather than covering all diseases in their country/area that each author try to relate types of diseases to factors such as past, present and perhaps future nursery cultural practices, tree species being grown, the overall importance of diseases, and then use one to three specific diseases to show how forest nursery diseases are managed in that locality. We are not looking for lists of chemicals that are used. Based on the outcome of our discussions and the written suggestions of prospective authors we will prepare a draft outline to guide authors in writing their contribution. Our goal is to have a final draft of the publication within 2 years and hopefully the publication will be available for distribution at our next meeting in 2002.

¹Consultant, 2325 McNeill Ave.Victoria, B.C.V8S 2Z1, Canada

² Forestry and Game Management Research Institute, Uherske Hradiste, 686 0 Kunovice, Czech Republic

MANAGEMENT OF FOREST INSECTS AND DISEASES IN CHINA

Ma Chuo¹

In order for sustainable forestry to be achieved in China it is necessary to develop strategies and tactics for managing those insects and diseases which damage forests. Since It is no longer acceptable to depend on single options for managing specific pests integrated pest management (IPM) schemes are being developed which rely on ecologically sound principles. Pest management practices derived from research on forest pests must comply with sustainable forest development. Priority areas for research include: (i) use of classical tree improvement and molecular biology techniques to develop trees which are resistant to pests, (ii) development of pest management techniques which maintain forest biodiversity, (iii) the study and use of naturally-occurring organisms for bio-control of forest pests, (iv) development and use of technology for early detection of pest outbreaks, and (v) development of new biocides.

¹ The Chinese Academy of Forestry, No. 12-1-301, An Yuan, An Hui Bei Li, Chaoyang District, Beijing 100101, China

SOME NEW FOREST TREE SEEDLING DISEASES IN CZECH REPUBLIC

Vlastislav Jancarik¹ and Zdenka Prochazkova¹

Recently several diseases, which were formerly absent or rare, have appeared in forest nurseries and plantations in the Czech Republic. The most conspicuous of these has been a severe (near-epidemic) outbreak and spread of the rust *Coleosporium* sp. on Scots pine, *Pinus sylvestris* L. Until lately this disease was confined to mountainous areas, however, since the late 1970s and early 1980s it has spread to and within lower elevations and in 1998 it became epidemic in several regions of the country. To date the effect of this disease on the overall health of even heavy-infected seedlings has not been determined. Also another rust, *Melampsora pinitorqua* Rostrup, which previously occurred only in a few restricted areas, has appeared throughout the country, killing Scots pine seedlings in forest nurseries.

Pine needle cast, caused by *Lophodermium* sensu lato, has also severely damaged pine seedlings in forest nurseries and plantations. Rather recently the predominant species was *L. pinastri* (Schrad). Chev. while *L. seditiosum* Minter, Staley et Millar was known only from a few localities. However, the latter fungus is now spreading throughout the country where it is severely damaging pines in forest nurseries and plantations. Fungicides, which were previously effective against this pathogen, are now proving to be ineffective. Additional threats are posed by two diseases from the North America, i.e., *Mycosphaerella dearnessii* (Cooke et Harkn.) Lindau and *M. pini* Rostrup so far have not been found in the Czech Republic. Another potential threat is the Scleroderris canker fungus, *Ascocalyx abietina* (Lagerb.) Schlapfer, which although detected on several earlier occasions appears to have spontaneously disappeared.

Another problem is an unexplained and dramatic loss in vigour of pine seedlings. Initially the seedlings seem healthy, with green and well-developed roots and buds, but they die soon after bud flush and initiation of new growth. An additional problem affects European beach, *Fagus sylvatica* L., seedlings in forest nurseries or soon after outplanting to reforestation sites. It has been shown that such seedlings suffer from subliminal, poor

growth and tracheomycotic fungi such as *Verticillium* and *Ophiostoma* sp. fruiting bodies occur on both the stems and leaves of diseased seedlings. Most likely initial infection occurs in the nursery prior to outplanting in the forest. There is an overall concern that these new diseases, which although known from other European countries, could cause very important losses in Czech forestry.

¹ Forestry and Game Management Research Institute Uherske Hradiste, 686 04 Kunovice, Czech Republic

B. Le Bihan¹, M.L. Soulas¹, R. Perrin¹ and P. Camporata¹

Cylindrocladium scoparium has been isolated from damped-off seedlings of different coniferous species grown in acid soils French nurseries. This pathogen had never been mentioned before in forest nursery in France and in Europe. *C. scoparium* can cause some damages to coniferous seedling during the first 30-45 days after emergence (damping-off) but also can cause some root rot later particularly if seedlings have been damaged from transplantation. Nevertheless this pathogen does not occur very frequently and the severity of damping-off and root rot it could produce in French forest nurseries remains very low.

¹ Station de Recherches sur la Flore Pathogène du Sol INRA BV 1540 17 rue Sully 21034 Dijon cedex France

PHYTOPHTHORA CACTORUM: DIVERSITY AND IDENTIFICATION

Jarkko Hantula¹, Eeva J. Vainio¹, Heikki Nuorteva¹ and Arja Lilja¹

We used genetic fingerprinting to show that *Phytophthora cactorum* isolates from birch and other host species tested are genetically different. The pathogenicity tests suggested that isolates of *P. cactorum* would be more virulent to their original hosts than to other plants. Genetic fingerprints also revealed that *P. cactorum* population on strawberry is much more variable in North America than in Europe. In order to develop a recognition system for *P. cactorum* we cloned and sequenced an efficiently amplifying RAMS-marker, and designed primers for it. In specificity tests amplification products with expected size were obtained only using template DNA from *P. cactorum* or *P. idaei*. The primers were used to successfully amplify DNA from a diseased birch and strawberry, and to show that *P. cactorum* in birch nurseries is soilborne.

¹ Finnish Forest Research Institute, Vantaa Research Centre, P.O. Box 18, FIN-01301 Vantaa, Finland

EXPERIENCES WITH DIFFERENT DIAGNOSTIC TECHNIQUES TO DETECT *PHYTOPHTHORA* SPP. IN WATER

Karin Themann¹ and Sabine Werres¹

Today in container-grown nurseries recirculation of the surplus and drainage water is usual because of economic and environmental reasons. This always contains a risk of spreading pathogens with the irrigation water, like the fungus of the genus *Phytophthora*. *Phytophthora* species are known to be one of the most damaging pathogens in nurseries. They are well adapted to the water, which they need to produce motile zoospores for dissemination.

There are only a few methods to detect fungal structures of *Phytophthora* in water and sediment samples and little or no information about their reliability. Therefore the following methods were compared to detect *Phytophthora* spp. under laboratory conditions and with samples from nurseries: The serological method DAS-ELISA (*double antibody sandwich enzyme-linked immunosorbent assay*), microbiological methods (selective media) and bait plants (apples, lupin seedlings, *Rhododendron* leaves). The specifity and sensitivity of each method was investigated with different *Phytophthora* species. Fungal structures (e.g zoospores) produced on solid and in liquid media served as test material.

In addition water and sediment samples from nurseries that recycle their irrigation water were examined using the same methods. Samples were taken regularly over a period of 14 months from different places in nurseries of different ages. The methods were compared e.g. for their ability to detect *Phytophthora* spp. in these samples. Some results are presented.

¹ Biologische Bundesanstalt für Land und Forstwirtschaft, Institut für Pflanzenschutz im Gartenbau, Messeweg 11/12, D - 38104 Braunschweig, Germany

REPORTS OF PHYTOPHTHORA CINNAMOMI FROM ITALY

S.O. Cacciola¹, A. Pane², E. Motta³, G. Magnano di San Lio⁴

Phytophthora cinnamomi is a quarantine plant pathogen in Italy. During the last twelve years, however, it has been found on several woody plants in agricultural crops, in forest ecosystems as well as in nursery stocks of ornamental and forest shrubs and trees (1). The first record of *P. cinnamomi* in Italy dates back to 1986 (2), when the pathogen was found on chestnut trees in Latium. Subsequently, it was isolated in chestnut coppices from trees with symptoms of ink disease, in various regions of central Italy. On these trees P. cinnamomi occurred together with other Phytophthora species, such as P. cambivora and P. citricola. Presently, the list of host plants from which P. cinnamomi has been isolated in Italy includes, beside chestnut, various species of *Rhododendron* and Chamaecyparis, walnut, avocado and myrtle. All the isolates were A2 mating type. The pathogen has been reported from various regions of central and northern Italy as well as from main islands, Sicily and Sardinia, and there is evidence to suggest that nursery stocks are a major source for its spread. In Sardinia e.g., P. cinnamomi has been isolated, together with P. nicotianae, P. cactorum and P. cryptogea, from containergrown myrtle plants in nursery. Myrtle is a typical shrub of the Mediterranean flora and it is often used for reafforestation of slopes and coastal areas in southern regions and main islands of Italy. In Sardinia, it is a typical species of the underbrush in forests of cork-oak, which has been reported to be susceptible to root and crown rot incited by P. cinnamomi (3).

References: (1) S.O. Cacciola *et al.* Informatore Fitopatologico 49: 23-29; *(2)* C. Parrini *et al.* Informatore Fitopatologico 47: 31-35; *(3)* S.M. Mircetich *et al.* Pl. Dis. Reptr. 61: 66-70.

¹ Istituto di Patologia Vegetale, Università di Palermo, Italy.

² Dipartimento di Scienze e Tecnologie Fitosanitarie, Università di Catania, Italy.

³ Istituto Sperimentale per la Patologia Vegetale, Roma, Italy.

⁴ Dipartimento di Agrochimica ed Agrobiologia, Università di Reggio Calabria, Italy.

STATUS OF FOREST NURSERY DISEASES IN INDIA AND EMERGING TRENDS IN SEEDLING DISEASE MANAGEMENT

C. Mohanan¹

The total forest cover in India is estimated to be 633,397 Km², which constitutes 19.8% of country's geographical area. The forest area in recent past has not changed much because of its diversion for non-forestry purpose has been more or less compensated by large-scale afforestation and reforestation activities. Of the 320 timber species available in India, 65 are of major importance and 106 have a fair demand. Many of these indigenous and exotic trees have been raised on a large-scale in production forests, treelands and village forests to meet the demand from the traditional and modern sectors. Seedlings of both broad leaved and conifer species are still raised by conventional methods though, rootrainer technology has been very recently introduced in forestry sector. Maintenance of seedlings in forest nurseries ranges from 3 to 18 months or more depending upon the tree species, climatic conditions as well as planting techniques followed. Raising and maintaining disease-free healthy planting stock for large-scale planting program is often risky and failures of nurseries due to outbreak of disease are not uncommon. Forest seedlings raised in warmhumid tropical zones are more often prone to disease hazard than those in warm-temperate zones. Fungi are the most predominant nursery pathogens, while bacteria, viruses and phytoplasma cause diseases in seedlings to a lesser extent. Among the fungal pathogens, Rhizoctonia solani has emerged as the most potential and widespread pathogen in forest nurseries in India, causing various diseases such as damping-off, web blight, collar rot, stem and foliage infection and root rot at different growth phases of the seedlings. Both broad leaved and conifer species are equally vulnerable to this pathogens. Cylindrocladium is the other important nursery pathogen, which causes epiphytotics in eucalypts nurseries raised in high-rainfall areas. At least 12 species of Cylindrocladium have been reported from India associated with the seedling diseases. Sclerotium rolfsii, Fusarium, Pythium, Macrophomina, Colletotrichum, Alternaria, Phoma, Phomopsis, Dreschslera, Bipolaris are the other potential pathogens in forest nurseries. Disease management in forest nurseries is at a crossroad. Due to repeated application of broad-spectrum fungicides in forest nurseries

over the years, many of the pathogens exhibit resistance to pesticides. *Rhizoctonia solani* exists in different anastomosis groups with varying virulence and fungicidal resistance. *Cylindrocladium* species also shows resistance to carbendazim, the most effective chemical being used for decades. Recently more emphasis has been given to cultural control measures in forest nurseries viz. regulation of shade, soil water regime, microclimate, seedling density, etc. integrated with biological measures, by soil solarization and introduction of indigenous antagonistic organisms, along with prophylactic fungicidal application. The paper highlights the results accomplished during the past two decades on forest nursery diseases and new trends in disease management.

¹ Kerala Forest Research Institute Peechi 680 653 Kerala India

DISEASES OF THE MOST IMPORTANT TREES GROWING IN EGYPT

Ahmed A.A. El-Settawy¹

The most common nursery diseases in Egypt fall into four groups, namely, damping off, leaf spots, powdery mildews and root diseases. The primary cause of damping off is *Fusarium solani* that infects wide range of tree species. It has been isolated from rot cuttings of *Populus* spp., seedlings of *Pinus* spp. and *Taxodium distichum*. The two most important leaf spot fungi are *Alternaria alternata* that occurs on *Eucalyptus camaldulensis* and *Populus* spp., and *Pestalotiopsis magniferae* on *Eucalyptus* spp. The most common hosts of powdery mildews are *Acacia saligna*, *E. camaldulensi* and *Quercus rubra*. *Rhizoctonia solani* and *Olpidium* spp. are the most prevalent root pathogens of several tree species. Pathogenicity tests have been completed for the foregoing pathogens. The conditions, other than poor sanitation and stress that may result in nursery diseases are discussed.

¹ Forestry and Wood Technology Dept., Fac. Agric. Alex. University, El-Shatby, Alexandria, Egypt

INSECTS EFFECTS ON TECTONA GRANDIS FOREST IN NIGERIA

Ademola Fola Bolumole¹

¹International Society of Tropical Forester, Nigeria, Chapter:No.1, Fola Bolumole Street, Off Abiola Way, Ring Road, Ibadan, Nigeria.

A REVIEW OF FOLIAGE DISEASES IN NURSERY STOCKS AND THEIR CONTROL IN SHIMANE PREFECTURE, JAPAN

Yasuo Suto¹

Several foliage diseases damage forest nursery stocks in Shimane Prefecture, Japan. *Cercospora* needle blight in *Cryptomeria japonica*, *Pestalotia* disease in *Chamaecyparis obtusa*, brown needle disease in *Pinus densiflora* and *P. thunbergii*, and frosty mildew in *Quercus acutissima* are serious problems. Etiological and ecological characteristics of the causal fungi of these diseases were examined in relation to control of these diseases. Effective measures of chemical application are discussed based on the field experiments.

¹5-11-46, Agenogi, Matsue-shi, Shimane 690-0015, JAPAN

SPECIES SPECIFIC PCR-PRIMERS AS A TOOL TO DETECT LATENT SHOOT AND NEEDLE DISEASE

Elna Stenström¹

Shoot and needle diseases can cause serious problems in forest nurseries. Many of these diseases have a latent period between infection and appearance of symptom and fruit body formation. For example *Lophodermium seditiosum* and *Phacidium infestans* infect seedlings during summer – autumn but the first symptoms of infection do not appear until the following spring, while fruit bodies are formed some months later. For *Gremmeniella abietina* the latent period could be even longer.

Because of such latent periods, it is sometimes impossible to know if seedlings are infected or not. Therefor many nursery managers treat their seedlings with fungicides, without knowing if they are infected or not, just to be sure. To create healthier seedlings for outplanting and to decrease the use of fungicide, nursery managers in Sweden emphasised the need to detect and identify the pathogens during their latent periods.

Species specific PCR primers have been designed against *L. seditiosum* after sequencing the ITS region of the ribosomal DNA. These primers could now be used to detect latent infection of *L. seditiosum* in young pine seedlings before visible symptoms of infection appear.

Dep. of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 07 Uppsala, Sweden.

IDENTIFICATION AND CHARACTERICATION OF *RHIZOCTONIA SOLANI* USING PCR/RFLP AND PINE BIOASSAY

P. Camporata¹, M.L. Soulas¹ and R. Perrin¹

A total of 255 isolates from various origins were first classified within anastomosis groups and sub groups according to the method described by Parmeter et al 1969. Then we developed specific primers RS1 and RS4 based on the ITS sequences of the nuclear rDNA. The PCR products were digested with seven restriction enzymes : Ase I, Ava II, Bse GI, Hinc II, Mse I, Bspl 1, and Cfr 13 1. There is a perfect correspondence between anastomosis classification and molecular systematic. The PCR/RFLP method revealed as very discriminant and allow detection and identification of *R.solani* in naturally infected pines seedling at very early stage of attack.

The pathogenicity of the strains belonging to the main anastomosis groups occurring in France express easily within a pine bioassay we developed.

¹ Station de Recherches sur la Flore Pathogène du Sol INRA BV 1540 17 rue Sully 21034 Dijon cedex France

MONOCLONAL ANTIBODIES TO GREMMENIELLA ABIETINA

S. Vartiainen¹, K. Koistinen¹, K. Ehrbar¹, E.O. Kajander¹ and R.-L. Petäistö²

Monoclonal antibodies are practical tools for the detection and component analysis of pathogenic fungi. A library of monoclonal antibodies was produced to conidial spores of Gremmeniella abietina A- and B-types. Thirty five independent monoclonal antibodies were characterized for binding to spore and mycelia antigens from known races of G. abietina and 13 common fungi belonging to Ascomycetes, Deuteromycetes, Zygomycetes and also a *Penicillium* sp. endophyte isolated from pine seedlings. Using ELISA, 26 of the monoclonal antibodies recognized spores and mycelia epitopes, three recognized mycelia only and six detected mainly or exclusively spores. Only three of 35 antibodies recognized spores of *Rhizopus* or *Trichoderma*, or both. Five monoclonal antibodies recognizing G.abietina spores were selected for further immunoassay development. A14-1 and B106-1 showed a strong competition with each other but not with B20-1, consequently two different surface epitopes could be detected. These epitopes existed in A-, B-, and Alpine amplitypes. Specificity was detected in ELISA, Dot Blot and immunofluorescence staining. Antibody B106-1 recognized a very specific macromolecular epitope and was used to develop a prototype capture-ELISA immunoassay for the specific detection of G. abietina spores from natural samples. Its detection limit was one spore and it recognized spores from European, North American and Asian races and tested types of G. abietina. Antibody A14-1 was used to develop a sensitive immunofluorescence detection method with a detection limit of one spore. These results indicate the unique nature of G. abietina spore epitopes and, more generally, the special nature of spore antigens. This unique finding was used successfully to detect air-borne spores collected by spore trapping and shows the potential use of such antibodies in monitoring for the pathogen.

¹ Department of Biochemistry, University of Kuopio, P.O.B 1627, FIN-7021, Kuopio, Finland

² Finnish Forest Research Institute, Suonenjoki Research Station, Juntintie 40, FIN-77600 Suonenjoki, Finland

ECTOMYCORRHIZAL FUNGI AND BIOLOGICAL CONTROL OF SOIL-BORNE PATHOGENS IN FOREST NURSERIES

Karoliina Niemi¹, Helvi Heinonen-Tanski² and Arja Lilja³

In Finland, production of container seedling has to a great extent replaced that of bareroot seedlings. Today 95 % of Scots pine (*Pinus sylvestris* L.) and 77% of Norway spruce (*Picea abies* L. Karst.), which are the main conifer species produced in forest nurseries, are containerized. Under natural conditions they both live in ectomycorrhizal (ECM) symbiosis in which the fungus encloses the roots with the hyphal mantle and grows among the epidermal cells of the roots. However, in nurseries the seedlings of these conifers are often non-mycorrhizal as the results of the high nutrient concentrations, especially nitrogen, in the growth media.

Mycorrhizal symbiosis improves the uptake of both water and nutrients by the plant and good nutrient status of the host plant improves defense against pathogens. Furthermore, ECM fungi compete for infection sites on the host root and for nutrients in the rhizosphere, impeding the growth of pathogenic fungi. By growing in the root cortex the ECM fungus activates plant defense reactions such as production of polyphenols. The ECM fungus itself may produce antibiotics, which together with the root metabolites form an effective biochemical defense system. The compact hyphal mantle around the root may act as a physical barrier. Efficient protection by mycorrhizae depends, however, largely on the mycorrhizal fungus, on the pathogen and on soil conditions.

Despite the general positive influences of ECM fungi on the host plant, artificial inoculation is seldom used in nurseries and the results of the suppression of pathogens have been variable. Although ECM fungi reduce the growth of soil-borne pathogens in the laboratory the pathogens are often more vigorous in nurseries. So that ECM fungi can outcompete pathogenic fungi, seedlings should be inoculated with rapidly growing mycorrhizal symbionts. This allows the ECM fungus to come into contact with the roots before or at the same time as the pathogen. Furthermore, new suitable isolates need to be continuously tested because repeated *in vitro* culturing of ECM fungi, for periods as short as 2 years, may result in their losing the ability to form mycorrhizae. The high cost of artificial inoculation and testing of new ECM fungi have restricted large-scale application of these fungi in nurseries.

Recently greater attention has been paid to the co-inoculation of ECM fungi and so-called helper bacteria plus the simultaneous application of both chemical and biological agents in nurseries. In some studies specific ECM isolates have been shown to tolerate quite high concentrations of fungicides. To obtain more information on such an integrated, disease management approach we have started to investigate the role of specific ECM fungi and inhibitors of polyamine synthesis for control of *Rhizoctonia* sp. on Scots pine seedlings. These experiments are being done in conjunction with traditional inoculation tests.

¹ Dept. of Ecology and Environmental Science, University of Kuopio, P.O.Box 1627 FIN-70211 Kuopio, Finland

² Dept. of Environmental Sciences, University of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland

³ Finnish Forest Research Institute, Vantaa Research Centre, P.O.Box 18, FIN-01301 Vantaa, Finland

R. Perrin¹, B. Lebihan¹ and P. Camporata¹

During solarisation process the different combinations, maximum temperature/duration, have usually different effects depending on the pathogenic species hosted by the soil and to a lesser extent soil characteristics. Our field trials showed that the most sensitive species belonged to the genus *Pythium*. The lethal temperature is slightly higher for *Rhizoctonia* solani but the most resistant ones are the *Fusarium* species. The native mycorrhizal species of the nursery appeared as the less thermoresistant fungal inhabitants of nursery soils.

Simulation work showed that *Pythium* species cannot survive 49°C/2 hours 2 successive days whereas *Rhizoctonia solani* need higher temperatures and/or longer period to be killed (49°C/3 days or 53°C/2 days). Nevertheless such conditions are frequently encountered up to 20 cm depth under French environmental conditions during summer.

¹Station de Recherches sur la Flore Pathogène du Sol INRA BV 1540 17 rue Sully 21034 Dijon cedex France

POSTERS

RHIZOCTONIA DISEASE IN FOREST NURSERIES

M. A. El Bakali¹ and M.P. Martin²

Potting mixes and plants exhibiting symptoms of a root disease were collected from nurseries or obtained from the laboratory of plant health (DARP, Generalitat de Catalunya). Ten isolates of Rhizoctonia sp. were obtained by isolating from diseased plants, planting media or by isolating from roots of seedlings (*Pinus, Quercus, Abies,*). Hyphal cells of six isolates were binucleate and four isolates had multinucleate cells. The isolates did not anastomose with known binucleate or multinucleate *Rhizoctonia*. Certain binucleate isolates reduced the growth of wheat and tomatoes while certain multinucleate isolates decreased the growth of wheat and increased the growth of tomatoes. It is difficult to predict such damage due to the influence of inherent factors in the potting mix and variation in pathogenicity of the isolates present.

¹Dept. Bio. Veg, Biologia, University of Barcelona, Av. Diagonal, 645. 08028 Barcelona, Spain

²Real Jardin Botanico, CSIC. Pza. De Murillo, 2. 28014 Madrid, Spain

GROWTH, MYCORRHIZAL FORMATION AND AMMINO ACID CONTENT OF FUNGICIDE-TREATED, SCOTS PINE NURSERY SEEDLINGS

Tarja Laatikainen¹, Marja-Liisa Juntunen² and Helvi Heinonen-Tanski¹.

The growth responses of container-grown Scots pine (Pinus sylvestris L.) seedlings and their ectomycorrhizal fungi treated with the fungicide chlorothalonil and propiconazole were determined in a forest nursery and afterward when the seedlings had been outplanted to a reforestation site. The study started in the spring of 1995 and it was completed in the autumn of 1997. Scots pine seedlings were grown in paper containers (Lännen Tehtaan ekopotti PS 508) filled with low-humified sphagnum peat (Finnpeat M6, Kekkilä Ltd.) according to normal forest nursery practice. There were three test groups treated with fungicides and a control group without fungicides with four, $40 \times 60 \text{ cm}^2$, seedling containers per group. Each container held 149 seedlings per container, so the seedling growth density was 620 seedlings/ m^2 . Twelve weeks after seed sowing, the seedlings were removed from the greenhouse and the first fungicide application was made. This was repeated four more times each 2-3 weeks before samples were taken. The first group of seedlings was treated with chlorothalonil (Bravo 500) and the second with propiconazole (Tilt 250 EC). A third test group was first treated three times with chlorothalonil and then twice with propiconazole. The amounts of chlorothalonil and propiconazole in each application were 2 000 g/ha (a.i.) and 125 g/ha (a.i.), respectively. These rates are recommended for forest nurseries. The first samples were taken in October, 1995, at the end of the first growing season when the seedlings were 24 weeks old. The seedlings were overwintered (stored) outdoors in paper containers. The second samples were taken in May, 1996, before the rest of test seedlings were outplanted at a forest site at the Suonenjoki Research Station. Subsequent samples were taken from these outplanted seedlings in the autumns of 1996 and 1997.

The ergosterol content of roots, indicating ectomycorrhizal infection, increased with time, but there was no significant difference between test groups. Ergosterol concentrations varied greatly within each test group, however, in 1995 there was a significant correlation

between percent ectomycorrhizal infection and ergosterol content in the control group. During the first growing season chlorothalonil-treated seedlings were the shortest and their biomass was the lowest, whereas the propiconazole treated seedlings were the tallest and their biomass was the highest. In contrast, in 1997 (end of the third growing season), control seedlings had the highest biomass. The decrease in dry mass of the needles and shoots of chlorothalonil-treated seedlings persisted through 1997, indicating that the chlorothalonil had retarded the frost hardening of seedlings.

The fungicide-induced changes in the free amino acid content of needles was especially noteworthy. In 1995, the free amino acid assays showed accumulation of (i) arginine and glutamine in chlorothalonil-treated seedlings and (ii) accumulation of aminobutyric acid and decreases in glutamine and glutamic acid in propiconazole-treated seedlings. By September 1996 there were no differences in amino acid content among the various test groups. Generally, in pine seedling arginine accumulates when net protein synthesis is limited by factors other than nitrogen availability. The results of this study indicate some kind of stress-related growth reduction in peat-pot seedlings treated with chlorothalonil at the nursery, but this growth reduction is late dissipates once the seedlings are outplanted to the forest.

¹ Department of Environmental Sciences, University of Kuopio, P.O.Box 1627, FIN-70210 Kuopio, Finland

² Finnish Forest Research Institute, Suonenjoki Research Station, Juntintie 40, FIN-77600 Suonenjoki, Finland

Nobuo Ogura¹

A new species of steinernematid, *Steinernema kushidai*, was recovered from a soil sample collected in Hamakita City situated in central Japan. The nematode showed a significant lethal effect on several species of scarabaeid grubs. Application of infective juneniles (IJs) on the nematode onto soil in plastic cups, where the 3rd instar larvae of the cupreous charfer were individually reared, killed the larvae within 4 days. Lethal concentration (50 %) within 10 days was ca. 20 IJs. The nematode could be mass-produced in peptone and yeast extract-rich media. IJs, that had been stored at 5 C after recovery from artificial culture, did not survive well, with over 90 % dying within 10 days. When IJs were preconditioned at 10 C for over 8 days, a survival rate of over 50 % was seen 100 days after storage at 5 C. During this preconditioning, trehalose concentration in the IJs icreased. In application of the nematodes in field, more than 100 000-200 000 IJs per square meter, reduced injuries caused by Scarabaeid grubs. *S. kushidai* applied on a sweet potato field and on a nursery of the Japanese cypres persisted for at least 2 years.

¹ Forestry and Forest Products Research Institute, P.O. Box 16, Tsukuba Norin Kenkyu Danchi, Ibaraki 305-8687, Japan

TERPENES AND THE SCLERODERRIS CANKER RESISTANCE IN PINE

M. Vuorinen¹, P. Kainulainen², A.-M. Manninen², L. Sallas², J. Holopainen² T. Kurkela³ and M. Hanso⁴

Normally Scots pine, *Pinus sylvestris* L., is resistant to Scleroderris canker. However, when sufficient infecting spores of *Gremmeniella abietina* are present the disease can become epidemic on Scots pine following an abnormally cold, rainy growing season. We tested Scleroderris canker resistance in a trial using nine Scots pine provenances and three climatically-different locations. This protocol allowed us to simultaneously determine seedlings, which were either susceptible or resistant to Scleroderris canker. Pines synthesize relatively large amounts of secondary-metabolite terpenes, which possess antimicrobial properties and are considered to be part of the cellular defence system of the plants against microbial pathogens. These antimicrobial compounds can be divided into pre-formed compounds that are endogenous plant constituents and to post-infection metabolites, which are released in response to pathogen attack. The susceptibility of pine seedlings to Scleroderris canker was tested by artificial inoculation and a relatively high number of seedlings of some southern provenances became diseased in the harshest, northern test site. The concentration of terpenes was lower in susceptible than in resistant provenances.

¹ Finnish Forest Research Institute, Suonenjoki Research Station, Juntintie 40, FIN-77600 Suonenjoki, Finland

² University of Kuopio, Department of Ecology and Environmental Science, PO Box 1627, FIN-70211 Kuopio, Finland

³ Finnish Forest Research Institute, Vantaa Research Centre, P.O. Box 18, FIN-01301 Vantaa, Finland

⁴ Estonian Agricultural University, Facultu of Forestry F.R. Kreutzwaldi 5, EE-2400 Tartu, Estonia

TIMING FUNGICIDE APPLICATION FOR CONTROL OF SCLERODERRIS CANKER: IN VITRO EFFECTS ON CONIDIA, GERMINATING CONIDIA AND MYCELIUM AND DISEASE CONTROL ON INOCULATED SEEDLINGS

R.-L. Petäistö¹ and M.-L. Juntunen¹

Two sets of experiments were made to determine the effects of application timing of chlorothalonil (Bravo) and propiconatsol (Tilt) fungicides on the Scleroderris canker pathogen, *G. abietina, in vitro* and on disease control on inoculated Scots pine, *Pinus sylvestris*, seedlings. In the first experiment to determine fungicide effect on *G. abietina* conidia, germinating conidia and hyphae, the fungicides were added to liquid, V8 culture medium 0, 4, 8 and 12 days after inoculation with *G. abietina* conidia. Fungicide efficacy was determined by weighting the oven-dry fungus mats 16 days after inoculation, e.g. cultures that received the fungicides after 12 days incubation were exposed to fungicides for 4 days. The results showed that both fungicides killed all conidia immediately after exposure while germinating conidia were killed after 4 days exposure. Eight day-old hyphae were killed by cholorothalonil, but not by propiconatsol while neither fungicide killed 12 day-old hyphae.

In the experiment to determine the effect of fungicide timing on Scleroderris canker control on seedlings, the fungicides were applied to 1-year old, *G. abietina*-inoculated seedlings at intervals following inoculation (the experiments were carried out in 1994-95, 1996-97 and 1997-98). The results showed that disease control decreased with time following pathogen inoculation, i.e. control was best when the fungicides were applied shortly after inoculation and decreased thereafter, being best within the first 10 days following inoculation. Apparently conidia germination was slower on the host than in the V8 medium. Propiconatsol appeared to be much less effective than chlorothalonil in controlling *G. abietina*. Our results indicate that *G. abietina* damage in nurseries might be prevented by monitoring *G. abietina* spore occurrence and dispersal so that fungicides could be applied at the most effective time.

¹ Finnish Forest Research Institute, Suonenjoki Research Station, Juntintie 40, FIN-77600 Suonenjoki, Finland

GENE EXPRESSION DIFFERENCES BETWEEN GREMMENIELLA ABIETINA A, B AND NA

Marja-Leena Majuri¹

Using the mRNA differential display technique, we have identified cDNA clones from *Gremmeniella abietina* that may be involved in gene expression alterations between different types of that fungus. According to the analysis, the double stranded RNA (dsRNA) does not have a significant effect on *G. abietina* gene expression. The overall gene transcript accumulation in *G. abietina* seems to follow genetical rather than pathological relationships.

¹ Finnish Forest Research Institute, Vantaa Research Centre, P.O. Box 18, FIN-01301 Vantaa, Finland

INTRODUCTION OF ROOTRAINER TECHNOLOGY IN FORESTRY – IMPACT OF NURSERY DISEASE MANAGEMENT

C. Mohanan¹

Recently, rootrainer technology has been introduced in forestry sector in India to raise healthy planting stock for afforestation and reforestation programs. The conventional nursery method in vogue include raising seedlings in seedbeds and transplanting into polythene containers filled with soil or directly raising in polythene containers of different sizes and maintaining for 3-18 months depending on the species and planting techniques followed in different regions. In conventional forest nurseries diseases pose major threat and epidemic outbreaks of diseases viz. seedling blight caused by Cylindrocladium spp. and web blight caused by Rhizoctonia solani are common which often cause partial to complete failure of nurseries, especially in high rainfall areas. Even though the new technology requires high initial investments, it offers many advantages over the conventional methods of raising and maintaining the nurseries. However, information is scanty on incidence, severity and spread of diseases in rootrainer raised forestry species under the tropical humid climatic conditions. Disease monitoring in rootrainer nurseries as well as conventional seedbed and container nurseries situated in different Forest Divisions in Kerala State was carried out during 1997-'99. Tectona grandis, Eucalyptus tereticornis, E. grandis, Pterocarpus santalinus, Lagerstroemia speciosa, Swietenia macrophylla, Hardwickia binata, Gmelina arborea, Terminalia arjuna, Acacia auriculiformis, A. mangium, etc. raised in seedbed nurseries succumbed to various diseases, viz. damping-off, web blight, seedling blight, seedling wilt, collar rot, stem and foliage infection caused by different fungal pathogens at various growth phases of seedlings. However, soil-borne fungal diseases seldom occurred in rootrainer seedlings raised under the same microclimatic conditions. Eucalyptus seedlings raised in rootrainers in both the Central Nurseries (Nilambur and Kulathupuzha) were found totally free from *Cylindrocladium* seedling blight, the most dreaded disease of eucalypts in the State. Cotyledon rot and seedling wilt in teak (Tectona grandis) and eucalypts

caused by *Burkholderia solanacearum* (= *Pseudomonas solanacearum*) are the only soilborne diseases encountered in rootrainer raised seedlings. Foliage diseases of minor significance incited by the air-borne inoculum of the pathogens viz. Bipolaris, *Colletotrichum*, *Phomopsis*, *Phaeoseptoria*, *Cylindrocladium*, etc. and web blight caused by a hypovirulent aerial strain of *Rhizoctonia solani* were also observed in rootrainer raised seedlings. Though, the new nursery technology offers production of healthy planting stock, reduction of seedling maintenance period and minimization of the chances of disease hazards in forest nurseries, performance of planted out rootrainer seedlings of different tree species in many areas is not very encouraging, and outbreak of nursery transmitted diseases like bacterial wilt in plantations is not uncommon. The paper highlights the merits and demerits of the new nursery technology in forestry and suggests improvement upon depending on the local climatic conditions and tree species.

¹ Kerala Forest Research Institute Peechi 680 653 Kerala India

EPIDEMIOLOGY AND INTEGRATED MANAGEMENT OF WEB BLIGHT IN BAMBOO NURSERIES

C. Mohanan¹

Bamboo, the world's fastest growing and environment friendly giant grass has now gained international recognition as important non-timber woody resource. Their versatility, rapid growth and myriad of end-uses have made them as backbone of rural economy in many states in India. Planting of bamboo on a large-scale has been recently commenced in Kerala State and so far 4600 ha have been raised under different afforestation programs. Bamboos are vulnerable to various diseases, which affect them in nurseries, plantations and natural stands. Among the 13 nursery diseases recorded on bamboo, web blight caused by Rhizoctonia solani is the most potential and widespread disease in bamboo nurseries. The disease was influenced greatly by the microclimatic conditions in the nursery, viz. soil moisture, ambient temperature, relative humidity, incident light over the seedbeds and seedling density. Due to the high humid tropical climate prevailing in the State and existence of different virulent strains of R. solani resistant to fungicides, disease management becomes more difficult. Epidemiology of the disease was studied and to adopt an integrated strategy for disease management employing cultural, biological and chemical measures, studies were carried out. Characterization of R. solani isolates made from bamboo nurseries throughout the State revealed that R. solani exists in at least three anastomosis groups, AG1-IA, AG1-IC, AG2-2IV, which varied in cultural characters, growth, virulence, utilization of carbon and nitrogen sources, and also showed varying levels of fungicidal resistance. To achieve biological control using antagonistic organisms, Trichoderma harzianum and T. viride isolates from local nursery soil were screened against *R. solani* isolates employing different techniques and efficient candidate antagonists were selected for mass culturing and inoculum production. Ten fungicides were screened against different strains of R. solani in the laboratory and the most efficient ones were selected for the nursery trials. Biological control measures applied in nursery include solarization of the

seedbeds for 18 days and introduction of *Trichoderma harzianum* and *T. viride* by two delivery methods, soil amendment and seed coating. Cultural measures include regulation of soil water regime in the seedbed, shade over the seedbed and seedling density. Prophylactic fungicidal treatments were also given separately. All the treatments were made at different levels and disease monitoring and data on incidence, severity and spread of the disease were recorded. Area under disease progress curve (AUDPC) and growth rate of disease were recorded for each treatment. The study revealed that soil solarization and introduction of antagonists were not effective in controlling the disease. Optimizing the cultural measures by manipulating shade over the seedbeds (without shade), reducing seedling density (<165g m-2), and regulating water regime in the seedbed (10 1 m-2 per day), along with prophylactic application of fungicide (carboxin 0.2% a.i.) after one and three weeks of seedling emergence, web blight disease can be effectively managed. The paper highlights the epidemiology of web blight and integrated disease management in forest nurseries.

¹ Kerala Forest Research Institute Peechi 680 653 Kerala India

FUNGICIDE CONTROL OF LOPHODERMIUM SEDITIOSUM ON PINUS SYLVESTRIS SEEDLINGS IN SWEDISH NURSERIES

Elna Stenström¹ and Bernt Arvidsson²

Lophodermium seditiosum is a serious needle pathogen on pine, particularly in forest nurseries. A heavy infection can result in severe needle loss, which in turn can result in reduced growth or even death of young seedlings. Fungicide treatment with maneb/mancoseb is known to have good effect against the disease but it is no longer allowed for use in Swedish forest nurseries.

In this experiment we evaluated the effect of three fungicides against *L. seditiosum*; propiconazole which is labelled for use in Swedish forest nurseries, fluaziname which is used against fungal pathogens in e.g. potatoes and azoxystrobin which is newly registered for the control of fungal pathogens, mainly in cereals. Seedlings were treated with the fungicides during the summers of 1995 and 1996 and the number of infected needles as well as the number of ascocarps per needle were measured.

Ascocarps were found on almost all of the needles on the untreated control seedlings. Propiconazole had no or only minor effect on ascocarp formation. Fluaziname had no effect on ascocarp formation during the first year but the year after, the new needles did not show any signs of infection. Azoxystrobin had effect on ascocarp production already the first year to the extent that ascocarps were found only on one fourth of the needles. The year after, all new needles in this treatment were completely healthy.

Azoxystrobin is now registered for use against L. seditiosum in Swedish forest nurseries.

¹ Dep. of forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 07 Uppsala, Sweden.

² Svenska Skogsplantor AB, Box 33, 551 12 Jönköping, Sweden.

FOREST NURSERY EXTENSION – MEANS FOR ADVISORY IN PLANT PROTECTION

Marja Poteri¹

Disease and pest control in forest nurseries is one of the crucial issues that can benefit from organized educational activities. In co-operation with Finnish nursery companies and Finnish Forest Research Institute a nursery project was started with the aim to enhance the know-how of nursery staff by delivering regularly research notes and arranging tailor-made courses.

In 1998 two courses on seedling diseases and pests were organized for nursery workers. The contents of the courses have now been processed to an up-to-date identification manual for forest nurseries. A common problem in inspecting the quality of transplanted seedlings is to identify the origin of a disease or pest. In the becoming manual an attempt is made to present also diseases and pests attacking seedlings at regeneration sites and to distinguish nursery damages from these post-planting problems.

Modern seedling production has an increasing demand for environmentally accepted practices. This means that nursery managers must have information how to improve hygiene in the nurseries and how to develop control methods towards Integrated Pest Management (IPM) principles. In addition to the already existing knowledge, different regional research programs produce information for disease and pest control. Forest nursery extension has as a task to transfer relevant research results for decision making in plant protection in nurseries.

¹ The Finnish Forest Research Institute, Suonenjoki Research Station, Juntintie 40 FIN-77600 Suonenjoki, Finland

MYCORRHIZAS AND HOST PLANT CHEMICAL DEFENCE AS A POTENTIAL CONTROLLING AGENT FOR INSECT HERBIVORE DAMAGES ON SCOTS PINE

Manninen A.-M.¹, Holopainen T.¹, Vuorinen M.², Holopainen J.K.^{1,3}

In forest nurseries ectendomycorrhizas are prevailing fungal species that form mycorrhizae in the roots of conifer seedlings. After planting in forests, ectendomycorrhizas are gradually replaced by ectomycorrhizas. Different mycorrhizal stage of seedlings in nurseries and forests could be one explanation to the susceptibility of nursery-grown Scots pine seedlings e.g. to polyphagous tarnished plant bug (*Lygus rugulipennis*). The role of mycorrhizas in the control of herbivory could be associated with the form and concentration of nitrogen and also with the chemical defense of the host plants. In this study the importance of mycorrhizal status and host plant defense to the performance of generalist and specialist insect herbivores on Scots pine seedlings was determined. Mycorrhizal infection in the roots of Scots pine seedlings was either increased by inoculating mycorrhiza to sterile roots in the laboratory conditions or decreased by suppressing mycorrhizas with fungicides in the field conditions. In addition, different nitrogen availability were used in some experiments.

Differently mycorrhizal Scots pine seedlings were equally defended against insect herbivores with respect to concentrations of carbon-based secondary compounds in oleoresin. Increase in the concentration of some individual resin acids in the seedlings with higher mycorrhizal infection level did not indicate clear effect on insect herbivore performance. Changes in the nutritional quality of differently mycorrhizal Scots pine seedlings were inconsistent. Thus, better performance of the generalist tarnished plant bug (*L. rugulipennis*) on seedlings with reduced mycorrhizas and better performance of the specialist aphid (*Schizolachnus pineti*) on seedlings inoculated with mycorrhiza (*Cenococcum geophilum*) can not be explained by changes in analysed chemical compounds. On the other hand, different Scots pine provenances were not equally defended, because total terpene concentration increased towards the north and simultaneously resin acids decreased. However, chemical quality of needles did not explain

the rejection of one of the provenances by pine-feeding aphids. Because nitrogen availability in the growth medium did not affect the chemical quality of seedlings, no drastic changes in insect herbivore performance with respect to nitrogen were found either.

The importance of mycorrhizal status to the analysed chemical compounds and to insect herbivore performance is not drastic. However, generalist tarnished plant bug grew better if seedlings had reduced mycorrhiza and specialist aphid grew better on mycorrhizal seedlings than on non-mycorrhizal seedlings suggesting that no generalization of ectomycorrhizal effects on performance of plant sucking insects can be done. Oleoresin compounds appeared to be, independently from mycorrhizal status, important to the performance of both generalist and specialist insect herbivores. Selection of seed origins that have high concentrations of insect repelling compounds could thus be one tool to control insect herbivore damages in forest nurseries.

² The Finnish Forest Research Institute, Suonenjoki Research Station, Juntintie 40 FIN-77600 Suonenjoki, Finland

¹ University of Kuopio, Department of Ecology and Environmental Science, P.O. Box 1627, FIN-70211 Kuopio, Finland

³ Agricultural Research Centre of Finland, Plant Protection, FIN-31600 Jokioinen, Finland