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# Diseases and Insects in Forest Nurseries Proceedings of the 5<sup>th</sup> Meeting of IUFRO Working Party S7.03.04, May 6–8, 2003, at Peechi, Kerala, India

A. Lilja, J.R. Sutherland, M. Poteri and C. Mohanan (editors and compilers)



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#### Abstract

The fifth meeting of IUFRO Working Party (WP) S7.03.04 (Diseases and insects in forest nurseries) was held May 6–8, 2003, at Peechi, Kerala province, India. This electronic version of the proceedings contains full-text versions of the papers presented at the meeting.

### Keywords

nursery, diseases, insects, control

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## **Preface**

The fifth meeting of IUFRO working party (WP) \$7.03.04 (Diseases and insects in forest nurseries) was held May 6-8, 2003, at Peechi, Kerala province, India. The meeting was hosted by Dr. C. Mohanan of the nearby Kerala Forest Research Institute (KFRI). Originally the meeting was scheduled to be held at the same locality in 2002, however, resulting from world unrest it was postponed until 2003. Unfortunately just before the 2003 meeting an outbreak of SARS (severe acute respiratory syndrome) had a severe impact on attendance by non-Indian members of the WP with only four attendees coming from outside India, i.e. two from Finland and one each from Canada and the Czech Republic with the remaining approximately 25 participants all being from south India. In spite of this 15 excellent papers were presented during technical sessions in the forenoons of the first and third days of the meeting. On day two there was an all-day long field tour to the Chettikulam Central Nursery and related sites while most of the afternoon of the third day was spent touring the KFRI. The meeting concluded with a banquet on the third evening at the Trichur Towers Hotel. WP members are indebted to Dr. Mohanan for arranging the meeting and to the staff at the KFRI, especially the director Dr. Joyti Sharma, for being such gracious hosts. This electronic version of the proceedings contains full-text versions of the papers presented at the meeting. The next meeting of the WP will be held in the Czech Republic in the autumn of 2005.

Dr. Zdenka Prochazkova Forestry and Game Management Research Institute Czech Republic



# Disease problems in root trainer forest nurseries in Kerala State and their management

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Disease survey was carried out in forest nurseries in the Kerala State, India during 2000-2002. Disease incidence, severity and spread were recorded and causal agents were isolated and identified. A total of 27 forestry species raised in root trainer nurseries including Acacia mangium, A. auriculiformis, Cassia fistula, C. siamea, Dalbergia latifolia, Eucalyptus spp., Gmelina arborea, Pterocarpus marsupium, P. santalinus, Santalum album, Tectona grandis, etc. were found affected with foliage diseases. Since, soil-less or soil-free growing media were used in root trainers and general hygienic conditions were maintained in the nurseries, soil-borne diseases seldom occurred. However, Pseudomonas tectonae causing seedling wilt was recorded in teak and Sclerotium rolfsii causing foliage blight in Pterocarpus spp. The common nursery pathogens like Rhizoctonia solani, Cylindrocladium spp., Pythium spp., Fusarium spp., were rarely encountered. Often seedling congestion in root trainers led to foliage infection by pathogens like Colletotrichum gloeosporioides, Coniella spp., Pestalotiopsis spp., Alternaria alternata, Phomopsis sp., Guignardia sp. and Curvularia sp. In general, severity and spread of foliage infection caused by most pathogens was low in all the nurseries, except leaf blight caused by Phoma glomerata. T. grandis and C. fistula seedlings were highly susceptible to P. glomerata. Carbendazim (0.05% a.i.), carboxin (0.01% a.i.) and mancozeb (0.1% a.i.) were found very effective in controlling the foliage infections in nurseries. Bactericide (Streptomycin sulphate 90%w/w + Tetracycline hydrochloride 10%w/w) was found effective in managing the bacterial infections.

# Introduction

During the past few years, forest nursery practices in the Kerala State have undergone a tremendous modifications. Introduction of root trainers in forestry sector and thereby the technological changes in seedling production brought out a major impact on nursery management. Seedling health has been given more importance which further widened the scope of phyto-sanitary measures. Growing media suitable to the plant species have been developed and seedling production technology has been standardized. Maintaining the seedling crops under tropical climate is one of the major problems confronting the nursery managers. During this period, disease problems may occur in succession and if timely intervention is not performed the entire seedling crop may be devastated by one or other diseases. In root trainers, the seedlings require a maximum period of 90 days of growth and hence a rigorous management is possible.

Earlier, as disease hazards in forest nurseries have became very common which often upset the entire planting programme, systematic studies and management of economically important diseases were taken up during 1980s and 1990s and nursery management practices for prime forestry species, have been standardized (Sharma et al. 1985; Sharma and Mohanan 1992, Mohanan, 2001). The present study was undertaken to assess the disease situation in nurseries employing root trainer technology for raising seedlings and also to manage the disease(s), if any, occur.

## Materials and methods

Disease survey was carried out in Central nurseries located at Kulathupuzha (8°54'N, 77°44'E), Chettikulam (11°50'N, 75°41'E), Valluvassery (11°18'N, 76°16'E), and Cheruvanchery (11°50'N, 75°41'E). Besides, root trainer nurseries raised at Kerala Forest Research Institute Campus, Peechi and Forest Field Station at Palappilly, and KFRI Sub-Centre, Nilambur were also surveyed during 2000–2002. Disease incidence, severity and spread were recorded using a Disease scoring scale (Table 1) and disease specimens were collected and brought to the laboratory for isolation and identification of causal organisms.

Table 1. Disease scoring scale.

Disease severity	Disease severity code	Disease severity scale, percentage seedlings affected
Nil	0	0
Low	L	1–25
Medium	M	26–50
Severe	S	51-75 or > 25% seedlings dead

Potato Dextrose Agar (PDA) medium was used for isolating and sub-culturing fungal organisms, while Nutrient Agar (NA) medium was used for isolating and maintaining bacterial pathogens. Isolation and purification of causal organisms were carried out employing standard procedures and identification up to species level was made. As far as possible, confirmation of pathogenicity of most causal agents was made through inoculation experiments employing root trainer seedlings.

Fungicides and bactericide were screened against important fungal and bacterial pathogens using standard techniques and most effective fungicides/bactericides at appropriate dosage were recommended and applied in the nurseries for controlling the respective disease(s). Observations on the effect of chemical treatments against diseases were recorded from the nurseries. General nursery management practices followed in each nursery were recorded and data on growing media used, their composition and pH were also collected.

## Results and discussion

Disease survey conducted in nurseries located at different parts of the State revealed that root trainer seedlings are almost free from soil-borne fungal diseases like damping-off, collar rot and wilt irrespective of the conducive climatic conditions prevailed in the nurseries. However, most of the species raised in root trainers suffered from one or the other foliage diseases, mostly incited by air-borne inocula of pathogens, the severity of which varied from nursery to nursery depending on the nursery management practices and prevailing environmental conditions. The common nursery pathogens like *Rhizoctonia solani* Kühn, *Cylindrocladium* spp., *Fusarium* spp. and *Pythium* spp. which cause various diseases at different growth phases of seedlings were seldom recorded in root trainer nurseries. R. solani, the most potential pathogen in forest nurseries which exists in different Anastomosis groups (Mohanan 2001) and having a wide host range was not encountered in the root trainer nurseries during 2000 and 2002. The details on the diseases affecting the seedlings Acacia auriculiformis A. Cunn., A. mangium Willd., Aegle marmelos (L.) Corr, Albizia lebbeck (L.) Willd., Artocarpus heterophyllus Lam., Azadirachta indica A Juss., Cassia fistula L., C. siamea Lam., Casuarina equisetifolia Forst, Cinnamomum zeylanicum Garc. Ex Bl., Dalbergia latifolia Roxb, Delonix regia (Boj.) Rafin, Dysoxylum malabaricum Bedd. ex. Hiern, Eucalyptus citriodora Hook, E. grandis Hills ex Maiden, E. tereticornis Sm, Garcinia gummigutta (L.) Robs., Gluta travancoricus Bedd, Gmelina arborea Roxb, Grewia tillifolia Vahl., Lagerstroemia microcarpa Wt., Pterocarpus marsupium Roxb., P. santalinus L., Santalum album L., Tectona grandis L., Terminalia bellirica (Gaertn.) Roxb. T. crenulata Roth and their causal agents are given in Table 2.

Seedling congestion in root trainers was found to be the major factor for the incidence and spread of foliage diseases. Pathogens like Colletotrichum gloeosporioides (Penz.) Sacc., Pestalotiopsis spp., Alternaria alternata (Fr.) Kiessler, Phoma glomerata (Corda.) Wollenw. & Hochapf, P. eupyrena Sacc, Phomopsis variosporum Sharma & Mohanan were found associated with the foliage diseases of seedlings. In general, severity and spread of foliage diseases caused by most pathogens was low in all the nurseries, except the foliage blight caused by P. glomerata. The pathogen was found widespread in nurseries and caused severe foliage infection in teak. In teak seedlings, P. glomerata along with P. eupyrena caused severe damage to the seedlings. In teak, the pathogens cause dark greyish brown necrotic lesions on foliage, usually at the margin and tip of the leaves or at the base of the petiole which coalesce and spread to the entire leaf lamina. The infected leaves show an upward curling and become brittle and withered. The disease also affects the leaf petiole and seedling stem. Severe infection leads to seedling blight. In Cassia fistula, the pathogen caused severe leaf blotch which led to defoliation and seedling mortality. Eucalypts seedlings raised in all nurseries were found almost free from any major diseases, except foliage blight caused by Coniella spp., Cylindrocladium spp. and Kirramyces eucalypti (Cook & Massee) J.Walker, the major pathogens in eucalypt seedbed nurseries were rarely encountered.

As soil-less or soil-free growing media were used in root trainers and general hygienic conditions were maintained in nurseries, most of the soil-borne diseases were excluded from the nurseries (Mohanan 2000a,b). However, foliage blight caused by *Sclerotium rolfsii* was recorded in both *P. marsupium* and *P. santalinus*. Both the host species are very susceptible to the Sclerotium blight in seedbed nurseries (Sankaran et al. 1986). Inoculum of most of the nursery pathogens activates in presence of a susceptible host under conducive edaphic and environmental factors. However, in root trainers with soil-less growing medium, the inoculum potential of pathogen is considerably negligible and thus chances of seedling infection will be less even under conducive environmental conditions. Most of the soil-inhabiting, disease causing fungi subsist mainly on

Table 2. Diseases recorded in root trainer nurseries. Nursery, host plant, disease severity and pathogen(s) associated.

Nursery <sup>1)</sup>	Host plant	Disease	Disease severity <sup>2)</sup>	Pathogen(s) associated
C, CK,V, K, P	Acacia auriculiformis	Leaf blotch	Low	Alternaria alternata, Curvularia sp.
V,K,P	Acacia mangium	Leaf spot	Low	Glomerella cingulata, Pestalotiopsis uvicola, Guignardia sp.
Р	Aegle marmelos	Leaf blotch	Low	Phomopsis sp., Phoma sp.
K,CK	Albizia lebbeck	Leaf spot	Low	Colletotrichum capsici
N,K	Artocarpus heterophyllus	Leaf blotch	Low	G. cingulata
C,P,K	Azadirachta indica	Leaf & twig blight	Medium	G. cingulata, Fusarium sp., Phomopsis sp.
V,P	Cassia fistula	Leaf blotch	Medium	G. cingulata, Phomopsis sp., Curvularia pallescens, Phoma glomerata
V	Cassia siamea	Leaf spot	Low	Pestalotiopsis tecomicola
V	Casuarina equisetifolia	Leaf tip blight	Low	G. cingulata, Phomopsis sp.
Р	Cinnamomum zeylanicum	Leaf blotch	Low	G. cingulata, Sclerotium rolfsii
V	Dalbergia latifolia	Leaf spot	Low	Colletotrichum gloeosporioides, Phomopsis sp.
P,CK	Delonix regia	Leaf spot	Low	Guignardia sp., Phomopsis sp.
P,PP	Dysoxylum malabaricum	Leaf spot	Medium	C. gloeosporioides, Phomopsis sp.
K	Eucalyptus citriodora	Leaf spot	Low	Coniella minima, Phomopsis sp., Marsonina sp.
K,V,CH	Eucalyptus grandis	Leaf spot	Low	Coniella fragariae, Cylindrocladium quinqueseptatum
CH,CK,V, K,P,N	Eucalyptus tereticornis	Leaf spot	Low	C.fragariae, C. quinqueseptatum, C. gloeosporioides, Phomopsis sp., Kirramyces eucalypti
P,CH,N	Garcinia gummigutta	Leaf blotch	Medium	C. minima, G. cingulata
P	Gluta travancoricus	Leaf blotch	Medium	Pestalotiopsis sp.
CH	Gmelina arborea	Leaf blotch	Low	Pseudocercospora ranjita
K,P	Grewia tillifolia	Leaf blotch	Low	Phomopsis sp., Guignardia sp.
V	Lagerstroemia microcarpa	Leaf spot	Low	C. gloeosporioides
V,CK	Pterocarpus marsupium	Leaf blight	Medium	Sclerotium rolfsii
V	Pterocarpus santalinus	Leaf blight	Medium Low	S. rolfsii Phomopsis sp.
CK	Santalum album	Leaf spot	Low	G. cingulata, Guignardia sp.
CK,V,K,P	Tectona grandis	Collar rot	Low	Pseudomonas tectonae
. , ,	3	Wilt	Severe	P. tectonae
		Leaf blight	Medium	Phoma glomerata, P. eupyrena, C. gloeosporioides,
				Phomopsis variosporum
Р	Terminalia bellirica	Leaf spot	Low	Phomopsis sp., Guignardia sp.
Р	Terminalia crenulata	Leaf blotch	Low	Pestalotiopsis maculans

 $<sup>^{1)}</sup>$  C = Central nursery Cheruvanchery; CK = Chettikulam; V = Valluvassery; K = Kulathupuzha; P = nursery at Peechi; N = Nilambur; PP = Palappilly.

<sup>&</sup>lt;sup>2)</sup> Percentage of seedlings affected. Low = 1–25%; Medium = 26–50%; Severe = 51–75% or 25% seedlings dead.

dead organic materials and the presence of surplus, readily available nutrients in organic compost in root trainer cells makes less competition among the pathogens for the nutrients and thus least attractive for infection of seedlings. The compost prepared from forest weeds is the major constituent of the growing medium in root trainers and it is suspected that the sclerotium of the pathogen which is very resistant to environmental stress persisted in the compost and contributed to the development of disease. Susceptibility of the host (*Pterocarpus* spp.) is also a major factor for the development and spread of the disease.

Bacterial seedling diseases caused by *Pseudomonas tectonae* were also recorded in teak seedlings. The bacteria cause cotyledon rot, collar rot, seedling wilt, and foliage infection. The disease appears as water-soaked lesions on cotyledons and foliage and become dark greyish brown. The lesions spread and cause rotting of the affected tissues in cotyledon, seedling stem and foliage. The bacteria also cause seedling wilt in teak; the infection is systemic and produced symptoms characteristics of vascular wilt drooping of leaves, epinasty and wilting. As in conventional nursery beds, spread of disease through root contact is not occurring in root trainers, however, physical contacts of infected foliage to healthy seedlings in the root trainer blocks spread the disease. Moderate to severe disease incidence and seedling mortality in teak seedlings were recorded at Central Nursery, Kulathupuzha in 2000 and 2001. The source of inoculum may be either the potting medium or water. In the case of bacterial infection, all the affected seedlings are not killed outright. Often many seedlings may become carrier of bacterial pathogens, without showing any visible symptoms of disease. Hence, there is possibility of transferring the disease from nursery to the field through mildly infected planting stock. In seedbed nurseries, bacterial collar rot and wilt cause severe damage to the nursery stock. The seedbed nurseries raised in high rainfall areas were reported to be affected severely by the bacterial pathogen (Sharma et al. 1985, Mohanan et al. 1997).

In nurseries, application of fungicides, Carbendazim (0.05% a.i.), Dithane M45 (0.1% a.i.) against foliage diseases gave good results. For controlling seedling blight and foliage diseases caused by *Phoma glomerata* and *P. eupyrena*, application of Dithane M45 was found very effective. Application of a bactericide, Streptomycin (Streptomycin sulphate 90% w/w + Tetracycline hydrochloride 10% w/w) at the rate of 6 g per 8 l of water by drenching the seedlings gave good control of seedling infection caused by *P. tectonae*.

## **Conclusions**

In root trainer nurseries, soil-borne fungal diseases seldom occur mainly due to the use of soilless or soil-free growing media and maintaining the nursery in hygienic conditions. Foliage infections caused by air-borne fungal pathogens affect the seedling crops and seedling congestion may be the primary influencing factor for the incidence and spread of the disease. Among the fungal pathogens causing foliage infection, *Colletotrichum gloeosporioides, Coniella fragariae, Phomopsis* sp., *Phoma glomerata* and *P. eupyrena* are the important ones. Though, the new technology offers production of high quality healthy planting stock, application of proper fungicide(s) at proper time is required to control the foliage diseases. Otherwise, mild foliage infection may flare up and cause severe damage to the seedling crops.

# **Acknowledgements**

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# Preliminary observations on the occurrence of Ciboria batschiana (Zopf) Buchwald in the Czech Republic

## Z. Prochazkova<sup>1</sup>, A. Sikorova<sup>1</sup> and V. Peskova<sup>2</sup>

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The paper presents preliminary results on the occurrence of *Ciboria batschiana* (Zopf) Buchwald, in the Czech Republic, on acorns collected from the forest floor or from nets suspended above the forest floor for the years 2000–2002. The highest incidence of *C. batschiana*, but also the highest average germination and moisture content, was found on acorns collected in 2001, while the lowest number of infected samples were found in the 2000 crop. *Ciboria* infection of acorns differed by year of collection and by forest stand. There appears to be a positive relationship between the amount of acorn infection and abundance of rain in September of the collection year. Infection also occurred on acorns collected from nets suspended above the forest floor, but infection was lower than for acorns collected from the forest floor.

## Introduction

Ciboria batschiana (Zopf) Buchwald. [syn. Sclerotinia pseudotuberosa Rehm, anamorph Myrioconium castanae (Bainier) Morelet] is an Ascomycete fungus causing "black rot -mummification" of acorns of *Quercus* spp. This specialised parasite of *Quercus* and *Castanea* sativa seeds was described in 1878 by Zopf and in 1879 by Rehm (Dennis 1956) who later pointed out the risk (threat) to natural reforestation of oaks by this pathogen in Germany (Rehm 1989). Klika (1923) was the first to report the occurrence of the pathogen in Czechoslovakia. Initially research was oriented towards mycological studies, but since the 1960's the economic importance of stored seeds of sweet chestnut and oaks has been taken of prime importance. Urosevic (1957, 1961) reported up to 60 % of stored acorns were destroyed in 1955 and 1956 in Czechoslovakia. Later, in 1973 and 1974, about 90% of acorns stored for 3–8 months were killed by C. batschiana in France (Delatour et al. 1980). In the Czech Republic it is a common practice to sow acorns after their collection in the autumn and only a small amount of acorns are stored for one or more winters. However, from time to time the autumn sown acorns are damaged (mostly by abiotic factors; Procházková 1994) so the storage of certain quantities of acorns seems to be necessary. Ciboria spreads from affected to unaffected acorns even at low temperatures (-1 °C), thus the entire acorn crop may be destroyed during the first winter of storage (Urosevic 1956). Ascospores

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of the fungus can quickly infect acorns after they fall to the forest floor in the autumn (Neff and Perrin 1999), but also acorns on trees can be infected (Stocka 1994). The only effective control of black rot is hot water treatment (thermotherapy) of acorns which kills the fungus, but not the acorns (Neff and Perrin 1999). However, we have no information about the occurrence of infection in different crop years and different forest stands. Also, if acorns are infected mainly after their fall to the forest floor the use of nets suspended above the floor could decrease or prevent acorn infection.

The goals of research project QD0173 "Factors influencing quality of beechnuts and acorns during storage" are to determine the occurrence of *C. batschiana* on acorns of oaks (*Quercus*) in the Czech Republic in different years and the effect of collection method (forest floor versus nets suspended above the floor). This paper presents preliminary results of this research.

## Materials and methods

Acorns of different oak species were collected either directly from forest floor or from nets suspended below trees (to prevent the acorns from coming into contact with the forest floor) in oak stands around the country. In 2000, acorns also were collected on two dates from seven forest stands (Table 1). The occurrence of *C. batschiana*, other fungi and insect infestation was determined for acorns incubated in wet chambers (Petri dishes with 3 layers of filter paper) at kept at 15 (±2) °C for a maximum of 2 weeks. Moisture content (fresh weight basis) and germination (done together with health tests) of the acorns was also determined.

Table 1. Number of samples and collection technique in different years: Acorns of *Quercus robur*, *Q. petraea*, *Q. cerris*, *Q. rubra* and *Q. pubescens* were collected from early September to late October in 2000<sup>1</sup>; acorns of *Q. robur*, *Q. petraea* and *Q. rubra* from late September to early November in 2001 and 2002.

	2000	2001	2002
Total number of samples	100	62	47
Number of samples collected from forest floor	100	52	47
Number of samples collected from nets	None	10	None

<sup>&</sup>lt;sup>1</sup> Two collection dates in seven stands.

## **Results and discussion**

The highest incidence of *Ciboria batschiana*, but also the highest average germination and moisture content, was found on acorns collected in 2001, while the lowest number of infected samples were found in the 2000 crop (Fig. 1). The level of acorn infection with *C. batschiana* coincides with weather, especially precipitation in September. More rainfall occurred in 2001 than in 2000 and 2002 (Fig. 2) which facilitated the development of *C. batschiana* fruiting bodies and the

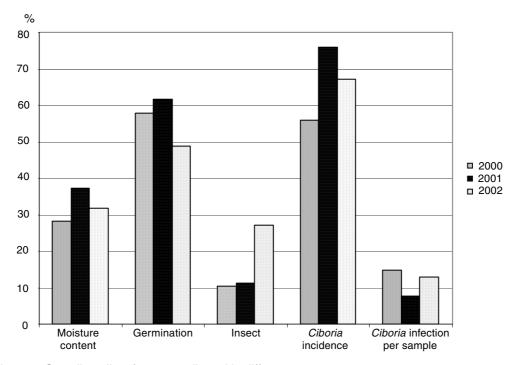


Figure 1. Overall quality of acorns collected in different years.

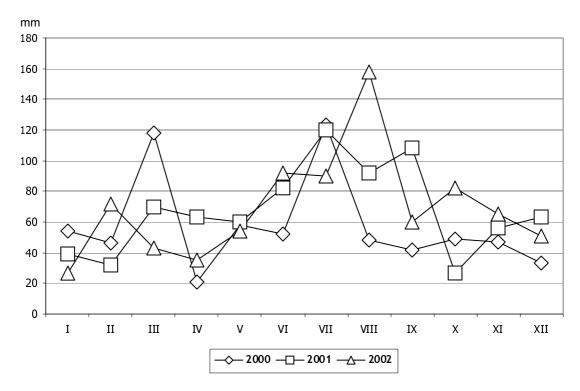


Figure 2. Average month precipitation in the Czech Republic during the study.

production and dispersal of ascospores, and consequently the infection of maturing acorns. In 2000, acorns matured and fell to the ground from early September to late October when both months were dry (about 50 mm of precipitation; Fig. 2). In 2001, acorn maturation was later than for the 2000 crop, i.e. maturation occurred in late September, October and early November. September was very wet (Fig. 2) and the conditions for development of *C. batschiana* were the best of all three collection years. Precipitation in 2002 was similar to 2000 but acorns matured later. The infection of acorns with *C. batschiana* varies depending on climatic conditions. In France in 1976 nearly total absence of fructification of *C. batschiana* was recorded because of dry weather even when the massive acorn crop in 1974 created favourable conditions for pathogen sporulation (Delatour and Morelet 1979).

Numbers of infected acorns from different, and in some cases adjacent, stands differed (Fig. 3). This finding supports the necessity of not mixing acorn collections from individual stands. Further supporting this practice is the suggestion by Delatour and Morelet (1979) that certain geographic areas might be more suitable for the pathogen.

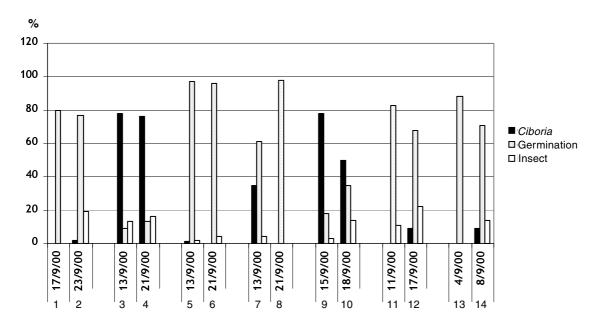


Figure 3. *Ciboria* infection of acorns from the seven stands and two collection dates in September 2000. Samples:1=207D10; 2=207D10; 3=207D15; 4=207D15; 5=212A15; 6=212A15; 7=212B14; 8=212B14; 9=307D11; 10=307D11; 11=308B10; 12=308B10; 13=312B12; 14=312B12

The highest infection was in acorns collected in stands of the same forest unit during September, 2000, specifically from about September 10 to September 20 (Fig. 4). However, infection varied among stands and collection dates.

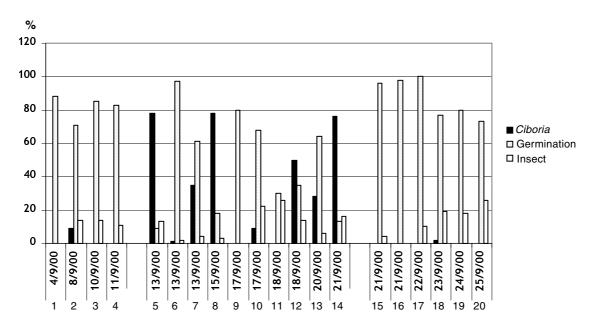


Figure 4. *Ciboria* infection of acorns from different stands of the same forest unit in September, 2000. Samples:1=312B12; 2=312B12; 3=212A9; 4=308B10; 5=207D15; 6=212A15; 7=212B14; 8=307D11; 9=207D10; 10=308B10; 11=211C12; 12=307D11; 13=306A9; 14=207D15; 15=212A15; 16=212B14; 17=206A11; 18=207D10; 19=217D13; 20=312A17

In forest stand 207 D15 with the highest *Ciboria* incidence in 2000 (Fig. 5), acorns were collected both from the forest floor and from nets in 2001. In total, the level of infection on acorns was higher for the 2000 crop than that for the 2001 crop. In 2001, acorns matured about a month later and perhaps this was the reason for the very low infection level. However, acorns collected by both techniques (i.e. from the forest floor and from nets) were infected with *Ciboria* even though the level of infection was higher on acorns from the forest floor. This confirms the finding of

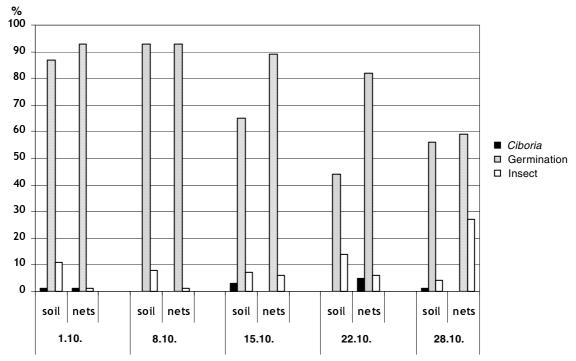


Figure 5. Quality of acorns collected from the forest floor and suspended nets in October, 2001, in Buchlovice, stand 207D 15.

Stocka (1994) that acorns on trees (before they are shed) can be infected by *Ciboria*. However, the use of nets for acorn collection can decrease pathogen infection but not prevent it.

## **Conclusions**

- 1. Ciboria infection of acorns differs by year of collection and by forest stand.
- 2. There appears to be a positive relationship between the amount of acorn infection and abundance of rain in September of the collection year.
- 3. Infection also occurs on acorns collected from nets suspended above the forest floor, but infection was lower than for acorns collected from the forest floor.

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# Control of nursery diseases and pests in Finnish forest tree nurseries

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Norway spruce (Picea abies), Scots pine (Pinus sylvestris) and European silver birch (Betula pendula) are the major tree species grown in Finnish forest nurseries where over 80 % of the seedlings are grown in containers in plastic-covered greenhouses. The change from growing bare root seedlings to container production has reduced the use of fungicides in Finnish nurseries. On Scots pine Scleroderris canker (Gremmeniella abietina), snow blight (Phacidium infestans) and needle cast (Lophodermium seditiosum) are controlled chemically, mainly by using propiconazole. Fungicides are applied during the growing season or in the case of snow moulds in autumn before permanent snow cover occurs. Root die-back (*Rhizoctonia* sp.) of container-grown spruce is controlled by improving air ventilation under the roots by elevating the seedling-growing containers. Another important factor in reducing root-die back involves the use of hot-water (+80 °C) washing of the reusable hard-plastic, growing containers. For winter stored seedlings sprayings against grey mould, Botrytis cinerea, may be needed. Tiophanate-methyl, iprodione or tolylfluanid are applied in autumn mostly before seedlings are packed for overwinter cold storage. Phytophthora shoot lesions on birch seedlings can also be controlled by hot water washing of the growing containers and during the growing season by using fosetyl-aluminium sprayings. Birch rust is usually controlled with triadimefon. If needed, pyretroids are used to control aphids and moths. One of the biggest challenges is to prevent B. cinerea damage in packed, winter-stored seedlings. Growers are encouraged to use cultural and integrated pest management techniques such as better nursery hygiene, including removing plant debris in nursery growing areas and hot water washing of containers plus removal of diseased, spore-producing trees adjacent to nurseries.

Keywords: forest nurseries, container-grown seedlings, fungicides, pesticides, nursery hygiene

## Introduction

Finland is situated in the Eurasian boreal forest zone and its forests are dominated by two conifers, *i.e.* Scots pine (*Pinus sylvestris* L. ) and Norway spruce [*Picea abies* (L.) Karst.]. European silver birch (*Betula pendula* Roth) is the most important hardwood. Fifty percent of the silvicultural forest area needing to be reforested each year is planted with nursery-produced seedlings while the remaining 50 % is regenerated using natural regeneration or direct sowing (Finnish Statistical... 2001).

Nowadays over 80 % of all forest tree seedlings are produced in containers (Fig. 1). The shift from bare root production to container production has occurred rapidly. Over the past decade about 150 million seedlings have been produced annually for reforestation (Finnish Statistical... 2001).

## Proportions of container seedlings

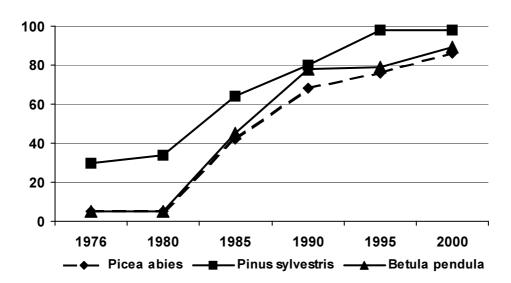


Figure 1. Container-grown seedlings as a percentage of total planting stock production.

# Seedling production in hard-plastic, growing containers

Seedling containers are made of hard plastic and the cavities are equipped with air-slits in order to obtain better root pruning and ventilation of root system. Containers are filled with pre-fertilized sphagnum peat and sowing is done by machines using single or double seeding. Fertilizers are applied via irrigation water which is mainly done using moving booms both in plastic houses and outdoor growing facilities. The minor part of bare root seedling production is minimal and consists mainly of 4-year-old Norway spruce.

The shift from bare root production to growing seedlings in containers has resulted in a significant decrease in the use of pesticides in Finnish nurseries. It is estimated that countrywide use of pesticides is nowadays only 5-10% of the amount which was used in bare root production at the end of 1970's (Juntunen 2001, Kangas et al. 1980).

In containers seedlings are grown in a peat substrate and thus the need for herbicides is significantly less than in bare root production. Growing seedlings in containers has decreased growing time in the nursery to 1–2 years instead of 2–4 years which was common with bare root seedlings. In containers seedlings can also be grown more densely (more seedlings per unit area) which has decreased total production area. Also, after seeding the seedlings are now germinated in plastic-covered (sheets) houses where they are grown for 2–4 first months. This practice decreases the need to control airborne diseases and pests.

# **Control of Scots pine diseases**

Under Finnish climatic conditions it is next to impossible to produce healthy and marketable Scots pine seedlings without using some fungicides. The most important diseases of Scots pine are Scleroderris canker [Gremmeniella abietina (Lagerb.) Morelet] and snow mould (Phacidium infestans P. Karst.) (Lilja et al. 1997). These pathogens infect seedlings during the growing season or in the autumn and remain latent during winter. All affected seedlings have to be discarded as both diseases kill seedlings. Infections caused by snow mould are visible immediately after the winter snow has melted. The symptoms of Scleroderris canker can also be seen in spring, but sometimes the symptoms may appear after bud burst has occurred and the new growth has started. Depending on springtime weather conditions, in some years it can be difficult to observe latent infections of Scleroderris canker before the seedlings are shipped from the nursery.

Pine seedlings in their first growing season are most susceptible to Scleroderris canker at the time of bud formation in August (Petäistö 1999, Petäistö et al. 1999, Petäistö and Juntunen 2000). At this time the seedlings are usually transferred from the plastic-covered greenhouses to outside, hardening-off areas where they are exposed to infecting spores. It has been found, however, that the amount of conidiospores of *G. abietina* is usually highest in June-July before bud formation has started (Petäistö and Heinonen 2003). Thus, it is important to start sprayings before the most susceptible seedling stage, if seedlings are kept outdoors. Early sprayings are also important for pine seedlings which are being grown on for a second year at the nursery where they remain outdoors all the time. According to the studies made in nurseries, the amount of *G. abietina* spores may vary dramatically depending on the year (Petäistö and Heinonen 2003).

Normally pine seedlings are grown the first 2–3 months in plastic-covered greenhouses and thus no chemical control against Scleroderris canker is needed. It is recommended to start sprayings immediately after the seedlings have been transferred from the greenhouses to the outdoor hardening-off areas. Propiconazole is used to control Scleroderris canker and sprays are applied every second week until the middle of September. Pine seedlings which are delivered as 2-year-old seedlings and thus start their second growing year outdoors in the nursery have also to be sprayed with the fungicide from June onwards.

Snow mould (*P. infestans*) of Scots pine is a problem in areas where permanent snow cover remains throughout the entire winter *e.g.* in central and north Finland. The fungus grows extensively among seedlings under the snow at below freezing temperatures. In spring, areas of diseased seedlings appear as usually round-shaped spots where all the seedlings have died. Snow mould is controlled by propinconazole and control is best when the material is sprayed on seedlings in late autumn just before permanent snow cover.

In south Finland *Lophodermium seditiosum* Minter, Staley & Millar may in some years heavily infect Scots pine seedlings and cause needle cast disease. Needle cast has a latent phase during winter and the symptoms appear late in the following spring or early summer. The disease can cause serious wilting and killing of seedlings if in spring non-symptomatic diseased seedlings are outplanted to the forest. *Lophodermium* needle cast is also controlled with propinconazole and the sprayings are made form June to September when the fungus sporulates.

Pine twisting rust, *Melampsora pinitorqua* (Braun) Rostr., is a disease which infects pine seedlings at the beginning of summer (June) and the symptoms appear 2–3 weeks later. The disease causes cankers and serious twisting of shoots and usually results in death of small seedlings. Aspen

(*Populus tremula* L.) is an alternate host for *M. pinitorqua*. Twisting rust can be avoided if there are no infected aspens growing in or around the nursery. If affected aspens are present and seedlings are not in a plastic-covered greenhouse, triadimefon sprayings are needed at the time of basidiospore liberation at the beginning of June.

# **Control of spruce diseases**

Most of the disease problems of Norway spruce seedlings have been to date avoided by using cultural practices for their management. By the 1980's when container production of spruce increased rapidly, a root die-back of Norway spruce appeared first in Norway (Galaaen and Venn 1979, Venn et al. 1986) and some years later in Finland (Lilja et al. 1992). The main causal agent in Finland proved to be a uninucleate *Rhizoctonia* (teleomorph *Ceratobasidium bicorne* J. Erikss. & Ryvarden) (Hietala et al. 1994, 2001, Lilja et al. 2000). A practical solution to the problem was devised by when seedlings were grown in containers which were elevated 20–25 cm above the greenhouse floor instead of placing them on the floor as was done before. Use of this procedure provided better air ventilation under and around containers and this in turn created unsuitable conditions for the fungus. Too, this practice enhanced the vigour of seedling root systems. Also, once it became known that a soil-borne pathogen was involved in root-dieback, hot-water washing of growing containers became a routine practice (Kohmann and Börja 2002). No chemical treatments are used to control root die-back.

Grey mould (*Botrytis cinerea* Pers. ex Nocca & Balb.) can be a problem during the growing season if the humidity is high, but mostly it is a storage problem. Recently too, storage of spruce seedlings at -2 to -4 °C has increased. For storage, seedlings are packed in closed, air-tight packages where humidity remains high. In these packages moulds can become a problem if the cooling and thawing of packages are not done properly and seedlings are kept in the sealed packages too long. Fungicide sprayings are mainly made in autumn before packing the seedlings, especially if autumn frosts have shifted with rainy seasons. Iprodione, thiophanate-methyl and tolylfluanid are used to control such moulds.

Recently, work has started to determine the distribution and infection biology of *Sirococcus conigenus* [(DC.) P. Cannon & Minter] on Norway spruce seedlings (Lilja et al. 2005). So far no control methods have been used against *Sirococcus* blight.

## Control of birch diseases

Birch rust [Melampsoridium betulinum (Kleb.) Fr.] has been an ongoing problem on growing birch seedlings including previously when seedlings were grown in bareroot nurseries. The disease causes early defoliation which weakens and retards the hardening of birch seedlings. Infected seedlings also grow less well after planting than healthy ones (Lilja et al. 1997). Triadimefon is applied against birch leaf rust when the first rust symptoms appear on the leaves in July. The severity of birch rust varies among years so the need for control varies each year.

A new birch disease caused by *Phytophthora cactorum* (Leb. and Cohn) Schr. was first found in Finnish nurseries in the 1990's (Lilja and Hietala 1994, Lilja et al. 1996). The pathogen causes stem and root collar lesions. The pathogen was introduced into Finland from Europe, most likely on plant material (Hantula et al. 2000). Nursery hygiene is important in controlling the disease.

This includes the removal of all dead plant debris such as leaves and shoots from the growing areas to prevent pathogen survival in the soil. During the growing season containers are also placed on an insulating cloth which is spread on the gravel ground cover (floor). The cloth prevents direct contact between the bottom of containers and the underlying soil. Attention must also be paid to regulating seedling irrigation and all excess water has to be avoided as soil water is a potential inoculum source. It is also important to wash and sterilize growing containers in hot (+80 °C) water. The resistance of birch seedlings to *Phytophthora* can be improved by fosetyl-aluminium spraying which has to be applied beforehand. In practise, just before birch seedlings are transferred from the plastic-covered greenhouse to outdoor hardening areas where they may encounter heavy rain.

# **Control of pest insects**

Because of the cold winter weather insects are not usually a big problem in Finnish nurseries. There are, however, some outbreaks occasionally which need control. Aphids, caterpillars [e.g. *Rheumaptera hastata* L. (Lepidoptera: Geometridae)], moths [e.g. *Croesus septentrionalis* L. (Hymenoptera: Tenthrediniae)] and the nymphs of *Lygus* bugs (Heteroptera: Miridae, mainly *L. rugulipennis* Poppius) are, if needed, controlled with pyretroids. In plastic-covered growing houses yellow, sticky trap papers are used to monitor insect populations, especially fungus gnats [*Bradysia* spp. (Diptera: Sciaridae)]. Attacks by *Lygus* bugs were a bigger problem in the growing of bare root seedlings than in container production. This is because container-grown seedlings are grown during their most susceptible stage in greenhouses and thus control is not usually needed. It is, however, important to use insect-proof screens on openings, e.g. doors and vents to prevent insects from entering the greenhouses.

Mites [Oligonychus ununguis Jacobi (Acari: Tetranychidae), Nalepella haarlovi var. picea-abietis Löyttyniemi (Acari: Nalepellidae)] are a problem mainly with bare root spruce seedlings, especially in hot, dry growing seasons. Mites are controlled, if needed, with oxydemeton-methyl. This miticide should be used only when urgently needed since the number of treatments must be kept at a minimum since mites tend to become resistant to miticides.

## **New risks**

There are some new techniques and production methods which may increase the risk of moulds in nurseries. In northern latitudes seedlings must be hardened in the autumn so that then do not suffer from freezing temperatures in winter. To facilitate seedling hardening off, nurseries have started using short day treatments (black-out) (Colombo et al. 2001). For this treatment seedlings are enclosed by black curtains to extend the dark night period. Since these treatments are made during the seedling growing season there is an increased risk of grey mould infections. Warm and humid conditions are readily created under the black cover which may favour mould fungi. At the same time seedlings may be weakened physiologically because of reduced photosynthesis and increased respiration.

Another new cultural practice which increases the mould problem is the packing of seedlings in air-tight packages in the autumn. Packed seedlings are stored over winter in cold, mainly freezing temperatures. As occurs with blackout the conditions within the packages are ideal for moulds and less favourable for the seedlings.

The practice of growing seedlings in containers in plastic covered greenhouses has shortened the time needed to grow a merchantable seedling and thus one plastic covered greenhouse can produce two crops in one growing season. Sowing in heated and artificially lighted, plastic-covered houses can be started 1–2 months earlier than normally. After the first crop is ready to be moved outdoors, a second crop can be sown in the same house 1–2 months later that the normal sowing time. To date no serious outbreaks of diseases or other pest problems have occurred, but one should be aware that the seedlings with 'abnormal' growing conditions might be more susceptible than seedlings being grown under more natural conditions. Conversely, it is also possible that seedlings being grown under such conditions may escape some diseases or pests.

## Possibilities for IPM in Finnish nurseries

The aim of integrated pest management is to reduce the use of pesticides and determine alternate control methods which can be used alone or in combination with chemical controls. In Finnish nurseries several fungicide sprayings are needed each season to control Scleroderris canker on pine seedlings. Recently, however, a method has been developed using monoclonal antibodies to detect and monitor the amount of *G abietina* spores in rain water (Koistinen et al. 2000). This method could be useful in timing the application of sprayings against Scleroderris canker.

In controlling grey mould it is important to keep humidity low and avoid excess water on plant surfaces. Inside plastic-covered houses air circulation is too poor to dry the shoots of irrigated seedlings and thus they may stay wet longer than seedlings which are grown outdoors where they are exposed to wind and direct sun light. To hasten the drying of irrigated seedling shoots some nurseries have attached 'ropes' to the irrigation booms. Such 'ropes' brush against the seedling shoots and remove excess water.

Nursery hygiene can be improved by removing diseased, spore-producing trees in and adjacent to nurseries. In the case of the diseases of pine seedlings, nursery sanitation to remove inoculum can reduce the amount of Scleroderris canker and snow mould. Alternate hosts, such as aspens for the pine twisting rust (*M. pinitorqua*), also form a risk if aspens grow close (less than 200 m) to pine producing nurseries (Kurkela 1973).

Growing areas should also be cleared of susceptible plant debris such as fallen leaves, needles and seedling remnants. Such measures are important in disease control *e.g.* in reducing the sporulation sites for grey mould and especially in the control *Phytophthora* on birch seedlings.

It is recommended to wash seedling growing containers in hot water (+ 80 °C) to kill propagules of soil-borne pathogens such as *Rhizoctonia* and *Phytophthora*.

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# Fungal diseases in forest nurseries in Shimoga district, Karnataka, India

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Five forest nurseries in Shimoga district maintained by the State Forest Department of Karnataka raise and supply seedlings of 30 economically important tree species for various purposes. Of these, 14 grow naturally in the Western Ghats region of Karnataka, seven are hardwood species and six are fruit yielding species. During routine survey of nurseries, at least 17 species were found infected by 15 fungal pathogens leading to leaf spot diseases during the rainy and winter seasons. Some of the important pathogens include species of fungi belonging to the genera *Cercospora, Colletotrichum, Diplodia, Fusarium, Gloeosporium, Myrothecium, Pestalotia, Pseudocercospora, Phoma* and *Septoria*. Over crowding often resulted in seedling blight. The incidence of fungal diseases in seedlings grown in all five nurseries during the two seasons are described, detailed in a table and constraints in seedling production are discussed.

Keywords: nursery diseases, forest nurseries, fungal diseases, Shimoga

## Introduction

India is one of the eighteen-mega diversity centers and consists of two 'Hotspots' – the Eastern Himalayas and the Western Ghats. The Western Ghats boasts of biologically diverse forests and consists of a variety of forest types and spreads over the states of Karnataka, Maharashtra and Kerala. Shimoga district in Karnataka is situated in the middle of Western Ghats and is an unique place for socio-cultural activities. The headquarters of the Shimoga Forest Department are situated in Shimoga town and is an important place for collection of seeds of forest trees and raising of seedlings of more than 30 tree species that grow in the Western Ghats. The seedlings raised here are supplied to the governmental and non-governmental organizations for the purpose of regeneration of forests and social forestry and raising vegetation in the barren and uncultivable lands.

A preliminary survey of nurseries in Shimoga division revealed the poor health of tree nurseries and as the result of poor management practices seedlings were often susceptible to diseases, which reduced their vigour. Often, diseases developed in epiphytic proportions. There is not much information available on the tree nursery diseases in Karnataka. The present study is an attempt to survey and document diseases prevailing in forest nurseries in Shimoga district, Karnataka.

## Materials and methods

### Location and identification of forest nurseries

Five forest nurseries located in Shimoga district were selected and visited two times in the winter, summer and rainy seasons during the year 2001–2002. The tree species were identified and data on their economic importance were collected.

## Collection and incubation of infected plant materials

Information on the number of infected seedlings in each species, the extent of damage caused, and disease symptomatology were collected. Diseased leaves, stems and roots were collected and brought to the laboratory where they surface disinfected (1.5% NaOCl solution, 5 minutes) and incubated on moistened sterilized blotter discs or on PDA medium contained in Petri dishes and incubated for 5 days at 23±2 °C and light-darkness cycle of 12/12 h. The incubated diseased materials were observed and the fungal species associated with disease symptoms were identified. The fungal species were identified based on their habit and colony characters and reproductive properties and confirmed by comparing with information given in standard manuals.

# **Pathogenicity testing**

Single spore isolations of fungal species that were associated with disease symptoms were made separately on PDA medium contained in Petri dishes. Further, fungal cultures obtained on PDA slants which were tested again and their identification was confirmed. Spore suspensions were prepared from seven-day-old cultures on PDA and the spore density was adjusted to  $10^5$ ,  $10^6$  or  $10^7$  spores per ml. Fifty  $\mu$ l of the above spore suspensions was placed on the upper surface of the leaves at a place that was previously pricked with a sterile needle. The artificially inoculated leaves were placed on moistened sterilized blotter discs and incubated for 3–5 days as described earlier.

# **Results and discussion**

There were three species of Acacia, i.e. A. auriculiformis A. Cunn., A. concinna (Willd.) DC., A. mangium Willd., four species of Ficus, i.e. F. bengalensis Linn., F. glomerata Roxb., F. religiosa L. and F. mysorensis Heyne ex Roth., two species of Madhuca, M. indica Gmel. and M. latifolia Macbride and three species of Terminalia, T. bellerica Roxb., T. tomentosa Roth. and T. arjuna (Roxb.) W. & A. while all other genera were represented by single species. They are Anacardium occidentale L., Artocarpus hirsutus Lam., Azadirchta indica A. Juss, Bambusa arundinacea (Retz.) Willd., Bauhinia variegata L., Bombax malabarica DC., Casurina equisitifolia Forst., Dalbergia latifolia Roxb., Eucalyptus globulus Labill., Grewelia robusta F., Lagerstroemia lanceolata Wall., Mangifera indica L., Michelia champaka L., Phyllanthus emblica L., Pongamia pinnata (L.) Pierre, Pterocarpus marsupium Roxb., Santalum album L., Sapindus trifoliatus L., Saraka asoka (Roxb.) De Wilde, Swietenia mahagony (L.) Jacq., Syzigium cumini L., Tamarindus indica L., Tectona grandis L. and Thespesia populinea (L.) Soland. ex Correa.

All the tree species are not grown in all five nurseries since culture of different species depends on their demand and importance. Disease symptom restricted mainly to the aerial parts and leaf spot/blight was the common symptom observed. Root rot was some times caused by *Fusarium* sp. particularly common on *T. indica*. Fifteen genera of fungi were recorded in seedlings of 17 tree species. The number of nurseries affected by each disease and occurrence of disease in different seasons is given in Table 1. *Pseudocercospora* leaf spot disease that occurred on *Eucalyptus* was prevalent at all the nurseries. The other species, *Alternaria* leaf spot on *T. tomentosa*, *Fusarium* leaf spot on *A. occidentale*, *Pestalotia* leaf spot on *S. cumini*, *Phyllachora* leaf spot on *P. pinnata* and rust caused by *Ravenelia* on *P. emblica* occurred in three nurseries. Other species of fungi either occurred in two nurseries or in one nursery.

Disease occurrence was severe in the rainy season followed by winter and summer seasons. About 10 tree species were found to have disease incidence of 50–100% during the rainy season (May–August) and almost an equal number of them expressed disease that ranged between 20 and 100% during winter (September–December). Only *Rhizoctonia* caused 100% infection during winter, 85% infection during the rainy season and during summer the incidence was less (20%). The organisms that caused severe diseases during the rainy season were *Cercospora* leaf spot on

Table 1. Occurrence of pathogenic fungi, symptoms caused and disease incidence in respective host seedlings grown in different forest nurseries of Shimoga district.

Fungal species	Disease symptoms	Host seedling	No. nurseries	Disease incidence, %		
			affected	Rainy	Winter	Summer
Acrosporium	Powdery	Tamarindus indica	2	33	23	9
(Oidium)	mildew	Azadirachta indica	2	32	19	3
		Tectona grandis	1	20	9	0
Alternaria	Leaf spot	Acacia concinna	1	27	22	9
	Leaf spot	Terminalia tomentosa	3	19	18	5
Cercospora	Leaf spot	A. indica	1	51	14	4
	Leaf spot	Bombax malabarica	2	57	45	15
	Leaf spot	T. grandis	2	38	26	5
Cladosporium	Leaf spot	Michelia champaka	1	60	45	13
Colletotrichum	Leaf spot	Bauhinia variegata	2	40	30	8
	Leaf spot	Eucalyptus globulus	1	65	46	11
	Leaf spot	Artocarpus hirsutus	3	19	6	1
	Leaf spot	Dalbergia latifolia	1	48	18	5
Diplodia	Leaf spot	Ficus glomerata	1	43	24	12
•	Leaf spot	F. mysorensis	2	51	26	6
Fusarium	Leaf spot	Anacardium occidentale	3	37	20	5
Myrrothecium	Leaf blight	Mangifera indica	1	45	28	8
Pestalotia	Leaf spot	M. indica	1	29	7	1
	Leaf spot	Syzigium cumini	3	29	12	5
	Leaf spot	Madhuca indica	1	41	25	7
Phyllachora	Tar spot	D. latifolia	1	39	28	16
•	Tar spot	F. religiosa	1	57	37	12
	Leaf spot	Pongamia pinnata	3	57	48	20
Pseudo- cercospora	Leaf spot	Eucalyptus sp.	5	61	39	15
Phoma	Black leaf spot	Madhuca latifolia	2	29	15	7
Ravenelia	Rust	Phyllanthus emblica	3	75	35	12
Rhizoctonia	Leaf blight	B. variegata	2	47	28	9
i ii ii200i0i iia	Leaf blight	Cassia fistula	1	85	100	20
Septoria	Leaf spot	F. bengalensis	2	38	23	20 12
<i>Θ</i> εριυπα	Leaf spot	F. religiosa	1	48	29	15

A. indica (51%), B. malabarica (57%), Cladosporium leaf spot on M. champaka (60%), Colletotrichum leaf spot on E. globulus (65%), Diplodia leaf spot of F. mysorensis (61%), Phyllachora tar spot on F. religiosa (7%), P. pinnata (57%), Pseudocercospora leaf spot on Eucalyptus species (61%), rust on P. emblica (71%) and Rhizoctonia leaf blight on C. fistula (85%) (Table1).

Disease symptoms on inoculated leaves were similar to those occurring on seedlings in the nurseries. The inoculum load required varied depending on the fungal species, but most organisms caused disease at an inoculum load of 10<sup>6</sup> and 10<sup>7</sup> spores per ml.

At least 11 fungal diseases that affect nine tree species are reported to occur on mature trees in India and only a few of them are reported to cause seedling diseases in nurseries. Some of the diseases that occur on nursery seedlings have been reported earlier to cause disease in the same species. For example, leaf spot of *F. bengalensis* and *F. religiosa* caused by *Septoria* (Joshi and Vashista 1959), leaf spot of *P. pinnata* caused by *Phyllachora pongamiae* and powdery mildew of *P. emblica* caused by *Ravenelia phyllanthus* (Rangaswamy et al. 1970), leaf spot of *Eucalyptus* species caused by the *Colletotrichum* state of *Glomerella cingulata* and *Psuedocercospora eucalyptorum* (Giri et al. 1996), leaf spot of *D. latifolia* caused by *Phyllachora dalbergiae* and the *Colletotrichum* state of *Glomerella cingulata* (Sharma et al. 1984).

Most nurseries in Shimoga raise more seedlings than are required. Oftentimes seedling numbers are large and the over crowding results in the creation of congenial conditions for transmission and establishment of fungal pathogens. Inquiry at nurseries revealed that plant production practices were followed by seed dressing with malathion and brassicol and spraying seedlings with blitox and agromine. Since brassicol is a narrow spectrum fungicide and blitox is a weak broad-spectrum fungicide, these appear to be inefficient plant production practices being followed in these nurseries. The study demonstrated that adequate plant production practices needs to be followed in order to avoid nursery seedling diseases.

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# Nursery pest problems on some native tree species in Kerala province, India

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Insect pest problems in forest nurseries of selected indigenous tree species viz., Calophyllum polyanthum, Dysoxylum malabaricum, Gmelina arborea, Garcinia gummi-gutta, Grewia tiliaefolia, Haldina cordifolia, Lagerstroemia microcarpa, Melia dubia, Vateria indica, Albizia odoratissima, Terminalia crenulata, Xylia xylocarpa and Pterocarpus marsupium was studied at Peechi in Kerala State. The most serious pest incidence was noticed on G. arborea (by the caterpillars of Epiplema fulvilinea); A. odoratissima (by the Psyllid Psylla oblonga) and Pterocarpus marsupium [by the psyllids Spanioneura (quadrimaculata group) and Arytaina sp.]. Moderate damage to seedlings of G. gummi-gutta by the leafhopper Busoniomimus manjunathi and by an unidentified lepidopteran leaf miner were also recorded. All the other seedling species were relatively free from major pest infestation. Also, data are presented on the nature and intensity of pest damage.

## Introduction

In Kerala, extensive forest plantations of a variety of tree species, both exotic and indigenous, have been raised in different area. Eucalypts, Paraserianthes falcataria, Lucaenia leucocephala, teak, Gmelina arborea, Ailanthus triphysa, Bombax malabaricum and Swietenia macrophylla are some of the tree species that have been raised in extensive plantations. Besides these, small-scale experimental plantations have also been raised, especially of some indigenous species such as Albizia lebbeck, A. odoratissima, Alstonia scholaris and Dalbergia latifolia among the moist deciduous species and Mesua nagassarium, Syzygium cumini, Toona ciliata and Vateria indica among the evergreen species. Although the performance of most of these species was satisfactory during the initial phases, in the long run these plantations were not successful. There is not much documentary evidence on the various factors that adversely affected the plantations of these species, but it is presumed that incidence of insect pests would definitely be one of the major reasons leading to failure of these plantations. The exotic species are now abandoned either due to severe pest attack or for ecological reasons. Thus, P. falcataria (attacked by the bagworm Pteroma plagiophleps (Psychidae); eucalypts (attacked by subterranean termites); L. leucocephala (attacked by the psyllid *Heteropsylla cubana*) and *S. macrophylla* (attacked by the phycitid borer *Hypsipyla* robusta) are currently not favoured for extensive plantation programmes due to these problems (given in parentheses). It should be noted that the psyllid *H. cubana*, attacking *L. leucocephala*, is an introduced pest while all the remaining pests are indigenous.

Teak, despite attack by its specific insect pests *Hyblaea puera* Cram. and *Eutectona machaeralis* Wlk. continued to be the most acceptable tree species since damage by these insects did not seriously affected its survival. However, being a valuable hardwood species, teak was not a substitute for cheaper softwoods to meet the many industrial and domestic requirements. It was in this context that attempts were made to select appropriate native species that are suited to various end uses. The suitability of various native tree species for such purposes was evaluated after careful assessment of their productivity and relative susceptibility to pests and diseases under field conditions. Recently, various trials have been made using native species such as *Calophyllum polyanthum*, *Dysoxylum malabaricum*, *Gmelina arborea*, *Garcinia gummi-gutta*, *Grewia tiliaefolia*, *Haldina cordifolia*, *Lagerstroemia microcarpa*, *Terminalia crenulata*, *Melia dubia*, *Vateria indica*, *Albizia odoratissima*, *Xylia xylocarpa* and *Pterocarpus marsupium* (Mathew 1993, Nair et al. 2002). Data generated on the nursery pest problems for these species are summarized in this paper.

## Materials and methods

Observations were made on seedlings raised in standard nursery beds (12x4 m) at Peechi. Five rectangular, grids 30x30 cm in size, selected along diagonal transects within each bed formed the sampling units. The number of healthy and affected seedlings within each grid and the nature of damage caused to them were recorded. Up to 15% infestation was ranked as low, up to 25% as moderate, up to 50% as high, up to 75% as very high and above 75% as severe. Observations were repeated fortnightly.

## **Results**

Details of the pests associated with the various seedling species and their nature of damage are given below.

## Calophyllum polyanthum (Guttiferae)

Moderate leaf feeding by an unidentified lymantrid caterpillar was observed in the seedlings grown at Peechi. Application of 0.1% Ekalux 25 EC (Quinalphos) was effectively controlled the caterpillar.

## Dysoxylum malabaricum (Meliaceae)

Mild attacks of a leaf webbing pyralid caterpillar, acridid grasshoppers and mealy bugs were noticed in the nursery. No control measures were undertaken, as the incidence of these insects was sporadic and not injuring the seedlings. In the out-planted seedlings also, mild feeding by caterpillars, grasshoppers and mealy bugs was noticed although the damage was very negligible.

## Garcinia gummi-gutta (Guttiferae)

Severe infestation of seedlings by the leafhopper *Busoniomimus manjunathi* was observed on nursery seedlings plus on saplings and trees (Viraktamath and Viraktamath 1985). The adults and nymphs of this sap-sucking insect clustered on tender shoots causing die-back. This insect was attended by the ant *Plagiolepis* sp., that also plays a role in the dispersal of this insect (Maicykutty et al. 2002).

Besides the above, up to 10% of the nursery seedlings of *G. gummi-gutta* were damaged by a dipteran leaf miner, which led to crinkling and subsequent withering of leaves. Also, mild attack of aphids in a few seedlings, sucking the sap of tender leaves was recorded. A few instances of root feeding by termites also occurred on seedlings in the nursery beds.

## Gmelina arborea (Verbenaceae)

Moderate to heavy attack by an epiplemid caterpillar *Epiplema fulvilinea* Wlk. was noticed on the nursery seedlings. It was controlled by applying a 3% solution of Econeem. Also, the chrysomelid larvae of *Calopepla leayana* Lat. were also found feeding on the leaves of nursery seedlings, destroying about 10% of them. Minor attack of leaf miners, aphids and jassids were also seen on leaves. Shoot die-back caused by the tingitid *Tingis beesoni* Drake and the scolytid *Xyleborus fornicatus* Eichh. has also been reported on seedlings and can be controlled by applying a 0.03% solution of Rogor (dimethoate) (Nair et al. 1986).

## Grewia tiliaefolia (Tiliaceae)

No serious pest problem was noticed in the *G. tiliaefolia* nursery except for mild leaf webbing by the tortricid *Archips* sp. and sporadic mild defoliation by the weevil *Myllocerus* sp. during May-August. Both of these insects are considered to be minor pests in the nursery. Leaf rolling by the pyralid caterpillar *Lygropia orbinusalis* Wlk. and gall formation by an unidentified psyllid are the pest problems were reported earlier *G. tiliaefolia* seedlings (Nair et al. 1986). The galls, of the pouch type, developed on both the leaf stalk and veins of tender foliage leading to distortion and drying up of leaves. The intensity of infestation was moderate. The psyllids can be controlled by applying Nuvacron 36 EC (monocrotophos).

### Haldina cordifolia (Rubiaceae)

No pest problems were noticed *H. cordifolia* nursery seedlings during the present study. However, incidence of the pyraustid leaf roller *Parotis vertumnalis* Guen. and the gregarious caterpillars of the epiplemid *Epiplema quandricaudata* are likely to build up in the nursery and young plantations (Nair et al. 1991), which can be controlled by the application of 0.1% solution of Ekalux 25 EC (quinalphos).

## Lagerstroemia microcarpa (Lythraceae)

Leaf feeding by the weevil *Indomias cretaceus* (Faust) and by the geometrid *Semothisa* sp.; leaf webbing by the phycitid *Phycita* sp. and an unidentified tortricid; sap sucking by aphids as well as presence of an unidentified mite (Acari) were observed in the nursery. Seedling damage resulting from these insects was moderate to heavy. The weevil, *I. cretaceus* (Faust) attacked the tender foliage of seedlings causing leaf withering. Damage by this insect was noticed from August-October. The cotyledons and tender leaves of root trainer seedlings were also eaten away by the looping caterpillars of *Semothisa* sp., which resemble a dry stem and thus often escape detection. The light reddish caterpillars of the tortricid webbed the tender leaves and shoots and fed from within. As feeding by this insect caused damage to the terminal shoot, growth was stunted on about 20% of the seedlings. Application of Ekalux 25 EC (0.1%) has been suggested for control of this pest (Nair et al. 1991). Incidence of an unidentified aphid that sucked sap from tender shoots was noticed on a few seedlings. This insect, which is spread by ants, is likely to develop into a potential seedling pest, if unattended. Infestation by a mite, which resulted in a fluffy overgrowth on the leaf surface causing seedling stunting, was also noticed during December. Fortnightly application of Dicofol (kelthane) at 0.05% gave good control.

## Melia dubia (Meliaceae)

Sap sucking by scale insects, leaf mining by an unidentified dipteran fly, leaf webbing by a pyralid caterpillar and top shoot boring by a phycitid caterpillar were observed. However, none of the above was serious and did not affect seedling. Also, very rare attacks by a mealy bug was noticed to cause mortality of nursery seedlings. It can be controlled by applying a 0.05% solution of Nuvacron 36 EC (monocrotophos).

## Vateria indica (Dipterocarpaceae)

The bagworm, *Pteroma plagiophleps* Hamp. was noted feeding on seedling foliage. This polyphagous insect has the potential to cause serious damage to *V. indica*. Also, the incidence of the thyridid leaf webber *Rhodoneura* sp. nr. *myrtaceae* Drury. was noticed in the nursery. It had been previously recorded as a pest of *V. indica* in natural stands (Nair et al. 1986). Incidence of the weevil *Indomias hispidulus* Mrshl., which feeds irregularly on the tender foliage of seedlings, occasionally caused minor damage to nursery seedlings. Leaf feeding by grasshoppers and root feeding by termites was also noticed on *V. indica* nursery seedlings.

## Xylia xylocarpa (Leguminosae)

No serious pest problems were noticed in the *Xylia* nursery except for damage caused by the weevil *Indomias hispidulus* Mrshl. which attacked the tender foliage of the seedlings leading to leaf withering. Incidence of this insect was noticed throughout the year and, depending upon the season, 12 to 41% of the seedlings were attacked.

## Pterocarpus marsupium (Leguminosae)

In the nursery, the psyllids *Spanioneura* sp. (*quadrimaculata* group) caused leaf vein galls and *Arytaina* sp. caused pouch galls resulting in the leaf crinkling and severe seedling stunting. Damage, when first noticed in September, occurred on 10.8% of the seedlings. This damage persisted until November of the next year when about 42% of the seedlings were affected. Kandasamy and Thenmozhi (1985) reported the latter as a pest of *P. marsupium*. Both the species of psyllids are considered as major nursery pests of this species and fortnightly application of 0.05% Nuvacron (monocrotophos) gave effective control. Also, incidence of the weevil *Indomias hispidulus* Mrshl., whose feeding punctures on leaves caused withering of the leaves. Its attack was observed from July-September when the new leaves appeared.

## Albizia odoratissima (Leguminosae)

Attack by the psyllid Psylla oblonga was the most serious problem leading to epicormic shoot formation and stunting and seedling die-back. In one observation block, about 40% of the seedlings were heavily affected. Within 8 months, the infestation level reached 98% of the seedlings and the infestation continued even when the seedlings were outplanted to the field. In the insecticide trials, fortnightly application of 0.05% Nuvacron (monocrotophos) effectively controlled this pest. Leaf webbing by the tortricid Rhesala moestalis Wlk. and sap sucking by the membracid bug Oxyrachis tarandus Fb. were the other pests associated with this seedling species in the nursery. R. moestalis is known as a defoliator of Albizias in India and can build up to epidemic proportions (Das and Sen Gupta 1960). About 25% of the seedlings were attacked by the leaf webber. Regarding the membracid bug, O. tarandus, a minor pest associated with various species of Albizias, usually affects saplings where it causes shoot stunting and die-back. This insect is tended by ants particularly *Crematogaster* spp. which also play a role in its distribution. Earlier, six species of insects including the bug, O. tarandus were reported from A. odoratissima in India (Browne 1968). No incidence of Albizia butterflies Eurema blanda Boisd. and E. hecabe Lin. was noticed during this study. Eurema spp. severely damage Albizias nursery seedlings and saplings in India (Browne 1968).

### Terminalia crenulata (Combretaceae)

Galls caused by the psyllid *Trioza* sp. severely damaged nursery seedlings. Immature stages of this bug develop on the stems and petioles, causing swelling and distortion. Growth was affected as the infestation occurred at the shoot terminus. As a result of infestation, epicormic shoots from which also become affected. Two species of psyllids *viz.*, *T. hirsuta* Crawf. and *T. fletcheri minor* Crawf. are known on *T. paniculata* (Mathur 1975). Infestation usually progresses and persists in the nursery and application of a systemic insecticide such as Nuvacron (monocrotophos) at 0.05% is effective. Other than the psyllid, incidence of an unidentified weevil and a grasshopper feeding on the foliage was noted. The damage was negligible.

## **Conclusions**

Sap sucking insects and defoliators are the major groups of nursery pests noticed in this study. Of these, infestation by plant bugs was the most serious problem with several species such as *Garcinia gummi-gutta*, *Albizia odoratissima*, *Lagerstroemia microcarpa*, *Terminalia crenulata*, *Grewia tiliaefolia* and *Pterocarpus marsupium*. In the former, a leafhopper *Busoniomimus manjunathi* that caused mortality of seedlings and saplings was a serious pest. Each of the remaining species was attacked by specific psyllid bugs which caused severe stunting and seedling mortality. On *L. microcarpa*, an aphid bug was noticed which could develop into a potential pest. With the other species, psyllids were the most serious problem. *A. odoratissima* was attacked by *Psylla oblonga*, *T. crenulata* by *Trioza* sp., *G. tiliaefolia* by an unidentified psyllid and *Pterocarpus marsupium* by *Spanioneura* sp. (*quadrimaculata* group) and *Arytaina* sp. The plant bugs, because of their life history peculiarities can develop quickly and cause seriously damage seedlings.

Among defoliating insects heteroceran caterpillars and curculionid/chrysomelid beetles were important seedling pests. Among the caterpillar pests, special mention should be made of the epiplemid *Epiplema fulvilinea* that severely defoliated *Gmelina arborea*. Similarly, the bagworm *Pteroma plagiophleps* found on *Vateria indica*, has the potential to develop into a serious pest and because it is polyphagous, it can spread to other species as well. Among the weevils, *Indomias hispidulus* and *Indomias cretaceous* are two closely related species associated with various tree species, but they are unlikely to develop into serious pests.

Another aspect that needs to be considered in species trials is the possibility of nursery pests becoming a problem in plantations. The plant bugs recorded on *G. gummi-gutta*, *A. odoratissima*, *L. microcarpa*, *T. crenulata* and *P. marsupium* as well as the bagworm noticed on *V. indica* are already known to attack saplings and trees and hence sufficient care should be taken to check their build up in plantations.

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# Psychids as major pests of nursery plants of Rhizophora mucronata, an important mangrove species along the West Coast

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Mangroves are one of the most productive ecosystems of the world. Nurseries, plantations and natural forests of mangroves along the West Coast were surveyed to document the incidence and pest status of insects infesting seedlings and in nurseries and naturally-germinated, saplings in mangrove tracts. *Rhizophora mucronata* Poir is a true mangrove species dominant in the mangrove ecosystems in Goa, Karnataka and Kerala. The bagworms (Lepidoptera: Psychidae) were found to cause considerable damage to saplings of *R. mucronata* in nurseries. Three species of bagworms *Brachycyttarus* sp., *Pteroma plagiophleps*, and *Metisa* sp. were recorded; *Brachycyttarus sp.* being more prevalent. More than 60% of the saplings were found to be infested by these psychids. The larvae feed on the leaves and build up enormous populations giving plants a blotched appearance. The nature and extent of damage and the seasonal occurrence of these insects are recorded. Possible methods of control are also discussed.

#### Introduction

Mangroves are unique plant formations growing in the highly stressed habitat along tropical and subtropical coastlines. They are subject to high salinity in the substrate and periodical water flooding of root system during high tides. They are also stressed by the oxygen poor conditions of the substrate. Mangrove species are characterized by numerous anatomical and physiological adaptations to survive in this environment. In India, mangroves occur on both the Western and Eastern coasts and the Andaman and Nicobar Islands, covering in total about 4871 sqkm (FSI 1999). In many places, they are highly degraded and according to the status report on mangroves (Government of India 1987), India lost 40% of its mangrove area in the last century. In 1976, recognizing the importance of the mangroves, the Indian government established the National Mangrove Committee which has recommended areas for research and development and for management of the mangroves. The challenges to mangrove forests include both natural hazards and destructive human activities, the gravity of which varies from place to place.

Veenakumari et al. (1997), recorded several insect herbivores on mangroves in the Andaman and Nicobar Islands. But very few pests have been reported from mangroves along the Indian mainland (Kathiresan 1993; Santhakumaran et al. 1995). The effect and impact of the pests in this forest

ecosystem has not been worked out for any of the mangrove patches in India, in spite of their importance.

During a recent survey of the mangroves of the West Coast, many nursery plants and saplings in afforested mangrove patches were found to be heavily defoliated by bagworms (Lepidoptera: Psychidae). The psychid, *Pteroma plagiophleps* Hamps (Lepidoptera: Psychidae) was first reported from mangroves along the Goa coast in 1995 (Santhakumaran et al. 1995). More than 90% of the saplings were found to be infested to varying degrees. During the present study, two more species of bagworms were found to seriously damage mangrove seedlings.

The infestation has reached serious proportions in some of areas, posing a threat to the management of plantations established under afforestation programmes. In this context, a detailed survey and study were made in the mangroves along the west coast to gather information on the extent of infestation, the biology, and possible control of these defoliators.

#### Materials and methods

A sample survey of the mangroves including the nurseries and afforested areas along the coasts of Goa and Karnataka was conducted from 2000–2003. Though efforts on conservation and afforestation of mangroves are in progress in these two states, an organised form of maintenance of mangrove nurseries with different mangrove species is not in vogue. *Rhizophora* was the predominant seedling species in the nurseries. In many places, the propagules are allowed to germinate on their own and then used for afforestation in other patches.

Mangroves on Charao Island in Goa, and Jalady in Coondapur of Karnataka were chosen for the pest surveys. In addition, the afforested patches of mangroves along the banks of the river Mandovi in Goa and Kodi mangrove patch in Karnataka were surveyed to note the percentage of infestation. Field trips were conducted to Kodi mangroves during different seasons to collect the insects and to determine their seasonal occurrence. The number of seedlings and saplings infested and the number of the larvae feeding was recorded. The saplings surveyed were 2–3 years old. About 200–250 leaves were collected from the 10 saplings infested by each species, from which 100 leaves were selected at random to measure the percentage of leaf area damaged by each species. Loss of leaf area was assessed using a leaf area meter.

Adults and larvae collected from the field were brought to the laboratory to study the biology. The larvae were reared in separate plastic containers maintained at room temperature by providing fresh supply of *R. mucronata* leaves. The parasites emerging from the collected larvae and pupae were also collected, identified and preserved.

# **Results and observations**

The field survey in the selected nurseries and afforestation sites of Goa and Karnataka revealed the presence of three species of bagworms on *R. mucronata*. These were *Metisa* sp., *Brachycyttarus* sp., and *P. plagiophleps*. Some of the seedlings were also found to be infested by the scale insect, *Aspidiotus* sp. (Hemiptera: Diaspididae).

#### Brachycyttarus sp.

This was the predominant species of bagworm causing defoliation of seedlings and saplings of *Rhizophora mucronata*. The larval bag was 1.4–1.6 cm in length and was made of combined pieces of vegetable matter. Females reared in the laboratory each laid 234–267 eggs, but the mortality rate of the larvae was very high under laboratory conditions. Whether the same is true under natural conditions is not known.

The larvae fed mostly on the tissues on the underside of the leaf leaving the characteristic brown blotches. The upper cuticle of the thick leaf was usually left uneaten and when it dried up and gave the characteristic brown colour to the leaf. Sometimes larvae were also feed on the bark of the plant.

The head capsules of the moulted larvae was attached to the head of the larval bag and a maximum of nine head capsules could be noted in a larva before the onset of pupation, suggesting the occurrence of nine larval instars. The seasonal incidence of this species is given in Fig. 1. The percentage leaf area destroyed was 0.08–67.95% of the total leaf area with a mean of 8.9%.

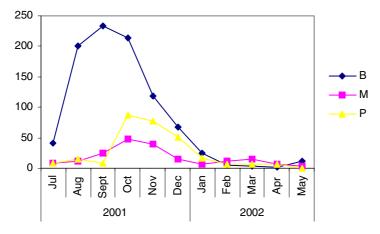


Figure 1. Seasonal incidence of bagworms on the saplings of *Rhizophora mucronata* in Kodi mangroves. B = Brachycyttarus sp.; M = Metisa sp.; P = Pteroma plagiophleps.

#### Pteroma plagiophleps Hampson

Many saplings of *Rhizophora* in Goa and Karnataka were infested by *P. plagiophleps* (Table 1). The infestation was greater for saplings in Goa. Each female lays 50–200 eggs. The larvae feed on the leaf mesophyll tissue and the epidermal layer shows brownish patches. During severe attack, the blotches look similar to that of *Brachycyttarus sp*. The larval period is 45–60 days. The pupal case hangs from a thick silk thread, which is attached to the leaf. Adults emerge after 19–21

Table 1. Percentage of *Rhizophora* seedlings and saplings infested by the three species of bagworms. B = Brachycyttarus sp.; M = Metisa sp.;  $P = Pteroma\ plagiophleps$ .

	Nursery Seedlings					
Locality	В	M	Р	В	М	Р
Goa	30	20	-	40	-	40
Karnataka	50	-	20	40	20	20

days of pupation. The insect was found throughout the year (Fig. 1). An ichneumonid, *Sinophorous* sp. was found parasiting the larvae.

#### Metisa sp.

The feeding marks of this species were similar to that of *Brachycyttarus* sp. The larval case was made of cut-mixed vegetable matter. The length of the larval case was 2.1–2.6 cm. We were unable to determine the number of larval instars. The damage caused by this species was found to be less than that of other psychids, *Brachycyttarus* sp. and *P. plagiophleps*.

# **Management options**

Though applying insecticides could be considered as management option, the risk of contaminating the marine environment has to be considered. Application of the insecticide Quinalphos at 0.1% is recommended for control of bagworms in isolated nurseries.

Parasitoids of the bagworms seem to be playing an important role in the control and management of the insects. Bagworms being polyphagous pests, silvicultural methods such as selection of other, non-susceptible species may help reduce the populations. Manual removal of the larvae feeding on the leaves can help to reduce the percentage of damage. Use of light traps to collect and destroy the adult moths is a feasible method of management.

# **Discussion**

These psychids, *Brachycyttarus* sp., *Metisa* sp. and *Pteroma plagiophleps* destroy a large proportion of photosynthetic material thus impairing the growth of seedlings and saplings and deteriorating the health of mangrove stands. Though *Pteroma* has been reported as occurring on mangrove saplings in Goa (Santhakumaran et al. 1995), this is the first report of *Brachycyttarus* sp., from the mangroves. Besides seedlings and saplings, large trees were also found to be infested by these species, though the percentage of infestation was small. Considering the loss of mangrove area resulting from other biotic interferences, the conservation aspects gains importance and control of these bagworms are indeed necessary for the establishment of this unique ecosystem. The major hindrance seems to be the difficulty in using an effective insecticide as it can contaminate the neighboring marine environment. Biological and ecological control seems to be the best possibility as of now.

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# Timing of fungicide control of *Gremmeniella* abietina on Scots pine seedlings

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In the study in 2002 in central Finland Scots pine seedlings were protected against natural infection of *Gremmeniella abietina* using chemical application every two-weeks during the whole growing season, or during shorter periods in the growing season. Of the chemical application treatments, the disease occurrence in the following spring was smallest when the countered number of conidia in the coexistently collected rain samples was the highest. The dispersal values according the conidia dispersal model correlated positively with the immunologically (ELISA) analysed number of conidia in the rainwater samples. In northern Finland in 2000, the modelled values and analysed conidia number showed weak, non-significant positive correlation with each other. Weaker correlation might be due to the that the spore collectors were not located as close to the conidia sources as in the experiment in central Finland. Both conidia analysis and the conidia dispersal model appeared to be useful for timing control, in addition to the earlier results about the susceptibility phases of the seedlings.

Keywords: spore dispersal, model, *Pinus*, growth phase, temperature sum, monoclonal antibody

#### Introduction

Gremmeniella abietina (Lagerb.) Morelet is a serious pathogen especially for pines. The fungus disperses by means of conidia and ascospores. The two types occurring in Fennoscandinavia are the A- and B-type. Both types produce spores, but conidia are more common especially in the A type. According to Petäistö and Heinonen (2003), rain and the temperature sum are the main factors predicting the dispersal of conidia. Monoclonal antibodies against conidia of *G. abietina* can also be used as a tool for following conidia dispersal (Koistinen et al. 2000).

The susceptibility of container pine seedlings in their first and second growing season has been studied using artificial inoculation with fixed amounts of *G. abietina* conidia in Central Finland. The second-year seedlings were most susceptible prior to a temperature sum of about 800 d.d., and the first-year seedlings after about 800 d.d (Petäistö and Kurkela 1993, Petäistö 1999, Petäistö and Laine 1999). A fungicide, chlorotalonil (Bravo), has been shown to decrease disease occurrence when applied during the first 10 days after inoculation (Petäistö and Juntunen 2000).

The aim of this study was to study the possibilities of timing the control of *G. abietina* in the nursery by using a conidia dispersal model and immunological conidia detection (ELISA analysis on rainwater samples), and by taking the seedling susceptible phase into account.

### **Material and methods**

#### Fungicide experiment

#### Seedlings and fungicide treatments

The experiment 2002 was performed at the Suonenjoki Research Station in central Finland. Scots pine, *Pinus sylvestris* L., container seedlings (Plantek-81F) were sown (local origin) on 29–30 April 2002, and moved outdoors on 24 June near 20-year-old Siberian cembra pines (*Pinus cembra* L.) that had diseased shoots bearing pycnidia of Scleroderris canker.

The seedlings were fertilized weekly with 0.1 % (1 July -29 July) and 0.05 % (5 August -26 August) Taimi Superex (NPK 19-4-20), 0.8 l per a tray. The height of the seedlings (the same sowing lot as the experimental seedlings) were measured in four trays, 9 seedlings/tray, on 24 June, 23 July, 7 August, 20 August and 6 September (Fig. 1).

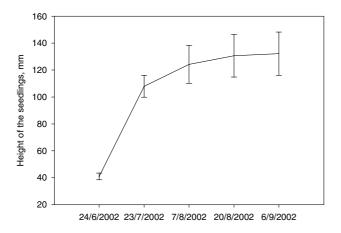


Figure 1. Height of the pine seedlings in summer 2002, Suonenjoki.

The fungicide chlorothalonil (Bravo®) was used (3 l/ha in 600 l/ha) to control Scleroderris canker infection during the summer. The five treatment (four trays per treatment) groups were:

- 1. fungicide treatments were performed on 26 June, 10 July, 24 July, 7 August, 21 August and 4 September and, at the corresponding times, and an additional four trays were treated with water,
- 2. fungicide treatments on 26 June and 10 July,
- 3. fungicide treatments on 24 July and 7 August,
- 4. fungicide treatments on 21 August and 4 September,
- 5. no fungicide treatment.

The effective control period for each fungicide treatment was calculated to be from one week before and to one week after the treatment (see Petäistö and Juntunen 2000).

The seedlings were examined on 7–9 May in spring 2003. They were classified as healthy, diseased (by *G. abietina*, brown needle base, dead bud, but also healthy needles), dead (by *G. abietina*, symptoms detectable), diseased by *Phacidium infestans* P. Karst. (mycelia found on seedlings in the spring, diseased seedlings in groups) and dead (cause not known). The number in each group was expressed as percent of seedlings.

#### Immunological conidia analysis on rainwater samples (ELISA)

*G. abietina* conidia are of the splash-dispersal type. The analysis using monoclonal antibodies was made on rainwater samples. Rainwater was collected using six plastic bottles, each with a funnel on top, placed around a diseased, about 20-year-old *P. cembra*. The funnels were covered with wire netting (mesh radius about 1.3 cm) to prevent larger litter from passing into the bottles. Rainwater was collected from the bottles and analysed weekly during 20 May to 4 November. The funnel and the bottle were rinsed with water (50–100 ml), which was added to the rainwater sample. The samples from the 6 bottles were combined to form one sample. The sample was passed through a tea gauze and stored in a plastic bag in a refrigerator until analysis by capture-ELISA (Koistinen et al. 2000).

#### Conidia dispersal model

Temperature (for calculating the temperature sum) and rainfall data were required for the conidia dispersal model (Petäistö and Heinonen 2003). Sensors connected to a minilogger at the experimental site measured air temperature and relative humidity during summer 2002. The rainfall data were obtained from the weather station of the Suonenjoki Research Station; temperature and relative humidity data were also available from this weather station.

#### Comparison of the conidia dispersal model and the ELISA results

As the conidia dispersal model was developed using data from central Finland, it was necessary to test whether it was applicable in more northerly areas. In 2000, the model was calculated using temperature and rainfall data from a more northerly area, Rovaniemi (weather station of the Finnish Meteorological Institute).

Rainfall samples were also collected in the Rovaniemi nursery in 2000 for ELISA conidia analysis. Rainfall samples were collected from 15 May to 28 August at five collection sites (two rainfall sample collection bottles at each point) at different points around the nursery. Weekly samples were packed in plastic bags and frozen, and the samples transported as frozen for the ELISA analysis. The samples from each of the five points were analysed separately. The results were combined for each collection time.

#### Data analysis

At the experimental site the trays and treatments were totally randomised. One-way ANOVA was used to test the disease occurrence between fungicide treatments. Correlation analysis was used to compare the disease occurrence as percent of seedlings (mean of tray means), values from the model and conidia numbers from the rainfall samples (ELISA).

### **Results**

#### Disease occurrence in spring 2003

About half of the untreated seedlings were diseased in spring 2003; occurrence of the disease was lowest in chemical application treatment 1. In general, the disease was identified very easily because pycnidia of *G abietina* had developed on the seedlings.

The treatments had a significant effect on the occurrence of the disease (Table 1). Treatment 5 (no fungicide) differed significantly (p<0.001) from treatments 1 and 2, but not from treatments 3 and 4 (Fig. 1). The lowest disease occurrence was in treatment 1 and 2.

Table 1. One-way ANOVA table of the effect of the fungicidal applications on the occurrence of disease expressed as a percent of diseased seedlings (mean of tray means).

	Sum of squares	df	Mean square	F	Sig.	
Between groups	7810.058	4	1952.515	19.671	.000	
Within groups	1488.903	15	99.260			
Total	9298.962	19				

#### Probability of conidia dispersal for summer 2002

The temperature and rainfall values during summer 2002 in Suonenjoki were needed for modelling the dispersal probability and the number of conidia (Petäistö and Heinonen 2003). In 2002 the main trend was that the dispersal probability values began to decrease after a temperature sum of 600 d.d., which occurred in the beginning of July (Fig. 2). The predicted daily conidia number and the dispersal probability values correlated 0.897, p<0.001, n=187). The sums of daily values were calculated for the effective periods of chemical application treatments 1–4 (Table 2).

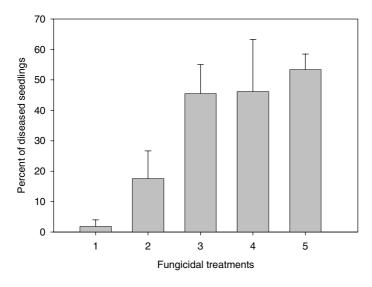


Figure 2. Percent of diseased seedlings in spring 2003 with fungicide treatments 1–5. The values are means of tray means. Vertical lines indicate standard deviation. Treatments with different letters differed statistically from each other.

Table 2. Summary of the results for Suonenjoki 2002. The effective period of the fungicidal applications, sum of the analysed conidia number (ELISA) and sum of the daily model dispersal probabilities (pm) and of the modelled number of spores (numbm) in the effective periods of fungicidal application and the disease occurrence in next spring (disease %).

Treatm	Effective period	dd-period	ELISA	pm	numbm	disease %
1	24.6.–11.9.	504–1434	17407406	32.14614	333.8778	1.86
2	24.617.7.	504-771	7935100	15.14175	159.7183	17.55
3	17.714.8.	771–1129	4203457	11.53306	109.0096	45.5
4	14.811.9.	1129-1434	5632134	6.436966	73.79788	46.15
5	no		17528620	40.4996	443.1463	53.37

#### Number of conidia in rainfall samples, ELISA

The number of conidia was calculated as the total number in the collected rainfall sample. During the period 20 May to 3 November the total number of conidia was 22 826 470. Half of this number was in the samples collected up to 30 June (543 d.d.), while 99 % of the conidia was collected so far 30 August (Fig. 2).

As the seedlings were moved outdoors to the experiment site on 24 June, the numbers of conidia measured from this day onwards were used in the experiment. The fungicide treatments were performed on Wednesdays. Rainfall samples were collected weekly, on Monday morning. When calculating the number of conidia for each chemical application treatment the mean conidia values for one day (from the weekly value) were used for those days that were not coexistent with the effective period days.

About 45 % of the conidia recorded during the experiment period (from 24 June onwards) occurred in the samples of the effective period of treatment 2, and 24 % and 32 %, respectively, from the samples of the effective periods of treatments 3 and 4 (Fig. 2, Table 2).

# Correlations between disease occurrence, dispersal probability and the ELISA results

During the period 20.5.–3.11., the weekly conidia number values (ELISA) correlated positively (0.4, p<0.05, n=24) with the weekly sum dispersal probability and the number of conidia (modelled).

In fungicide treatments 1–4, the disease occurrence (%) correlated negatively with the number of conidia (ELISA) (-0.9, p<0.06, n=4) of the corresponding effective periods and with the sums of the daily dispersal probabilities and the modelled number of spores during effective periods of the fungicide treatment (-0.9, p<0.10) (Table 3a and b).

Table 3a. Correlation between the weekly sums of the number of spores (ELISA), modelled dispersal probability (pm) and modelled spore number (numbm).

Suonenjoki, 200	)2	ELISA numb	summp	summnumb
ELISA numb	Pearson Correlation Sig. (2-tailed)	1	.399 .053	.407* .049
	N N	24	24	24
summp	Pearson Correlation	.399	1	.978**
	Sig. (2-tailed)	.053	_:	.000
	N	24	24	24
summnumb	Pearson Correlation	.407*	.978**	1
	Sig. (2-tailed)	.049	.000	
	N	24	24	24

<sup>\*</sup>Correlation is significant at the 0.05 level (2-tailed). \*\*Correlation is significant at the 0.01 level (2-tailed).

Table 3b. Fungicide applications 1–4, effective periods of these fungicide treatments, correlation between the sum of the number of spores (prevented ELISA) and sums of the daily modelled dispersal probability values (prevented pm) and sum of the modelled number of spores (prevented numbm) and disease occurrence in spring 2003.

Suonenjoki, 2002/2003		prevented ELISA	prevented mp	prevented mnumb	disease %
prevented	Pearson Correlation	1	.959*	.974*	910
ELISA	Sig. (2-tailed)		.041	.026	.090
	N	4	4	4	4
prevented	Pearson Correlation	.959*	1	.998**	911
mp	Sig. (2-tailed)	.041		.002	.089
·	N	4	4	4	4
prevented	Pearson Correlation	.974*	.998**	1	926
mnumb	Sig. (2-tailed)	.026	.002		.074
	N	4	4	4	4
disease %	Pearson Correlation	910	911	926	1
	Sig. (2-tailed)	.090	.089	.074	
	N ,	4	4	4	4

<sup>\*</sup>Correlation is significant at the 0.05 level (2-tailed). \*\*Correlation is significant at the 0.01 level (2-tailed).

#### Dispersal probability and ELISA results in northern Finland

The applicability of the model and the ELISA spore analysis was tested in Rovaniemi, North Finland, in 2000. According to the ELISA results, some of the spores were released already in week 22–28 May when the temperature sum was 54–83 d.d. The highest values occurred in the samples collected during 12–25 June and 17–23 July. The model gave a daily probability of over 0.1 for the first time in the last week of May.

The last conidia were found in the rainfall samples collected in 31 July - 7 August (651–715 d.d.) and, after this week, the model gave daily probabilities values of 0.3–0.8 (Fig. 3a and b). As the last rainfall samples were taken in the week 21–28 August, the possible number of conidia after this date could not be documented. The temperature sum reached its maximum value of 1100 d.d.

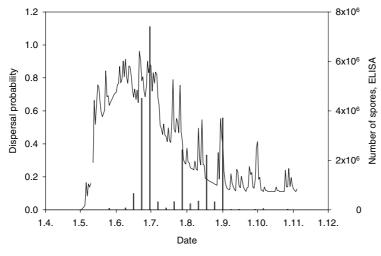


Figure 3a. The probability of conidia dispersal according to the model (Petäistö and Heinonen 2003) and the conidia number in the collected weekly rainfall samples (ELISA). Suonenjoki, summer 2002.

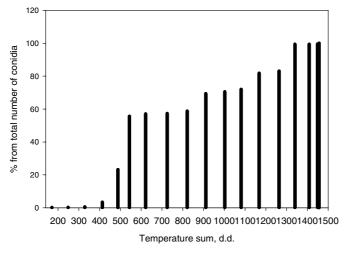


Figure 3b. Number of conidia as percent of the total number of conidia (ELISA) at different temperature sums. Suonenjoki, summer 2002.

in the middle of October, which was exceptional (about 20 days longer than the mean value) for the Rovaniemi area. The proportion of conidia out of the total number collected was 99.8% so far at a temperature sum of 590 d.d. (Fig. 4a and b).

The weekly sum conidia number (ELISA) correlated positively with the weekly sum of the dispersal probabilities 0.41, p <0.13 (n=5) and with the weekly sum of modelled number of spores 0.35, p<0.20 (n=15) (Table 4).

Table 4. Correlation between weekly spore number (ELISA), model dispersal probability (wprobabl) and model spore number (wnumber). Rovaniemi, 2000.

		ELISA	wnumber	wprobabl
ELISA	Pearson Correlation	1	.351	.407
	Sig. (2-tailed)		.199	.132
	N	15	15	15
wnumber	Pearson Correlation	.351	1	.814**
	Sig. (2-tailed)	.199		.000
	N	15	15	15
wprobabl	Pearson Correlation	.407	.814**	1
•	Sig. (2-tailed)	.132	.000	
	N ,	15	15	15

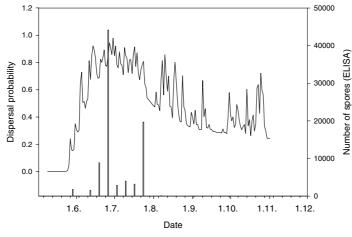


Figure 4a. The probability of conidia dispersal in Rovaniemi and the conidia number (ELISA). Summer 2000.

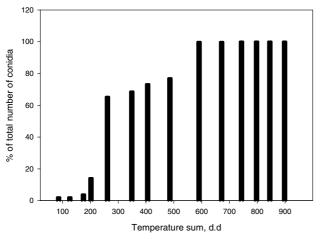


Figure 4b. Number of conidia as a percent of the total number of conidia in rainfall samples (ELISA) at different temperature sums. Summer 2000.

# **Discussion**

*G. abietina* infects plants via spores. Weather factors affect the dispersal of the spores, as well as fungal penetration and the incidence of damage in the host plant. The correct timing of application control in forest nurseries is important in order to achieve optimum prevention of the disease (Petäistö and Juntunen 2000). Being able to use fungicide only when it is necessary would decrease the amount of fungicide applied and thus reduce the negative effects of fungicide on the environment (Landis et al. 1991). Information about the spore dispersal time and the susceptibility of the plant helps in optimising the timing of chemical application.

The Bravo fungicide treatments, applied every two weeks during the outdoor period of the seedlings, gave the best preventive effect. These fungicide treatments covered the period when 99 % of the total number of recorded conidia fell on the seedlings in the experiment site. In this two-week-interval treatment, the diseased percent on trays was about 3 % from the amount of diseased seedlings in non-controlled trays.

According to Petäistö and Juntunen (2000), this kind of fungicide treatment (about two weeks prior and after inoculation) considerably decreased the incidence of fungal damage. In addition, Sletten (1971) reported, using another fungicide on three-year-old bare-root transplanted pine seedlings, better effectiveness with a two-week fungicide interval than with only monthly fungicide treatment.

For the treatments with two applications, the smallest proportion of diseased seedlings occurred when the two fungicide applications were performed on 26 June and 10 July. Both the conidia number analysis and the model showed that conidia dispersal was more frequent during the effective time of this application than in the effective periods of the other two applications. In addition, the temperature sum had reached almost 800 d.d. during this prevention period and the first-year seedlings had obviously started to become susceptible to the fungus (Petäistö 1999, Petäistö and Laine 1999, Petäistö and Juntunen 2000).

Both of the later two-application treatments (24.7. and 7.8.; 21.8. and 4.9) obviously prevented about 14–15 % of the total disease occurrence (non treated). The corresponding ELISA conidia number proportions out of the total number were 24% and 32%, the model dispersal probability sum out of the total sum 28% and 16%, and the modelled number of conidia out of the total number 25% and 17%. Thus the modelled values and the ELISA conidia number values in the latest period did not seem to be exactly compatible. The seedlings might be less susceptible in the latest period (1129–1434 d.d.) than in the earlier. To the correlation analysis on the relationship between the conidia number and modelled values and disease occurrence did not include factor of susceptibility phases, which is a very important factor. However, general information about the growth phases and stress factors (e.g. cold, shade) is important for interpreting the correlations.

The modelled values and the ELISA conidia numbers recorded in Rovaniemi 2000 did not correlate as well as those in Suonenjoki 2002. One possible explanation for this is that the rainwater collectors in Suonenjoki were located in the immediately vicinity of the diseased trees, but not in Rovaniemi. The collection of rainfall in Rovaniemi should have been continued for a longer period because the growing season in 2000 was extremely long.

In conclusion, use of the model and/or conidia number analysis seemed to be worthwhile during the general main conidia dispersal time and when the seedlings are susceptible.

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# Seedling diseases of some important forest tree species and their management

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Forest nurseries in Karnataka State provide planting stock for afforestation programmes. Consequently, to determine the kinds of diseases present in the nurseries, and the damage they cause an extensive survey was conducted for four important seedling diseases, i.e. (i) leaf spot and blight disease of Dendrocalamus strictus caused by Myrothecium roridum (ii) leaf spot disease of D. strictus caused by Cercospora apii, (iii) wilt disease of Hardwickia binata caused by Fusarium oxysporum and (iv) leaf blight disease of Terminalia catappa caused by Fusarium solani. Pathogenicity tests, made by inoculating seedlings with putative pathogens, produced disease symptoms identical to those observed in the field. Field evaluation of different fungicides at 0.2% concentration was carried out against three nursery diseases. Bavistin followed by Dithane M-45 and Captan reduced leaf spot and blight disease intensity of D. strictus caused by M. roridum. Both Bavistin and Benlate were efficient and could be recommended to manage leaf spot disease of D. strictus caused by C. apii. Bavistin followed by Captan and Dithane M-45 reduced wilt disease incidence of *H. binata* caused by *F. oxysporum*. In vitro evaluations of plant extracts, biological agents and fungicides were carried out against F. solani, the incitant of leaf blight disease on T. catappa seedlings. The plant extracts of Lantana camera and Azadirachta indica were efficient. Also, the biocontrol agents such as Trichoderma harzianum and T. viride were efficient while among the different fungicides tested, Captan and Dithane M-45 were efficient in inhibiting the mycelial growth of the fungus.

# Introduction

The forest cover in most tropical countries is declining at an alarming speed. A recent account of the world's forests, FAO (1997) shows that with the exception of India, where there has been a small improvement, all tropical countries have lost forest cover during the 1990–1995 period. The deforestation rate is highest in Central America and South East Asia amounting to 1.2 and 1.7% annually (Schmidt 2000).

Fungal diseases are a serious problem in forest regeneration and sometimes fungi can cause heavy mortality in nurseries. Many of the fungus pathogens are carried through seeds into forest nurseries and become established on seedlings. Apart from these seed-borne fungal pathogens, soil-borne fungal pathogens have also been shown to be devastating by attacking young seedlings in forest nurseries. These seedlings are particularly susceptible to several diseases because of their tender tissues and as they often have difficulty in establishing themselves. When such diseased,

substandard seedlings are used as planting stock, they further spread the disease to plantations and forests, causing heavy damage. Since seedlings grown in forest nurseries are the primary sources of planting stock, it is necessary to investigate the seedling diseases and apply control measures either before sowing the seeds or at the seedling stage. There is a strong need for better understanding of these microorganisms, the diseases they cause and their management.

### Materials and methods

In the present investigations four seedling diseases were investigated in forest nurseries in Mysore District, Karnataka state.

#### Leaf spot and blight disease of Dendrocalamus strictus

Out of 135 species of bamboo grown in India *Dendrocalamus strictus* (Roxb.) Nees is one of the important forestry species that is grown in plantations and agro-forestry systems. While surveying for nursery diseases of *D. strictus*, leaf spot and blight were commonly encountered among young seedlings (10-month-old) in a forest nursery in Kukkarahalli, Mysore. The disease resulted in defoliation and weakening of seedlings. Infected leaves showed characteristic pale brown spots and blight symptoms (Figs. 1 and 2), and when severe resulted in distortion and defoliation of leaves.

#### Leaf spot disease of Dendrocalamus strictus

This foliar disease was observed among 6-month-old seedlings of *D. strictus* in a forest nursery in Paduvaralli area, Mysore District of Karnataka State. Affected leaves showed characteristic brown spots (Fig. 3). When severe the disease resulted in drying and defoliation of leaves. Further, infection sometimes spread to stems causing seedling death.

#### Wilt disease of Hardwickia binata

Hardwickia binata (Roxb.) is an important hardwood tree well distributed in the tropical and subtropical regions of India. It is considered as one of the important forest tree species in afforestation programmes. During a survey of forest nurseries, wilt disease of *H. binata* was observed among young seedlings in Nagavala forest nurseries of Mysore District, Karnataka State. The wilt symptoms started from the tip of the plant and along the margin of the leaves (Fig. 8). Subsequently, the disease advanced downward causing defoliation and eventual death of seedlings.

#### Leaf blight disease of Terminalia catappa

Terminalia catappa L., is commonly called 'tropical almond' or 'Indian almond'. The seeds are eaten raw, the leaves are used as food for Tasar silk worms, the roots and bark are used for tanning, while the fruits are used as a source of dye (Nayer et al. 1994). Because of its multifarious uses, it has been given due importance in plantation programmes in nurseries. During a recent survey of forest nurseries, a severe leaf blight was observed on young seedlings of *T. catappa* (Fig. 9) in a forest nursery in Nagavala, Mysore.

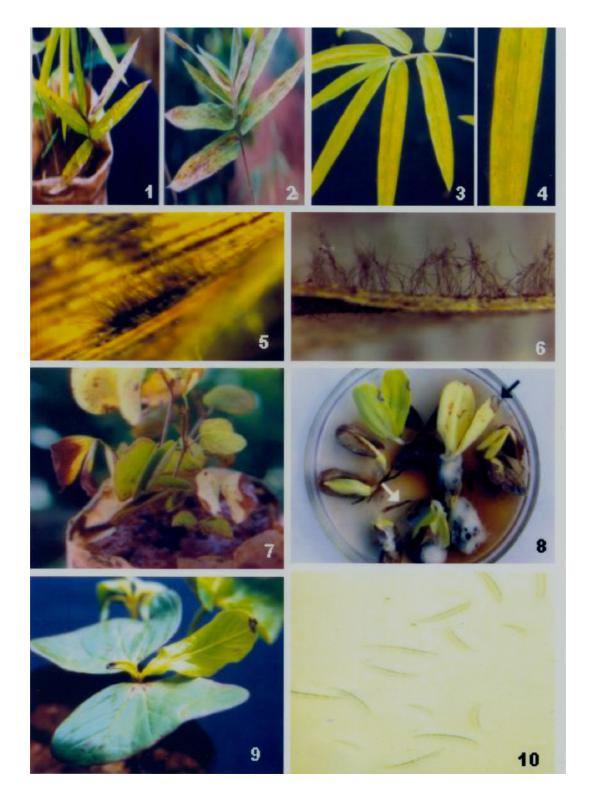


Figure 1. and 2. Seedlings of *Dendrocalamus strictus* showing leaf spot and blight disease.

Figure 3. and 4. Seedlings of *Dendrocalamus strictus* showing leaf spot disease caused by *Cercospora apii*.

Figure 5. and 6. Sporulation of *Cercospora apii* from the leaf spot of *Dendrocalamus strictus*.

Figure 7. Initiation of wilt symptom in seedlings of *Hardwickia binata* caused by *Fusarium oxysporum*.

Figure 8. Seedlings of *Hardwickia binata* showing wilt and root rot symptom on water-agar medium caused by *Fusarium oxysporum*.

Figure 9. Seedlings of *Terminalia catappa* showing leaf blight disease caused by *Fusarium* solani

Figure 10. Microconidia and macroconidia of Fusarium solani.

For the purpose of identifying the pathogen, diseased seedlings were collected in sterilised polythene covers, brought into the laboratory where they were washed thoroughly under running tap water to remove debris. Next, they were washed in distilled water and surface sterilised with 4% NaOCl. The diseased parts were then cut into 1cm-long pieces. One set of pieces was placed on three layers of wet blotters equidistantly in Perspex plates and another set plated onto PDA medium. Both sets were incubated for 7 days under 12h/12h alternate cycles of near ultra violet light and darkness at 22±2 °C. After 8 days of incubation the diseased bits were evaluated for the causal organism.

#### Isolation, identification and multiplication of the causal organism

The diseased parts of the leaves were analysed in the laboratory. The causal micro-organism was isolated and grown on PDA medium. To study the pathogenicity of the micro-organism, a spore suspension was prepared from 8-day-old sporulating colonies using sterile distilled water. The spore load was calculated haemocytometrically and its load was adjusted to  $5x10^6$  spores/ml of suspension. This spore suspension was inoculated to 50 young seedlings. The inoculated seedlings were incubated in a moist chamber and observed for disease symptoms.

#### **Transmission studies**

Transmission studies were carried out by using a water-agar and sand method. Four replicates of 100 seeds each inoculated with causal micro-organism were used to determine pathogenicity.

#### Management of seedling diseases

In vitro evaluation of Terminalia catappa

To determine the effect of fungicides, plant extracts and biological agents on the leaf blight causal organism of *Terminalia catappa*, an *in vitro* evaluation was carried out. The efficacy of the fungicides and plant extracts were tested using the Poisoned Food Technique (Grover and Moore 1962).

For *in vitro* fungicidal evaluation, 0.2 g of the fungicides Bayleton, Ridomil, Dithane M-45, Captan and Hadron were separately dissolved in 100 ml of sterile, distilled water, then 5 ml of fungicide solution was thoroughly mixed with 15 ml of PDA medium (v/v 5:15) at 45°C. This was poured to a series of sterilised glass Petri plates. After solidification of the medium the plates were inoculated with 3 mm culture discs of the test micro-organisms (Fig. 11).

For the *in vitro* evaluation of plant extracts, leaf materials of selected plants viz., *Bacopa monniera*, *Acalypha indica*, *Azardirachta indica*, *Lantana camara* and *Derris indica* were studied. Each plant extract was separately mixed with 15 ml of molten PDA (v/v 5:15) and poured into sterilised glass Petri plates. After solidification the plates were inoculated with 3 mm diameter culture discs of micro-organisms. The colony diameter of the fungus was measured after 4, 6 and 8 days of incubation and compared with the colony growth of the fungus in the control plates (Fig. 12).

The fungal antagonistic effect of putative biocontrol fungi such as *Trichoderma harzianum*, *T. viride*, *T. koningii* and *Gliocladium virens* were tested *in vitro* for their bio-efficiency in inhibiting the punitive pathogens using the 'Dual Culture Technique' (Dennis and Webster 1971).

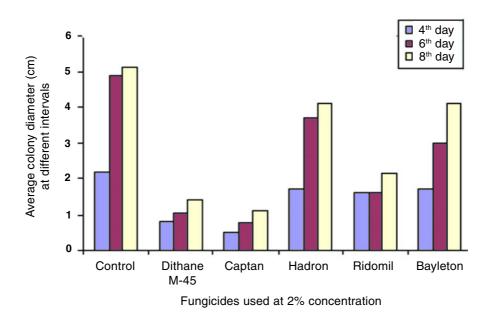


Figure 11. Effect of different fungicides on the colony growth of Fusarium solani in vitro.

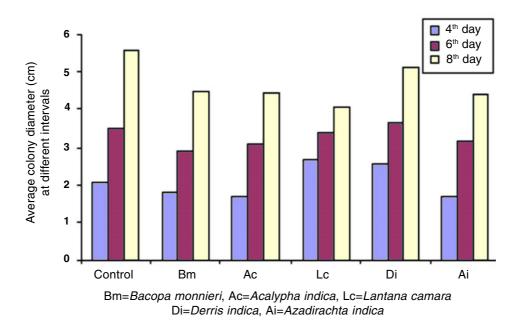


Figure 12. Effect of different fungicides on the colony growth of Fusarium solani in vitro.

#### Field evaluation of fungicides

Diseased seedlings grown in poly bags were used to study the effect of the different fungicides under field conditions. The same fungicides as used for *in vitro* studies were tested at 0.2% concentration. The fungicidal treatments were carried out by spraying the fungicidal solution onto the seedling and also by drenching the soil in which the seedlings were raised. These fungicidal treatments were applied twice at an interval of 10 days. For each treatment there were four replicates of 50 seedlings each. After 30 days of treatment, the effectiveness of the different fungicides was evaluated by calculating the percent disease incidence and percent disease reduction. For each test plant the total leaves and total diseased leaves were counted and the average of the 50 seedlings in four replicates was calculated.

Percent disease incidence (PDI) was calculated using the formula:

$$PDI = \frac{Number of diseased leaves on each plant}{Total number of leaves on each plant} X 100$$

Percent disease reduction (PDR) was calculated using the formula

$$PDI = \frac{PDI \text{ in control-PDI in treatment}}{PDI \text{ in control}} X 100$$

In all the cases seedlings without fungicide treatment served as the control.

# Results

#### Leaf spot and blight of *Dendrocalamus strictus*

Laboratory examination of diseased *D. strictus* leaves revealed a high incidence of the fungus *Myrothecium roridum*, both when incubated on wet blotters and also on PDA medium. Thirty days after inoculation of young seedling with *M. roridum* pale brown spots and blights symptoms (identical to those observed in the field) developed on seeding leaves. Re-isolations made from artificially inoculated leaves yielded the same fungus and confirmed its pathogenicity.

In the water-agar seedling test 76% of the seeds with severe infection failed to germinate due to infected embryos (Table 1). Out of the remaining seeds which germinated 11.5% showed post-emergence mortality. The fungus sporulated on dead and diseased seedlings. Some of seedlings were killed even before the first leaf opened. Sometimes there was browning and rotting of the roots. Overall, 5.5% of the seedlings remained healthy. Severely infected seeds were colonised by *M. roridum* and completely rotted. In the sand method test 85% of the inoculated seeds failed to germinate (Table 1). Pre-emergence mortality was greater for the sand method than for the water agar method. Eight percent of the seedlings remained healthy compared to 61% in the control.

The effectiveness of the different fungicidal treatments on percent disease incidence and on percent disease reduction are shown in Table 2. Among the different fungicides tested, Bavistin treated seedlings showed  $\sim 11.6\%$  disease incidence and 78.4% disease reduction followed by Dithane M-45 with 12.2% and 76.5%, Captan with 13.3% and 75.1% and Bayleton with 21.3% disease incidence and 60.4% disease reduction, respectively.

Table 1. Seed to seedling transmission of Myrothecium roridum on Dendrocalamus strictus.

Method	Treatment	Seed germination	Pre- emergence mortality or ungerminated seeds	Post- emergence mortality	No. of diseased seedlings	No. of healthy seedlings
		Mean <u>+</u> SE	Mean <u>+</u> SE	Mean <u>+</u> SE	Mean <u>+</u> SE	Mean <u>+</u> SE
Water-agar method	Control Inoculated	75 <u>+</u> 1.29 24.00 <u>+</u> 0.91	25.00 <u>+</u> 1.29 76.00 <u>+</u> 0.91	0.00 <u>+</u> 0.00 11.50 <u>+</u> 1.32	0.5 <u>+</u> 0.50 7.00 <u>+</u> 0.70	74.50 <u>+</u> 1.70 5.50 <u>+</u> 1.44
Sand method	Control Inoculated	62.00 <u>+</u> 1.08 15.00 <u>+</u> 1.47	38.00 <u>+</u> 1.08 85.00 <u>+</u> 1.47	0.00 <u>+</u> 0.00 3.25 <u>+</u> 1.10	1.00 <u>+</u> 0.70 3.75 <u>+</u> 0.85	61.00 <u>+</u> 1.63 8.00 <u>+</u> 0.40

Values are the means of four replicates. SE = Standard error.

Table 2. Field evaluation of fungicides against leaf spot and blight disease of *Dendrocalamus strictus* caused by *Myrothecium roridum*.

Treatments	PDI Mean <u>+</u> SE	PDR Mean <u>+</u> SE	
Control	54.43+2.52 <sup>e</sup>	0.00 <u>+</u> 0.00 <sup>a</sup>	
Captan	13.31+1.13 <sup>a</sup>	75.09 <u>+</u> 3.31 <sup>₫</sup>	
Bavistin	11.62+0.54 <sup>a</sup>	78.44 <u>+</u> 1.68 <sup>d</sup>	
Dithane M-45	12.50+2.62 <sup>a</sup>	76.49+5.54 <sup>d</sup>	
Bayleton	21.26+1.36 <sup>b</sup>	60.35+4.39°	
Ridomil	31.06+1.30 <sup>d</sup>	42.84+1.93 <sup>b</sup>	
Fungihit	33.81 <u>+</u> 1.69 <sup>d</sup>	37.60 <u>+</u> 3.65 <sup>b</sup>	

The values are the means of four replicates. In each column, values followed by the same letter are not significantly different at  $P \le 0.05$  level when subjected to Duncan's multiple range test. PDI = percent disease incidence; PDR = percent disease reduction; SE = Standard error.

#### Leaf spot disease of *Dendrocalamus strictus*

Laboratory analysis of the disease parts of the leaves of *D. strictus* affected with leaf spot indicated a high incidence of the fungus *Cercospora apii* (Figs. 5 and 6), when incubated both on wet blotters and on PDA medium. *C. apii* when inoculated to young seedlings resulted in the development of identical spots within 25 days. It was confirmed that *C. apii* causes leaf spot disease with characteristic brown spots among young seedlings of *D. strictus*. In the water agarseedling symptom test, 69% of the seeds showed pre-emergence mortality and 11% of the seeds suffered post-emergence mortality (Table 3). Based on germinated seedlings, 9.75% showed disease symptoms of characteristic brown spots. The diseased parts of the leaves showed sporulation of fungus. Although there was 31% germination, 11% of the seedlings could not survive as the sprouted seedlings were killed by the pathogen. In the sand method there was remarkably more pre-emergence mortality of 81% than post-emergence mortality of 9.5% when compared with the 44 and 1%, respectively for the un-inoculated control. In this method about 8.25% of the seeds resisted infection and developed as healthy plants. Inoculated seeds in the water agar method test showed more diseased seedlings than in the sand method.

Only fungicides were considered in the field evaluation of the different treatments against leaf

Table 3. Seed to seedling transmission of Cercospora apii on Dendrocalamus strictus.

Method	Treatment	Seed germination	Pre- emergence mortality or ungerminated seeds	Post- emergence mortality	No. of diseased seedlings	No. of healthy seedlings
		Mean <u>+</u> SE	Mean <u>+</u> SE	Mean <u>+</u> SE	Mean <u>+</u> SE	Mean <u>+</u> SE
Water-agar method Sand method	Control Inoculated Control Inoculated	63.00±3.41 31.00±1.29 56.00±1.77 19.00±1.29	37.00±3.41 69.00±1.29 44.00±1.77 81.00±1.29	2.75±1.10 11.00±1.47 1.00±0.40 9.50±1.04	1.00±0.70 9.75±0.85 0.75±0.75 1.25±0.75	49.25±7.23 9.75±1.49 45.25±3.68 8.25±0.85

Values are the means of four replicates. SE = Standard error.

spot disease. Table 4 gives the effectiveness of the different fungicidal treatments on percent disease incidence and percent disease reduction. Among the fungicides tested, Bavistin-treated seedlings showed ~11.7% disease incidence and 76.3% disease reduction followed by Benlate with 15.3% and 69%, Bayleton with 19.8% and 59.9% while Dithane M-45 showed 20% disease incidence and 59.5% disease reduction.

Table 4. Field evaluation of fungicides against leaf spot disease of *Dendrocalamus strictus* caused by *Cercospora apii.* 

Treatments	PDI Mean <u>+</u> SE	PDR Mean <u>+</u> SE	
Control Bavistin Benlate Bayleton Dithane M-45 Fungihit Hadron	49.43±0.41° 11.68±0.95 <sup>b</sup> 15.31±1.24° 19.81±1.53 <sup>a</sup> 20.00±0.64° 25.93±0.73 <sup>d</sup> 27.00+0.88 <sup>d</sup>	0.00±0.00° 76.32±2.16° 69.00±2.54° 59.94±2.96° 59.52±1.39° 47.48±1.90° 45.36+1.91°	

The values are the means of four replicates. In each column, values followed by the same letter are not significantly different at  $P \le 0.05$  level when subjected to Duncan's multiple range test. PDI = Percent disease incidence; PDR = Percent disease reduction; SE = Standard error.

#### Wilt disease of Hardwickia binata

Diseased seedlings analysed in the laboratory had a high incidence of the fungus *Fusarium oxysporum*. The diseased parts showed heavy sporulation of the fungus when incubated both on wet blotters and on PDA medium. In the water agar seedling symptom test ~45.3 % of the seeds showed pre-emergence mortality (Fig. 7). The other 54.8% of the seeds germinated out of which 29% showed post-emergence mortality (Table 5). Diseased seedlings showed rotting of the roots and characteristic wilt symptoms on the leaves. The ungerminated seeds showed rotting due to heavy colonisation by the fungus. Among inoculated seeds, 9% developed into healthy seedlings. Un-inoculated healthy seeds produced healthy seedlings. In the sand method 68.5% of the inoculated seeds showed germination failure and ~13.8% showed post-emergence mortality when compared to 27.8% and 3.3% of the uninoculated healthy seeds (Table 5).

Table 5. Seed to seedling transmission of Fusarium oxysporum in Hardwickia binata.

Method	Treatment	Seed germination	Pre- emergence mortality or ungerminated seeds	Post- emergence mortality	No. of diseased seedlings	No. of healthy seedlings
		Mean <u>+</u> SE	Mean <u>+</u> SE	Mean <u>+</u> SE	Mean $\pm$ SE	Mean $\pm$ SE
Water-agar method Sand method	Control Inoculated Control Inoculated	81.50±4.03 54.75±0.75 72.25±1.65 31.50±0.64	18.50 <u>+</u> 4.03 45.25 <u>+</u> 0.75 27.75 <u>+</u> 1.65 68.50 <u>+</u> 0.64	0.75±0.75 29.00±0.40 3.25±1.49 13.75±2.01	0.5±0.50 13.75±1.31 1.25±0.75 3.00±1.08	80.00 <u>+</u> 4.52 9.00 <u>+</u> 1.87 67.75 <u>+</u> 2.42 14.75 <u>+</u> 2.09

Values are the means of four replicates. SE = Standard error.

The effectiveness of the different fungicides under field conditions is given in Table 6. The field evaluations revealed Bavistin to be the most effective fungicide in controlling the disease showing ~18.8% disease incidence and 70.4% disease reduction followed by Captan, which showed 20% disease incidence and 67.6% disease reduction. Dithane M-45 was effective, with a disease incidence of 24.5% and percent disease reduction of 60.3 compared to 62.5% disease incidence and no percent disease reduction in the control.

Table 6. Field evaluation of fungicides against leaf spot disease of *Hardwickia binata* caused by *Fusarium oxysporum*.

Treatments	PDI Mean + SE	PDR Mean + SE	
Control Captan Bavistin Dithane M-45 Bayleton Ridomil Fungihit	62.50±2.72° 20.00±1.08° 18.75±3.77° 24.50±1.70° 27.00±2.41° 35.00±2.54° 39.00+3.24°	$0.00\pm0.00^{a}$ $67.62\pm2.42^{cd}$ $70.43\pm5.00^{d}$ $60.32\pm3.82^{cd}$ $56.49\pm4.33^{c}$ $43.74\pm4.44^{b}$ $37.86\pm2.83^{b}$	

The values are the means of four replicates. In each column, values followed by the same letter are not significantly different at  $P \le 0.05$  level when subjected to Duncan's multiple range test. PDI = Percent disease incidence; PDR = Percent disease reduction; SE = Standard error.

#### Leaf blight disease of Terminalia catappa

Diseased seedling screened for the causal organism revealed the presence of the fungus *Fusarium solani* (Fig. 9). When this fungus was inoculated onto young seedlings it produced identical symptoms within 30 days. Re-isolations from the inoculated leaves yielded the same fungus and proved its pathogenicity.

Among the different fungicides tested *in vitro* against the mycelial growth of *F. solani*, Captan and Dithane M-45 showed high fungitoxicity and inhibited the growth of the fungus to a considerable extent followed by Ridomil and Bayleton (Fig. 11). Hadron was the least effective fungicide.

Out of five leaf extracts tested, those of *Lantana camara* followed by *Azadirachta indica*, *Acalypha indica* and *Bacopa monniera* were found to be effective in inhibiting the growth of *F. solani in vitro* (Fig. 12).

All the fungal antagonists tested were found to be very effective in inhibiting the growth of *F. solani*, with *Trichoderma harzianum* being the most efficient followed by *T. viride*, *T. koningii* and *Gliocladium virens*.

# **Discussion**

The present investigation revealed *M. roridum* to be the causal agent of leaf spot and blight on young seedlings of *D. strictus*. The pathogenic nature of *M. roridum* has been reported by several workers. This fungus is seed-borne, causing heavy seedling mortality in tomato (Srivastava and Tandon 1966), mungbean (Nath et al. 1970) and cotton (Srinivasan and Kannan 1974). Dake (1980) and Shivanna (1989) have also reported *M. roridum* causing reduced germination in cotton and cluster bean, respectively. Although there is no report of this fungus affecting forest tree species, it has been reported to cause leaf spot in *Bombax ceiba* (Sharma et al. 1985) and reduce seed germination in *Pterocarpus marsupium* (Ali and Sharma 1996).

The seedling symptom test showed both pre and post-emergence mortality of seedlings. The disease symptoms manifested themselves in the form of leaf blight, browning and rotting of root, revealing the bi-directional systemic movement of the fungus in the seedlings from the seeds. Earlier workers have also reported the pathogenic nature of *M. roridum*, i.e. it has been reported to inhibit seed germination, and cause seed rot and seedling mortality (Dake 1980, Sharma et al. 1985, Shivanna, 1989, Ali and Sharma 1996). Leaf spot and blight observed in the present study can be attributed to the pathogenic nature of *M. roridum*.

Chemotherapy is an effective strategy for controlling serious fungal diseases in forest nurseries. In the present study Bavistin followed by Dithane M-45 and Captan reduced the disease intensity under field conditions. Bavistin is an effective fungicide against many fungal pathogens and is being extensively used to control many fungus-caused diseases of forest tree specie *viz*, leaf spot and blight of *Michelia champaca* caused by *Rhizoctonia solani* (Mehrotra 1992) and root rot and leaf blight of *Boswellia serrata* caused by *Macrophomina phaseolina* (Mehrotra 1996). Apart from Bavistin, Dithane M-45 and Bayleton have also been widely used to control fungal diseases. Dithane M-45 is reportedly effective against leaf spot disease of *Ficus religiosa* caused by *Colletotrichum gloeosporioides* (Dadwal and Jamaluddin 1992) and Bayleton against *Melamspora larici-populina* on *Populus* in nurseries (Pandey et al. 1996), which agree with our results. Our studies show too that spraying with 0.2% concentrations of Bavistin or Dithane M-45 can be recommended against leaf spot and blight disease of *D. strictus* caused by *M. roridum* in forest nurseries.

In the present study the pathogen that caused many characteristic brown spots over the entire leaf blade of *D. strictus* was identified as *Cercospora apii*. Several reports are available on the leaf spot disease in forest tree species caused by *C. tectonae* reportedly causes leaf spot disease in *Tectona grandis* (Spaulding 1961), *C. wrightii* in *Wrightia tinctoria* (Siddaramaiah et al. 1980a), *C. subsessilis* in *Azadirachta indica* (Sankaran 1986), *C. bombacina* in *Bombax ceiba* (Sharma et al. 1988) and *C. dehradunii* in *Grevellia pteridifolia* (Misra 2001). Apart from forest tree species, *Cercospora* species also attacks, e.g. groundnut and safflower (Suryanarayana 1978; Siddaramaiah et al. 1979). Our results agree with those of these workers.

Among the different fungicides evaluated in the field for reducing the disease incidence, Bavistin and Benlate were the most effective. Bavistin is effective against a wide range of fungal pathogens and has been used to control many diseases such as leaf spot of *W. tinctoria* caused by *Cercospora wrightii* (Siddaramaiah et al. 1980a), leaf spot and blight of *Michelia champaca* caused by *Rhizoctonia* sp. (Mehrotra 1992) and post-emergence damping off of *Eucalyptus* hybrid caused by *Verticillium* sp. (Harsh et al. 1992). Siddaramaiah et al. (1980b) have recommended Bavistin and Benlate for managing *Cercospora* leaf spot of *Carthamus tinctorius*, which agrees with our results. Bayleton and Dithane M-45 are also effective in reducing the disease incidence of some diseases. The fungicides are effective against rust disease of poplars caused by *M. larici-populina* (Pandey et al. 1996) and leaf spot disease of *F. religiosa* caused by *C. gloeosporioides* (Dadwal and Jamaluddin 1992). Among our different fungicide treatments, 0.2% Bavistin or Benlate can be recommended to manage the leaf spot disease of *D. strictus* incited by *C. apii* in forest nurseries.

In the present investigation wilt disease was observed among young seedlings of *Hardwickia binata*. Laboratory analyse revealed *Fusarium oxysporum* as the causal organism. *F. oxysporum* also causes wilt in seedlings of *Dalbergia sisoo* (Harsh et al. 1992) and chick pea (Gupta et al. 1986). Using the water agar method, inoculated seeds showed pre emergence mortality, rotting of the roots and characteristics wilt symptoms. Ungerminated seeds were diseased and rotten. Most of the seedlings that were diseased did not survive. In sand method, seeds inoculated with this fungus showed more pre- and post-emergence mortality compared to the corresponding control. *F. oxysporum* has been reported on several tree seeds where it causes seed decay, germination reduction and seedling wilt (Ali and Sharma, 1996; Mamatha et al. 2000). In the present study among the different fungicidal treatments considered for field studies Bavistin, Captan and Dithane M-45 reduced disease incidence. Bavistin, Captan and Dithane M-45 have been reported to be effective against many plant diseases (Dey and Debata 2000, Gupta et al. 1989).

In our study, *F. solani* incited leaf blight disease of young, *T. catappa* seedlings in forest nurseries. *F. solani* is known to cause root rot and seedling blight of *Azadirachta indica* (Sankaran et al. 1986; Shukla 1992) and wilt of seedlings of *Albizzia falcataria*, *Eucalyptus camaldulensis* and *Paraserianthus falcataria* (Sharma and Sankaran 1987, Kumar and Vishwanath 1993; Sankaran and Sharma 1996).

Among the fungicides tested *in vitro* growth of *F. solani*, Captan and Dithane M-45 were highly fungitoxic and inhibited the growth of the fungus to a considerable extent followed by Ridomil and Bayleton. The effectiveness of Captan and Dithane M-45 against fungal pathogens is known, e.g. Gupta et al. 1989; Dadwal and Jamaluddin 1988. Dithane M-45 is effective against many fungal pathogens, being recommended for management of fungus-caused diseases like seedling blight of *A. falcataria* (Srivastava and Soni 1993), leaf spot disease of *Populus deltoids* caused by *Alternaria alternata* (Dey and Debata 2000), and leaf spot and blight of *Syzygium cumini* caused by *Cylindrocladium quinqueseptatum* (Mehrotra and Mehrotra 2000). Followed by Captan and Dithane M-45, Ridomil and Bayleton were effective against *F. solani*. The efficiency of Ridomil and Bayleton against fungal pathogens has been reported by Rathore and Pathak (2002) and Pandey et al. (1996).

Out of five leaf extracts tested, those of *Lantana camara* followed by *A. indica*, *Acalypha indica* and *Bacopa monniera* inhibited the growth of *F. solani in vitro*. Several reports describe the fungitoxicity of *L. camara*. Leaf extracts of *L. camara* exhibit maximum toxicity against spore germination of *Curvularia tuberculata* (Srivastava and Lal 1997). Leaf extracts of *Azadirachta indica* also effectively inhibited mycelial growth of *F. solani*. Many researchers have reported

fungal inhibitory property of *Azadirachta indica* (Nair and Arora 1996, Singh and Dwivedi 1990), which agrees with our findings. Apart from the above, *Acalypha indica* and *B. monniera* also exhibit some fungitoxicity. These results can be attributed to the fact that several plants possess chemicals toxic to different micro-organisms, which serve as chemical protective barriers against the invasion of different micro-organisms.

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# Potential of plant products for the management of whiteflies in nurseries

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Whiteflies are tiny sap-sucking insects belonging to the family Aleyrodidae. They injure plant in a variety of ways. Among them the spiralling whitefly *Aleurodicus dispersus* and the babul whitefly *Acaudaleyrodes rachipora* are highly polyphagous attacking important tree species both in nurseries and plantations. Experiments were conducted to study the efficacy of a dye obtained from the bark of *Persea macrantha* against *A. dispersus* on *Michelia champaca* seedlings. The results showed that the 1% concentration of dye dissolved in ethanol and teepol were very effective in containing the population of the nymphs of *A. dispersus* on *M. champaca*. Further foliar application of neem seed oil alone at 5% concentration and basal application of deoiled neem cake at 1 gm/ polybag gave effective control of the babul whitefly *A. rachipora* on important tree species of arid and semi-arid zone of India. The findings are discussed in this communication.

Keywords: Whiteflies, Aleyrodids, Persea macrantha

#### Introduction

Whiteflies are tiny sap-sucking insects belonging to the family Aleyrodidae. In recent years, whitefly pests have become a major problem, almost world wide. They injure plant in a variety of ways (David and Subramanium 1976). Among the whitefly pests the spiralling whitefly Aleurodicus dispersus Russell and the babul whitefly Acaudaleyrodes rachipora (Singh) are highly polyphagous attacking important tree species both in nurseries and plantations. High population of whiteflies feeding on nutrients of plants affect the plants physiological process, ultimately causing leaf shedding and reduced growth rate. Chlorotic spots appear at feeding sites on leaf surfaces. Vast amount of honey dew produced by nymphs leads to mould development on leaves and adversely affects photosynthesis (Sundararaj et al. 2000). All the life stages of whiteflies are hard to control with conventional insecticides because of rapid multiplication, their preferred habitat on the under surface of leaves, thereby not being easily targeted by direct hit of spraying insecticides (Sood et al. 2003). Further with the growing evidence of the adverse effects of conventional pesticides on health and environment, the need for safer methods of pest management has become inevitable. Use of plant products is now emerging as one of the prime means to protect any plant. This communication deals with the possibility of managing A. dispersus and A. rachipora on some forest tree species.

# **Material and methods**

#### Experiments for the control of A. dispersus

A solid colouring matter obtained (yield 6 to 7%) from the bark of *Persea macrantha* (Nees.) Koster. by hot extraction with alcohol and repeated purification using ethanol and chloroform, was assessed for its pesticidal properties against *A. dispersus* on the seedlings of *Michelia champaca* L. The dye was dissolved separately in 50% ethanol and 0.1% teepol and two sets of experiments were conducted, one for ethanol and the other for teepol based solutions. Each treatment consisted of 10 seedlings. The spray solutions were applied by using a hand sprayer. The nymphal population was taken by counting the nymphs on a leaf from the middle canopy from three plants at random per treatment. The data collected were pooled and means were computed for statistical analysis.

# Experiments for the control of A. rachipora

Series of experiments were conducted with neem based products along with other biofertilizers and conventional pesticides and fertilizers for the management of *A. rachipora*. The effect of basal application of freshly prepared neem cake alone and in combination *Rhizobium*, VAM, Single Superphosphate against the incidence of the *A. rachipora* was evaluated in one month old seedlings of forest tree species of Indian arid zone *viz.*, *Acacia nilotica* (L.) Willd., *A. senegal* (L.) Willd., *A. tortils* L., *Prospis cineraria* Ronjh, *P. juliflora* D.C. and *Albizia lebbeck* (L.) Benth. Foliar spray of neem seed oil alone or and in combination with conventional insecticides *viz.* Chlorpyriphos and Monocrotophos were evaluated on heavily infested three month old seedlings of *A. nilotica*, *A. senegal* and *A. tortilis* against *A. rachipora*. From the results of the experiments conclusions were drawn for the management of *A. rachipora* on important forest seedlings of Indian zone.

# Results and discussion

The nymphal population of *A. dispersus* observed in different treatments with dye of *P. macrantha* dissolved in ethanol is shown in Table. 1. The pretreatment count varied from 88 to 123 nymphs per leaf in different treatments and the nymphal population invariably affected in all the treatments except control. The effect was maximum with minimum nymphal population being 0 to 7.3 nymphs per leaf at 1% concentration and it was significantly less than all other treatments at all the observation days after treatment.

Table 1. Nymphal population of *Aleurodicus dispersus* on *Michelia champaca* in different treatments with dye of *Persea macrantha* (Solvent: Ethanol 50%).

Concentration		Mea	an no. of nym	ohs/leaf at diff	erent DAT*		
of dye (%)	0	1	2	3	7	10	14
1.00	105.33	1.66	1.33	0.66	0.00	2.00	7.33
0.50	88.00	42.33	18.66	33.33	40.66	41.66	46.66
0.25	111.66	39.66	35.66	30.66	39.33	41.66	46.33
0.13	114.33	59.00	64.66	49.00	57.33	60.66	65.66
0.05	83.33	62.33	56.33	53.00	57.66	60.00	69.33
0.02	99.33	82.00	75.00	65.00	80.33	82.00	84.33
Control	123.00	97.00	89.33	84.66	90.33	93.33	97.00
CD (P=0.05)	NS	12.76	11.04	13.09	17.43	20.65	19.76

<sup>\*</sup>DAT= Days after treatment

The pretreatment count of nymphal population per leaf ranges from 89.3 to 112.3 in different treatments with dye of *P. macrantha* dissolved in teepol (Table 2). In this experiment too the treatment with 1% dye recorded lowest number of nymphal population being 8 nymphs per leaf at one day after treatment to 20 nymphs per leaf at 14 days after treatment. In general treatment with 1% dye recorded significantly less number of nymphs than control in all the days of observations. The study showed that the solid colouring matter from the bark of *P. macrantha* contains insecticidal principles effective against *A. dispersus*. The present record of effectiveness of dye of natural origin on whitefly is in agreement with the findings and advocations of Reddy et al. (1985), Vir (1990) and Reddy and Venugopal (1993). To date 1079 plants are reported to have pest management properties. Of them 866 plants against insects, 150 plants against nematodes, 30 plants against mites, 20 plants against rodents and 13 plants against snails (Prakash and Rao 1996). The study indicated that the solid dye from the bark of *P. macrantha* contains insecticidal principles which can be used in the management of *A. dispersus*.

Table 2. Nymphal population of *Aleurodicus dispersus* on *Michelia champaca* in different treatments with dye of *Persea macrantha* (Solvent: Teepol 0.1%).

Concentration		Me	an no. of nym	phs/leaf at dif	ferent DAT*			
of dye (%)	0	1	2	3	7	10	14	
1.00	89.33	8.00	12.33	9.66	18.66	16.00	20.00	
0.50	112.33	35.66	29.66	36.00	42.65	51.33	60.66	
0.25	106.00	41.33	52.33	61.66	59.00	73.33	82.66	
0.13	112.33	76.66	61.33	55.66	72.33	119.66	122.33	
0.05	101.66	78.66	109.00	111.33	122.00	148.66	121.33	
0.02	98.33	103.00	121.33	115.33	102.66	111.66	123.33	
Control	107.00	112.66	117.66	128.00	115.33	109.66	132.33	
CD (P=0.05%)	NS	23.45	18.67	23.54	18.76	22.94	21.90	

<sup>\*</sup>DAT= Days after treatment

In the other set of experiments conducted on seedlings of different tree species from the Indian arid zone, the seedlings treated with neem cake alone and in combination with other nutrients recorded lower number of eggs and nymphs of *A. rachipora*. This trend was observed for 3 months after application in the tested tree species. Experiments of foliar spray of neem seed oil alone or in combination with synthetic insecticides demonstrated that the neem seed oil at 0.5 % alone is significantly superior to monocrotophos 0.2 % and endosulfan 0.2 % in reducing egg and nymphal populations and its combination with either 0.1 % monocrotophos or endosulfan is on par with neem seed oil alone to control this whitefly. In general, neem seed oil at 0.5% and its combination with monocrotophos and endosulfan resulted in fewer eggs and nymphs while monocrotophos and endosulfan alone were less effective in reducing the incidence of egg and nymphal population of *A. rachipora*. Similar findings were reported by Coudrict et al. (1985) on Sweet Potato whitefly *Bemisa tabaci* and Sundararaj et al. (1996) on *A. rachipora*. The studies revealed that by basal application of neem cake and foliar spray of neem seed oil the incidence of *A. rachipora* on the seedlings of important tree species of Indian arid zone can be managed.

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# Pests and diseases of sandalwood plants in nurseries and their management

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Sandalwood, the highly valued tree of global fame, grows naturally under suitable conditions in different parts of India. Relating to conservation efforts and also to promote sandalwood as a commercial species, many sandal nurseries are maintained in different states of India. Reconnaissance surveys were conducted in the sandalwood growing areas in the states of Karnataka, Tamilnadu, Kerala, Andhra Pradesh, Orissa and Madhya pradesh during 1994–1999 to study the occurrence and distribution of insect pests and diseases of sandalwood plants in the nurseries, plantations and natural forests. Defoliators and sapsuckers were the most devastating pests. An account of the more important ones and their natural enemies is given in this paper. Seedling diseases (damping off and wilt) were found to take a heavy toll (up to 100%) in the nurseries. The causal organisms were identified as *Fusarium oxysporum* and *Phytophthora* spp. and nematodes. Control measures were standardized for pest and disease management in the sandalwood nurseries.

# Introduction

Sandalwood Santalum album L. (family Santaleaceae) is distributed all over India and is a tree of great economic importance because of its fragrant heartwood and oil. Large-scale plantation programs have necessitated a demand for planting stock, for which nurseries have been established in different states. Good quality seeds and grafts of superior clones are used for the production of plants. Sandal wood seedlings and grafted plants face problems from insect pests and diseases, which take a heavy toll and sometimes the whole stock is wiped off.

More than 150 insects are known to occur on *S. album*, but only a few have been recorded as serious and bearing economic importance. These include defoliators, sapsuckers, stem borers and termites. The role of sap sucking pests belonging to the family Coccidae is very deleterious to the normal health, growth and reproduction of sandalwood plants. The adult beetle, *Mylabris pustulata* Thunberg. (Coleoptera: Meloidae) was reported as a general feeder on the floral parts of sandalwood plants. (Sivaramakrishnan 1984). The weevil *Sympiezomias cretaceus* Faust belonging to a polyphagous group was recorded as defoliating several trees of forestry importance including sandalwood seedlings in nurseries (Sivaramakrishnan et al. 1987). Two species of bugs, *Saissetia nigra* Nietner and *S. coffeae* Walker were recorded damaging immature fruits of sandal, which fall off and do not germinate (Sivaramakrishnan et al. 1987). The lac insect, *Kerria lacca* Kerr. has been reported as causing mortality of the plants (Remadevi et al. 1997). *Ceroplastes ceriferus* 

Anderson has been observed either singly or in groups on sandalwood trees causing leaf drop, reduction in plant vigor, leading to dieback of plants (Remadevi and Sivaramakrishnan 1997). The coccid, *Inglisia bivalvata* Green was observed to cause dieback of branches and in severe cases, death of saplings and young sandalwood trees (Remadevi and Raja Muthukrishnan 1998). Sandalwood seedling disease has been identified as a serious threat to the raising of sandal wood plants in nurseries (Nayar et al. 1980, Sivaramakrishnan et al. 1984).

A survey of the sandalwood growing areas in the different states helped to delineate the occurrence and distribution of the different insect pests and diseases of sandalwood plants. The paper gives an account of important pests and diseases of sandalwood plants in the nurseries and the methods of their management.

#### Materials and methods

Reconnaissance surveys were conducted during 1994–1999 under a World Bank aided FREE (Forestry Research Education and Extension) project, in the different sandalwood growing areas, plantations and nurseries in the states of Karnataka, Tamilnadu, Kerala, Andhra Pradesh, Orissa and Madhya Pradesh for recording the incidence of different insect pests and diseases. The different insects and the damaged parts of sandalwood seedlings were collected and brought to the laboratory for identification. Field and laboratory observations on the bio-ecological aspects of the pests and diseases were made. The assumed biocontrol agents of the different pests were collected, identified and preserved. Experiments on the control measures were conducted and standardized in the nurseries at Gottipura, Nallal, Yelawala and the IWST campus.

# Results and discussion

#### **Nursery pests**

An account of the important pests encountered in the nurseries in the different states in India are presented in Table 1 and 2.

#### **Defoliators**

Sandalwood seedlings in the nursery are subjected mainly to attack by defoliators and sapsuckers. *Cryptothelea cramerii* Westwood (Psychidae: Lepidoptera) cuts off the young seedlings, almost at ground level. A bag is constructed of small pieces, of the seedling stem, and these are placed side by side so as to form a cylindrical bag open at both ends. The sandalwood seedlings ultimately dry up. The weevil Sympiezomias cretaceus Faust (Curculionidae: Coleoptera), a polyphagous weevil feeds on the leaves from the edges towards the midrib. Sometimes the distal half of leaves gets cut off as a result of circular holes made in a line by the adult weevils. Feeding occurs at night. During the day adult beetles hide on the under surface of leaves or inside curled leaves or between webbed leaves.

The nymphs and adults of the grasshopper, *Holochlora albida* Kirby (Locustidae: Orthoptera) are green, resembling a leaf. They are well concealed among the young seedlings and they can only be detected when they move. These hoppers usually gnaw on tender shoots of sandalwood seedlings.

Table 1. Name, family and order of defoliators present in nurseries and plant part affected by these insects.

SI. No	. Name	Family	Order	Plant part affected
1	Sympiezomias cretaceus	Curculionidae	Coleoptera	Leaves
2	Holochlora albida	Locustidae	Orthoptera .	Shoots
3	Teratodes monticollis	Acrididae	Orthoptera .	Leaves
4	Letana inflata	Tettigonidae	Orthoptera	Leaves
5	Cryptothelea cramerii	Psychidae	Lepidoptera	Shoots & leaves
6	Acanthopsyche moorei	Psychidae	Lepidoptera	Leaves
7	Pteroma plagiophleps	Psychidae	Lepidoptera	Leaves

The nymphs and adults of the grasshopper, *Letana inflata* Brunner are slender and resemble the stem of sandalwood seedlings. They feed voraciously on the foliage. The eggs are laid in longitudinal slits in succulent sandalwood seedling stems and the slit swells and cracks laterally, damaging the seedlings.

The nymphs and adults of the grasshopper, *Teratodes monticollis* Gray (Acrididae: Orthoptera) are dull green, brighter under the wings. The pronotum is produced into a sharp hood over the body, giving it a striking appearance. Its green color and appearance camouflages well with the sandalwood seedlings. They have been observed as a serious defoliator of sandalwood seedlings.

The bagworm, *Acanthopsyche moorei* Heyl (Psychidae: Lepidoptera) carries its bag upright at right angles to the stem or leaf of the seedling, but in the later instars the bag is heavy and is carried in a pendant position. The black caterpillar was seen defoliating the sandalwood seedlings giving a burnt appearance to the leaves.

The attack of bagworm, *Pteroma plagiophleps* Hampson (Psychidae: Lepidoptera) results in total defoliation and drying up of sandalwood seedlings. Defoliation by these bagworms also impart a burnt appearance to leaves.

#### Sapsuckers

About 50 species of sap sucking insects were recorded on *Santalum album* by Mathur and Singh (1960–61). Out of this, only a few are considered injurious to the sandalwood seedlings (Remadevi et al. 1998). New coccid species on sandalwood seedlings were also recorded during the present survey. Most of the coccids attack both sandalwood seedlings and trees. Use of sandalwood twigs for grafting purposes, when affected by coccids, has led to the failure of grafted plants. As the coccids produce honeydew, badly infested plants get completely covered by sooty molds, which reduce photosynthesis the vigor of sandalwood seedlings.

Two species of coccids, *Saissetia coffeae* and *S. nigra* were first recorded in Coimbatore on Sandal (Ayyar 1929). The adult female of *S. coffeae* Targioni-Tozzetti is elliptical in outline, convex, brown and shinny. *S. nigra* Nietner females are black and larger. The nymphs move and settle on the under part of the sandalwood plant. These scales feed on the sap of leaves and tender shoots of sandalwood seedlings, causing die back. The coccid, *Aspidiotus* sp. was observed as a minor pest causing wilting and yellowing of the leaves of nursery plants.

Table 2. Name, family and order of sapsuckers present in nurseries and plant part affected by these insects.

SI No.	Name	Family	Order	Plant part affected
1	Saissetia nigra	Coccidae	Hemiptera	Shoots and leaves
2	Saissetia coffeae	Coccidae	Hemiptera	Shoots and leaves
3	Pulvinaria psidii	Coccidae	Hemiptera .	Shoots and leaves
4	Pulvinaria maxima	Coccidae	Hemiptera .	Shoots and leaves
5	Ceroplastes actiniformis	Coccidae	Hemiptera .	Shoots and leaves
6	Inglisia bivalvata	Coccidae	Hemiptera .	Shoots
7	Tachardina lacca	Coccidae	Hemiptera .	Shoots
8	Aspidiotus sp.	Coccidae	Hemiptera .	Leaves

The coccid, *Pulvinaria psidii* Mask is a common and destructive polyphagous insect in many sandalwood nurseries. The coccid, *P. maxima* Green is a new record on Sandalwood seedlings and is found very common on Neem. Sometimes it is considered to cause considerable damage to young sandalwood trees. The leathery pale brown adult females cover the tender shoots and stems in numbers and the white male scales are generally found conspicuously on the sandalwood leaves. The ovisacs are prominent and comparatively very long. The infestations leads to premature fall off of the leaves.

The coccid, *Ceroplastes actiniformis* Green has a thick pale white or pink waxy spherical test and the marginal area is divided into eight portions, which enclose a central cone area. Being sapsuckers, they cause severe sap drainage and sooty mold formation on the leaves below. The sap drainage leads to die back and ultimate death of sandalwood seedlings in nurseries. Spraying with monocrotophos (0.02–0.05%) kills this coccid.

The lac insect, *Tachardina lacca* Kerr. was observed on nursery plants and seedlings along with severe attack on trees. Quinalphos (Ekalux 20 AF) 0.5% or Dimethoate 0.2% mixed with 0.05% sticker was sprayed for effecting the control.

Feeding of sap by the coccid, *Inglisia bivalvata* Green caused browning of the leaves and withering. When the attack was severe, saplings succumb to the infestation. These scale insects are attached to twigs. Female scales look like bivalved shells. While adult males are winged, females are wingless and sedentary. Nymphs move out from underneath the scales of females and settle on tender branches to suck the sap. This insect occurs throughout the year. Parasites and predators help in the biological control of this pest. Spraying of 0.2–0.3% Chlorpyriphos or Quinalphos was effectively controlled this pest. The natural enemy complex of the different coccids was also studied. Parasitism varied from 4–10 % in the different coccids. We found the parasites in the coccids: *Ceroplastes actiniformis* Green, *I. bivalvata*, *T. lacca*, *Aspidiotus* sp. and *Saissetia* sp. (Table 3).

Table 3. Name and family of parasites found in the coccids *Ceroplastes actiniformis*, *Inglisia bivalvata*, *Tachardina lacca*, *Aspidiotus* sp. and *Saissetia* sp.

Host	Name of the parasite	Name of family
Ceroplastes actiniformis	Signiphora sp. nov. Coccophagus cowperi Girault Coccophagus ceroplastae (Howard) Metaphycus sp. nov. Bothriophryne pulvinariae Agarwal Encyrtus aurantii (Geoffroy) Cheiloneurus basiri Hayat Cephaleta anupama Narendr. & Mini Aprostocetus sp. A. Scutellista caerulea (Fonscolombe)	Signiphoridae Aphelinidae Aphelinidae Encyrtidae Encyrtidae Encyrtidae Encyrtidae Encyrtidae Encyrtidae Pteromalidae Eulophidae Pteromelidae
Inglisia bivalvata	Anicetus sp. nov. inglisiae Philosindia sp. nov. inglisiae Microterys sp. nov. Coccophagus bivittatus Compere Marietta leopardina Motschulsky Aphanogmus sp. Aprostocetus sp. B. Philosindia sp. Coccobius sp. Anagyrus mirzai Agarwal & Alam Anicetus sp. nov. inglisiae	Encyrtidae Encyrtidae Encyrtidae Aphelinidae Aphelinidae Ceraphronidae Eulophidae Encyrtidae Aphelinidae Encyrtidae Encyrtidae Encyrtidae Encyrtidae
Tachardina lacca	Ooencyrtus sp. nov. kerriae Aprostocetus sp.	Encyrtidae Eucyrtidae
Aspidiotus sp.	Thomsonisca pakistanensis (Ahmad) Aphytis sp.	Encyrtidae Aphelinidae
Saissetia sp.	Philosindia sp. nov. inglisia	Encyrtidae

Based on our studies on pests of sandal, chemical control measures have been standardized which are presented in Table 4.

Table 4. Control of different sapsuckers on sandal.

Insect pests	Prevention/control measures
Ceroplastes ceriferus	Spraying of Monocrotophos (0.02–0.05%)
Saissetia sp.	Spraying 0.5% Quinalphos
Inglisia bivalvata	Spraying 0.2-0.3% Chlorpyriphos or Quinalphos
Tachardina lacca	0.5% Quinalphos (Ekalux 20 AF) along with 0.05% sticker sprayed thoroughly on the affected parts. Initial stages can be controlled by spraying 0.1% Rogor or 0.04% Cypermethrin.

#### **Nursery diseases**

Damping off and seedling wilt were the most serious diseases recorded during the survey in different states. High incidence of disease was recorded in Karnataka and Tamilnadu destroying the entire nursery stock during damp seasons. Mortality in seedlings was recorded in all three stages during their growth- due to pre-emergence blight, post-emergence mortality, root rot and wilting of older seedlings.

The diseases are caused by a several fungi and nematodes. Species of *Fusarium, Rhizoctonia*, *Phytophthora* and *Pythium* were found most commonly in infected seedlings. *Fusarium oxysporum* Schlecht. was common and the most virulent fungus found in all infected sandal seedlings affected by pre-emergence blight and vascular wilt. In vascular wilt, nematodes attacked seedlings along with *Fusarium* causing serious problems to seedlings. The fungus spreads rapidly in the tissues and the seedlings either wilt completely or rot off at ground line. Fungus attack on succulent root tips in older seedlings and transplants are usually not fatal. If soil moisture is favorable, new roots develop and the seedlings continue to live though with less vigor and poorer growth. If such damage is followed by drought or by excess soil moisture, both of which discourage formation of new roots, seedling mortality may eventually occur.

Wilt is a systemic disease in sandal wood seedlings, where the entire individual or its parts exhibit wilting of the foliage in acropetal succession up to the shoot. The leaves become yellow, lose turgidity and fall off. The affected plant or the branch soon dies. Dwarfing, stunting and necrosis was also a common symptom found in seedlings. In all cases translocation of water and nutrients was adversely affected. Characteristic symptoms of vascular discoloration in the outer layers of the seedlings were evident.

F. oxysporum isolates from the infected seedlings were grown on slants of PDA and inoculated to the seedlings raised in sterile soil under controlled conditions. Wilt symptom and chlorosis developed 7–10 days after fungus inoculation. With the onset of symptoms, plants were treated with different doses of fungicide and further disease manifestation was controlled.

To formulate a package of practices for the raising healthy seedlings in nurseries, experiments were conducted with different treatments. Sandalwood seedlings were grown with well drained sterilized and non-sterilized soil; pre-treatment of seeds with copper fungicide and without treatment; controlled watering and excess of watering, soil application of nematicides and no application. Based on the different experiments, which were closely monitored, control measures were standardized and are given in Table 5.

Table 5. Control of different type fungal diseases.

Type of disease	Casual organisms	Control measures
Fungus attack on seeds	Fusarium, Alternaria, Aspergillus	Store the de-pulped and dried sandal seeds with organomercuric compound (Cerasan/Agallol) dressing.
Seedling disease 1. Pre-emergence rot 2. Damping off	Fusarium or Phytophthora or Rhizopus spp.	Selection of seeds, which are free of fungi, seed dressing with organomercuric compound. Controlled watering, good drainage in containers.
3. Fusarium wilt	Fusarium or Phytophthora species followed by nematodes	Drench the potting medium with copper fungicide and nematicide (Blitox/Bordeaux mixture and Quinalphos or Phorate). Controlled watering of plants.

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# Improvement of seedling production system in forestry sector and its impact on seedling health

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More than 120 forestry species are being utilized in India for various afforestation programmes. Among these, teak (*Tectona grandis*), eucalypts (*Eucalyptus grandis*, *E. tereticornis*), acacias (*Acacia auriculiformis*, *A. mangium*), and poplars (*Populus* spp. are exploited on a large-scale and the annual planting rate is about 1.78 Mha. Production of planting stock and stand establishment are affected by various limiting factors, of which diseases play a major role. The introduction of root trainer technology has made a tremendous impact on forest nursery seedling production. The technology offers production of large number of healthy, uniform-sized planting stock within 90 days. Using the technology also improves the quality of seedlings and clonal plants since it allows for growers to better manipulate mycorrhizae, biofertilizers, biopesticides and micronutrients. The other advantage over seedbed and polythene container nurseries is that root spiraling of seedlings can be avoided. However, the technology needs further optimization and growing media have to be developed and standardized depending on the seedling crop, local climatic conditions, and the planting technique to be followed. This paper highlights the modernization of planting stock production system and its impact on nursery management.

## Introduction

Even though, forest tree seedling production systems has been revolutionized in many countries, in India, production of planting stock is still largely depended upon conventional methods. In forest nurseries, from 1970 onwards polythene containers of different sizes have been used on a large-scale for raising seedlings and for transplanting bare root seedlings. The technology in vogue is economical, however, spiraling or coiling of roots in seedling crops, especially in eucalypts, is one of the disadvantages. Since 1985, various forest industries and research institutions have tested different types of containers for growing seedlings. However, very recently root trainers were introduced in the forestry sector and presently this technology is being widely used for growing planting stock of selected forestry species. Due to the tropical, warm-humid climate in many parts of the country, diseases and insect pests cause major havoc and they are often a limiting factor for raising and maintaining nursery stock. Under conducive microclimatic conditions, seedling crops of any forestry species may succumb to one or more diseases and pests. In India, climatic conditions range from temperate in the north to tropical warm-humid in the north-eastern and southern peninsula and hence incidence of diseases and pests in nurseries exhibits tremendous variation. Disease and pest outbreaks in forest nurseries in high rainfall areas often become drastic and most seedling species raised become seriously affected. As the

disease and pest hazards in forest nurseries became very common they often affected entire planting programmes. Consequently, systematic studies and management of economically important diseases and pests were taken up during the 1980s and 1990s and nursery management practices for important forestry species were standardized (Mohanan 1997 and 2001, Sharma et al. 1985, Sharma and Mohanan 1991). Recently, Mohanan (2000) made a comparative account of the disease situation in root trainer and conventional nurseries. The study showed the tremendous improvement in seedling quality, and thereby stand establishment, resulting from using the root trainer, seedling production system.

## **Planting stock production**

Forest nurseries play a vital role in all afforestation programmes. The quality of planting stock warrants not only successful field establishment, but also subsequent growth performance and high yield. Raising high quality seedling requires technical skills including careful planning for all the major components such as quality seed, appropriate growing media, root trainers/containers, nursery hygiene and protection. In India, forest nurseries become operational either throughout the year, as in the case of production of bare root seedlings (teak) for preparation of stumps, or during December to June, i.e. for raising fast-growing species like eucalypts and acacias. The seedling production system can be broadly grouped as either conventional and modern.

## Conventional system

In this system, bare root seedlings are raised in seedbeds and polythene containers. The nurseries are established and maintained by the state forest departments in suitable sites which are close to the planting area in each year during December–July or at least 6 months before the proposed planting operations. Seedlings are grown in seedbeds of standard size (12 x 2 x 30 m) and for the first 45 to 60 days, a shade panel of either coconut leaves or other broad leaves is provided to protect the seedlings from sun scorch. The seedbeds are watered regularly and regulation of shade, water and seedling density is done depending up on the seedling crop. When seedlings reach a height of 10 to 15 cm, they are pricked out into polythene containers (18 x 12 cc) filled with forest soil. This occurs during February–March and for the first 2 to 3 weeks the container plants are kept under shade. The container plants are maintained till they are planted out during June–July (south–west monsoon). In general, seedling crops need to be maintained for at least 6 months; however, bare root, teak seedlings need to be maintained for 12 to 18 months for preparation of stumps, which are planted out directly during May–June.

In India, since 1970 polythene containers have been widely being used for raising forest nursery seedlings. Polythene containers of different sizes are used for either direct seeding or transplanting the bare root seedlings. Though, the polythene containers are handy and economical, they have the inherent problem of causing root coiling or spiraling. Seedling roots grow geotropically, but if they do not meet any physical obstruction, they may tend to grow laterally around the side of the container. Usually, root spiraling will not adversely affect growth of the seedlings in containers, but it can seriously affect stand establishment. Even though, spiraling can occur in almost any type of container, root spiraling is commonly observed in flat-bottomed, smooth walled polythene containers.

## **Root trainer system**

Improvement of the planting stock production system, especially the introduction of root trainers and establishment of permanent nursery facilities in the forestry sector was initiated under a World Bank aided forestry programme. Central forest nurseries with facilities for raising and maintaining many quality seedlings, clonal multiplication facilities, composting and growing media development units were developed in different parts of each state to meet the need of annual planting programmes. Reusable type root trainers with different growing-cell sizes and capacities were used. The root trainer is a specially designed cylindrical container made up of opaque material with two open ends of which the lower end tapers gradually with a smaller open end, to provide favourable condition for the root development. Inside the root trainer, four to six ridges or ribs run longitudinally from one end to another, to prevent root coiling. When a root starts to touch ridge it immediately changes its course and grows downward thus avoiding coiling.

The principles of root trainer technology include: (i) providing appropriate environment to attain rapid development of primary roots and subsequent secondary roots, (ii) allowing early natural pruning of primary tap root and induce secondary root system so as to attain 'forced multiple taproots', and (iii) maintaining acute angle of secondary and tertiary root tips and its subsequent pruning, so as to keep downward movement to attain network of massive root system. The above principles aim at training the root in a desirable direction and enhancing the surface area for absorption with little or no injury or disturbance to the tender roots. Appropriate training of the root system is achieved by providing a porous, easily penetrable, nutrient rich growing medium with good drainage and proper aeration. Limiting the quantity of the growing medium induces root competition and thereby optimum utilization of the medium in the cells. Natural pruning of primary and secondary roots occurs by exposing the growing tips to sunlight and air, and thus induces strong vigorous multiple tap roots. Maintaining the angle of inclination in such a way that lateral roots should tend to develop downward, and providing taper in the bottom of root trainers results in a downward development of the roots.

Even though different types of root trainers are available for grow seedlings, reusable trays containing cells from which seedlings or ramets (clonal plants) can be removed at the planting site are the most preferred. Single cell and styrofoam blocks or composite trays are being widely used. Both seedlings and ramets are raised on a large-scale in root trainers. The size of the container for a particular seedling crop depends on both biological and economical features. Usually, cell volume in the root trainers for raising broad-leaved species ranges from 150–300 cc. However, optimum container size varies according to many different factors, including growing density, seedling species, size of seedling desired, type of growing medium, environmental conditions, and length of the growing season. The distance between the individual cells in the block influences seedling growing density, one of the most important container characteristics affecting seedling growth. The spatial arrangements of cell within the block also has economical implications, however, tree seedlings require a certain minimum amount of growing space, which varies with species and age. In general, root trainer seedling quality increases with a corresponding decrease in growing density. Since root trainer technology has only recently been introduced in India, several factors are yet to be determined for the optimization of the technology to suit particular climatic conditions, seedling crops and planting techniques.

The root trainer nursery certainly has many advantages over the seed bed and polythene container nurseries, not only in the production of quality seedlings but also in overall nursery management. Some of the advantages include ease of: (i) filling the growing cavities with growing medium, (ii)

regulating the moisture regime in the container, (iii) applying fertilizers, micronutrients, biopesticides and biofertilizers, (iv) and other factors such as casualty replacement and handling and transport. Also, air pruning of roots produces actively growing rot tips and uniform sized hardy planting stock (seedlings/ramets). There is less shock to seedling resulting from transportation/outplanting and the nursery growing period is no more than 90 days.

## **Clonal nurseries**

Clonal forestry on a large-scale was initiated in Andhra Pradesh State by ITC Paper Boards Ltd. during the 1980s. *Eucalyptus tereticornis* (hybrid) plus trees (healthy, resistant against pink disease) were selected and ramets were prepared on a large-scale for raising plantations for farm forestry and regular forestry programmes (Lal 1993). In Kerala State, clonal forestry has been initiated recently and many disease resistant clones of *E. tereticornis* and *E. grandis* were identified and ramets were prepared on a large-scale for fulfilling the needs of the State Forest Department (Balasundaran et al. 2000).

In root trainers, eucalypts seedlings and ramets can be raised, however, the method of production of ramets differs slightly on account of use of coppice cuttings as reproductive material. Single cell root trainers of 150 cc are usually used for raising the ramets. Various growing media such as vermiculite, perlite and soil/sand mixtures are being used. Rooting hormones usually employed include indole acetic acid (IAA), indole butyric acid (IBA), naphthalene acetic acid (NAA), and 2,4 dichlorophenoxy acetic acid (2,4 D). Of these, the most commonly used is IBA and it is used as a dip method, quick dip method and dry-dip smear method. Of these, the dry dip method is most commonly used for eucalypts and IBA (4000 ppm) is mixed with inert talcum powder and applied to stimulate root formation. The root trainers with the cuttings are initially transferred to the mist chamber and subjected to intermittent mist for 4 to 5 weeks until a good root system is developed and shoot growth starts. After initiation of sprouting, the root trainers are transferred to hardening units provided with sprinklers and shade nets to reduce sunlight and temperature. Nutrients (DAP and NPK mixture) are supplied to the ramets and an average plant height of 30 to 45 cm is maintained. The technology offers production of uniform sized, disease-free, healthy planting stock leading to higher and more uniform wood yield from the plantations.

# **Growing media**

The common organic materials used as growing media include for example coir pith, sugar cane waste, saw dust, tree bark, forest weeds, paddy straw, wheat straw, and water weeds. Most of these organic materials benefit from composting and balances the ratio of carbon to nitrogen in the material. Among the inorganic components, used in the potting mix, for improving drainage and aeration are vermiculite, perlite, pumice, and sand. Composted organic material has all the chemical and physical properties of an ideal growing medium. Even though, any organic material is suitable for composting, the most vital factor for compostability is its C/N ratio, i.e. the ratio of carbon to nitrogen present in the raw material. The optimum range is 25–30:1 and at C/N ratio above 30, nitrogen must be added in the form of nitrogenous fertilizers such as urea or ammonium sulphate. In different states, forest weeds (*Chromolaena odorata*, *Lantana camera*, *Andropogon sp.Combretum* sp.,), wheat straw, coir pith, water weeds, and tea and coffee waste are used for composting. Usually, Berkeley's method is followed for making the compost. Depending upon the state, a slight modification of the process is being made in accordance with the availability of

the compostable organic materials. Usually, the succulent shoots of desirable forest weeds are cut manually and transported to the chopping site where a manually-operated chopper machine equipped with double blades chops the materials to a size of 5–10 mm. Prior to placing the chopped material in the compost shed, 50% water by weight of total weight of the mixed material is added, to give a homogenous mass and to expedite microbial activity. Then the mixture is placed in the compost shed layer by layer and sprinkled with water. After a week when the temperature of the heap reaches about 55 °C, the heap is turned over and thoroughly mixed. In no case is the temperature allowed to exceed 60 °C as this would result in death of the microorganisms. Length of the period for completing the process depends on the plant species and the organic matter used. However, usually for forest weeds, within 30 to 45 days composting will be completed. The final product is dark brown and feels greasy and smells earthy. Depending on the tree species potting media are prepared by mixing the compost with soil and sand in different proportions.

## Impact on seedling health

Introduction of root trainer growing practices and technological changes in producing planting stock has had a major impact on nursery management. As soil less or soil free growing media are used in root trainers, common soil-borne diseases like damping-off, seedling blight, and wilt seldom occur. However, the conventional nursery system which caters to the larger part of the requirement of planting stock, still suffers severely from the diseases. Rhizoctonia solani and Cylindrocladium quinqueseptatum, the major pathogens, pose threats to the seedbed nurseries. Under the conducive tropical climate, maintaining the nursery crop for a longer period is one of the most serious problems confronting nursery managers. During this period, diseases and pest problems occur in succession and if timely intervention is not done the entire seedling crop may be devastated by one or other diseases and pest attacks. However, in root trainers, seedling crops require a maximum period of 90 days of growth and hence rigorous management is possible. In conventional nurseries, seedlings have to be maintained in the seedbeds or in polythene containers at least for 6 months. During this period, diseases caused by different fungi and bacteria affect the seedling crop and often epidemic outbreak occurs (seedling blight caused by Cylindrocladium spp.) devastating the entire seedling crop. In root trainers, even if foliage disease occurs, the affected seedlings can be easily removed from the blocks and replaced with healthy seedlings, thereby avoiding the spread of the disease in the nursery. Similarly, seedlings showing poor or stunted growth or deformity can be easily replaced or corrected by application of appropriate nutrients. Since, root trainer seedlings exhibit uniform growth performance, prophylactic pesticide treatment, if required, and maintenance of seedling quality are easier than in the conventional nursery system.

Under planting stock improvement programmes, disease resistant and fast-growing ramets can be produced on a large-scale employing the root trainer technology. Screening of efficient clones for disease resistance at the nursery level can also be performed very efficiently employing the new technology. The new technology is very efficient and suitable for planting stock improvement using mycorrhizal, biofertilizer and biopesticide manipulation, since the root trainer technology gives more emphasis to healthy root system of the seedlings. Recently, in eucalypt, root trainer nurseries, planting stock improvement using *Pisolithus tinctorius*, an ectomycorrhizal fungus, has been carried out (Mohanan 2002a). Application of a fungal spore slurry and mycelial and spore pellets was easy and handy in this system. In teak (*Tectona grandis*), planting stock improvement through arbuscular mycorrhizal (VAM) fungal manipulation using root trainer seedlings also showed promising results (Mohanan, 2002b) and the root trainer technology offers

quick assessment of mycorrhization of root system. As far as other nursery parameters like seedling density, shade over the nursery and water regime are concerned the system offers sufficient flexibility in operations. Seedling density can be controlled at the desired level at various seedling growth phases by emptying the cells in blocks in a non-destructive way. Similarly, seedlings of different tree species can be kept under shading of different intensity and duration. Control over water and nutrient regimes is also easy and handy in this system. Moreover, seedling health and quality can be assured by rigorous nursery management.

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# Seed health problems in tropical forest tree seeds and their impact on seedling production

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Recently, studies were undertaken to standardize the seed technology of tropical forestry seeds under the World Bank aided Forestry Projects and data were generated on seed technology of 88 broadleaved forestry species in the Kerala State. In the present paper, seed health problems in Tectona grandis, Albizia lebbeck and Dalbergia sissoides are dealt with. Sample trees were selected in different seed zones in the State and phenological data were collected. Seed crop assessment was carried out and seeds/fruits samples were collected during 1998–2001 and seed/fruit characteristics were studied. To overcome the seed coat dormancy and to enhance the seed germination potential, cold and hot water treatments, and acid (H2SO4) treatment were carried out. Seed microflora was assessed by employing standard techniques (ISTA) and identification of spermoplane microorganisms was made. Seedling growth, vigour and seedling diseases were studied in seedbed and root trainer nurseries. The causal agents from diseased seedlings were isolated and identified. Seed dressing fungicides, Hexathir, Hexacap and Carbendazim were screened for their efficacy under seed storage. Results showed that seeds of all the three forestry species harboured large number of fungi. These fungi play a role in causing seed rot and poor germination. Even though, storage molds like Aspergillus, Penicillium and Trichoderma were the predominant ones, field fungi like Colletotrichum, Bipolaris, Phomopsis and Phoma were also encountered. Treatments to break the seed coat dormancy in seeds of all the three tree species increased percent seedling emergence as well as reduced the spermoplane fungal flora and thereby the risk of rot caused by them. Seed treatment with fungicides was effective in reducing the spermoplane fungal flora.

# Introduction

Despite the advances in seed technology over the past several years, little effort has been made of them in India to improve the quality of tree seeds and thereby the planting stock. Teak (*Tectona grandis* L.), the prime forestry species in the country, has been raised extensively in plantation since 1850s. Germinability of teak seed is usually found to be low (<50%) which is largely attributed to emptiness (Troup 1921, Murty 1973, Ghosh 1977); the percentage of emptiness in teak fruits varies from area to area (Gupta and Kumar 1976). Although, several studies have been conducted, the factors responsible for low percent germination is not fully revealed. For the causes of poor seed germination, so far, studies have been directed towards seed maturity, seed dormancy, size, etc. (Dabral 1976, Gupta and Pattanath 1975, Joshi and Kelkar 1971), however, little attention has been paid to study the deterioration of seed quality by microorganisms.

With the increasing demand on indigenous species over exotics (eucalypts and acacias) to reforest the degraded areas, many lesser exploited tree species are being tried under various planting schemes. *Albizia lebbeck* (L.) Willd. and *Dalbergia sissoides* Grah. are among those species, however, information on their phenology, fruit/seed characteristics, seed health problems and seed pretreatment requirements is meager. The present study was taken up to investigate the seed health parameters and standardize technology to raise quality seedlings in nursery.

## **Materials and methods**

#### Selection of trees and collection of seeds

Stands of *Tectona grandis*, *Albizia lebbeck* and *Dalbergia sissoides* in various localities belonging to different agro-climatic zones in the State (Prasad and Kandya 1992) were selected for the study. Ten trees of respective species were selected in each stand, marked and observations on flowering, fruiting and seed maturation were recorded and seed crop assessment carried out. Seeds/fruits were collected during 1998 and 1999 seeding seasons from the selected stands of respective tree species. Seeds collected from individual trees in a locality were mixed together and composite samples made. Seed/fruit characteristics, pod size, seed size, number per pod and number of locules were studied. Seed samples were brought to the laboratory, extracted, purified and seed weight and moisture level were assessed. Seeds were sun or air dried to reduce the moisture content to 10–15% and stored. Composite samples of each tree species from each localities were stored separately in cloth bags at 25±2 °C.

Working sample from each composite sample was drawn and seeds were further categorized into different groups of apparently healthy, discoloured, deformed and biodeteriorated. Status of fungal and insect infestations was assessed by dry seed examination method using sterioscopic binoculars. The percentage of discoloured, poorly filled, shrunken and deteriorated seeds in each sample was assessed separately. Seed moisture content was measured by oven drying method. Weight of seeds from each category as well as from pooled sample was determined separately (ISTA 1985).

#### Seed pre-treatment

To overcome the seed coat dormancy and thereby enhancing the germination potential of seeds, various treatments were carried out. These include: i) cold water soak - soaking the seeds in water at room temperature for 24 hours; ii) hot water soak - soaking the seeds in boiling water and keeping them until the water cools down; iii) acid treatment - soaking the seeds in concentrated sulphuric acid for 5 to 20 min and washing thoroughly with water.

#### Seed health testing

Spermoplane microflora was assessed by employing standard blotter technique (ISTA 1985). Both sterilized and non-sterilized seeds were screened. Agar plate method (ISTA 1993) was employed to detect the seed borne fungus and bacteria. Working sample of 200 to 400 seeds of each species was drawn from each composite sample and tested. Seeds were plated at equal distance in sterile plastic dishes ( $\emptyset$  90–140 mm) lined with three moistened germination paper discs (blotter). Both surface sterilized and non-sterilized seeds were tested. Surface sterilization

was carried out with 0.01% mercuric chloride for 2 min and thoroughly washed with sterile water. The number of seeds incubated per Petri dish varied with the size of the seeds. The set ups were incubated at 25±2 °C in a Seed germinator fitted with fluorescent lights adjusted at 12 hr dark and light cycle. The incubated Petri dishes were removed from the Seed germinator after 12 days and observations on germination, microbial association and infection on emerging seedlings recorded. Potato dextrose agar medium was used for assessing the spermoplane microflora by agar plate method. Seeds surface sterilized with 0.01% mercuric chloride and then washed with sterile water were plated on PDA medium in petri dishes and incubated for 7 to 10 days. Microbial colonies developing from the seeds were isolated, purified and identified. Identification of spermoplane microorganism was attempted up to generic level or species level in certain cases and percent incidence of each microorganism was deduced.

To assess the effect of spermoplane microorganisms, some of which may also be seed-borne, on the germinability of seeds, growing on test was carried out. Steam sterilized perlite was used as the growing medium. Plastic trays ( $60 \times 30 \times 20$  cm) were filled with sterilized perlite and the seeds of respective tree species were sown, watered and maintained. Observations on seedlings emergence, incidence of disease on seedlings etc. were recorded up to 30 to 45 days of emergence. The diseased parts from the seedlings were plated aseptically on PDA medium and causal organism isolated and identified.

## Fungicidal seed dressing

Fungicides, thiride (Hexathir), bavistin (Carbendazim) and captan (Hexacap), were evaluated for their efficacy as seed dressing chemicals. Cleaned seeds of each species were treated with fungicides at the rate of 2 g/kg of seeds in polythene bags (18 x 12 cm) and stored for three weeks. The treated seeds were tested employing blotter method.

#### Results and discussion

#### Phenology and fruit/seed characteristics

#### Tectona grandis

Teak trees generally flowers during June to August-September and fruits ripen from November to January. However, early flowering from May to July was also observed in all the three localities in the State (Table 1). Fruits were available till March in Wayanad and Nilambur, while in Chinnar, fruits were available for collection during May also. Fruits characteristics vary from locality to

Table 1. Fruit characteristics of teak from different localities.

Locality	Seed sub- zone	Fruit witho Length	ut calyx, cm Breadth	Mean no. of locule/fruit	Empty locule/fruit	Sound seeds/fruit
Kuppadi	Wayanad	1.54	1.42	3.50	1.64	1.20
		(0.04)	(0.03)	(0.84)	(1.67)	(1.03)
Nedunkayam	Nilambur	1.24	0.94	3.40	1.60	0.70
•		(0.15)	(0.07)	(0.69)	(1.50)	(0.67)
Churulipetty	Chinnar	`1.13 <sup>′</sup>	`1.09 <sup>′</sup>	`3.81 <sup>′</sup>	2.52	`0.90
. ,		(0.01)	(0.09)	(0.40)	(1.34)	(0.94)

Figures in parenthesis are SE of mean value.

locality (Table 1). In teak seeds, number of locules varied from 2 to 4 and sound seeds varied from 0 to 2. Seed lot from Wayanad showed the highest number of sound seeds.

Teak fruits collected from Wayanad, Nilambur and Chinnar were severely infested by insects which made tunnels in the mesocarp and endocarp from the pedicel part of the fruit. Insect infestation ranged from 9 to 18.5 percent and the highest per cent insect attack was observed on seeds from Wayanad. Fungal hyphae, fructifications and sclerotia were also observed on the fruits. Discolouration and shriveling was associated with about 12.5% of the fruits from Chinnar. Results on extraction of seeds from teak fruits revealed that emptiness is very common in teak fruits from Chinnar (Table 1). Moreover, ill-filled and shriveled seeds were more in seed samples from Chinnar than from the other two localities. Seeds inside the locules were also found deteriorated and covered with fungal mycelium.

### Dalbergia sissoides

*D. sissoides* trees generally flowers during December to January and up to February in Dhoni and Wayanad. Seeds are available for collection in February. At Chinnar, mature fruits are available in May. Fruits characteristics showed variation in seedlots from different localities (Table 2). Seeds were found moderately affected with spermoplane microorganisms and insects. The seeds from each locality could be categorized into apparently healthy, discoloured, and deformed seeds. The percentage of seeds belonging to such categories was 78, 13 and 9 respectively in seedlot from Chinnar, 67, 21 and 12 respectively in seedlot from Dhoni, 74, 17 and 9 respectively in seedlot from Wayanad.

Table 2. Caharacteristics of seeds of D. sissoides.

Seed characteristics	Localities					
	Chinnar	Dhoni	Wayanad			
Length of pod (cm)	6.16	6.30	5.80			
Width of pod (cm)	1.42	1.68	1.78			
No. of locules per pod	3.50	4.40	5.00			
No. of seeds per pod	3.20	4.00	4.60			
% discoloured and deformed seeds	22.00	33.00	26.00			
Wt. of 100 seeds (g)	3.00	3.25	3.07			
% MC	16.66	15.00	20.00			

#### Albizia lebbeck

A. lebbeck trees flower mostly during January to March. But in Chinnar and Palakkad flowering occurs during August to September also. Pod collection period is from November to March. The fruit characteristics length and width of pod, number of locules per pod, and number of seeds per pod varied considerably (Table 3). Percent moisture content of seeds collected from different localities ranged from 10.62 to 12.15. A. lebbeck seeds collected from the three localities Chinnar, Kuzhalmannam and Palakkad were found severely affected with microorganisms. The percentage of discoloured and deformed seeds was very high and it ranged from 50 to 60 (Table 3). Severe infection of seeds inside the pod as well as germination of intact seeds were also noticed. The weight of 100 seeds from the pooled samples from the three localities ranged from 11.2 to 12.0 g. Dry seed examination revealed fungal mycelial mats, fructifications, as well as insect infestations. Comparatively deformed, shriveled and infested seeds were high in seedlot from Chinnar than those from other two localities.

Table 3. Pod/seed characteristics A. lebbeck.

Seed/fruit characteristic		Localities	
	Chinnar	Kuzhalmannam	Palakkad
Length of pod (cm)	19.76	23.75	21.72
Width of pod (cm)	3.79	4.53	4.08
No. of locules per pod	6.50	11.80	9.00
No. of seeds per pod	6.00	10.60	8.40
Length of seed (cm)	0.95	0.91	1.10
Width of seed (cm)	0.78	0.64	0.66
Thickness of seed (cm)	0.20	0.22	0.22
% discoloured and deformed seeds	50.00	52.00	60.00
Wt. of 100 seeds (g)	12.00	11.20	11.90
Wt. of 100 discoloured seeds (g)	5.79	9.50	9.80
% MC	12.12	11.00	19.62

#### Seed microflora

### Tectona grandis

A rich microflora comprising a total of 18 fungal genera, together with mycelia sterilia (black and white), bacteria and actinomycetes was detected on seeds of *T. grandis* from different localities. Of these seed lot from Wayanad harboured more number of microorganisms and showed their highest frequency of occurrence (Table 4). The most frequent fungal genera were *Aspergillus*, *Botryodiplodia*, *Fusarium* and *Trichoderma*. Though, bacteria were found in all the three seed lots tested, their highest frequency (33%) was observed in seed lot from Wayanad. As expected, number of microorganisms and their percent incidence were higher in non-surface sterilized seeds (NSS) than surface sterilized (SS), and acid pre-treated (A) seeds. By surface sterilization, most spermoplane microflora except bacteria was excluded. Sulfuric acid treatment was also equally

Table 4. Spermoplane microorganisms detected on *T. grandis* (Wayanad seedlot).

SI. No.	Microorganism	Blotter me	ethod, % ind	cidence	Agar plate method
	•	NSS	SS	Α	% incidence
1	Alternaria alternata	2			2
2	Aspergillus spp.	1			
3	Aspergillus niger	16	1	4	4
4	Botryodiplodia theobromae	2			4
5	Chaetomium sp.	3			
6	Colletotrichum gloeosporioides	6			
7	Curvularia sp.				4
8	Drechslera sp.	1			
9	<i>Fusarium</i> sp.	20			12
10	Mucor sp.			14	
11	Paecilomyces sp.	8			
12	Penicillium sp.	7			
13	Pestalotia sp.	4			
14	Phoma sp. ·	5	4		
15	Trichoderma sp.	12	4	4	
16	Verticillium sp.	3			
17	Sterile mycelium (black)		8	8	2
18	Sterile mycelium (white)	2			
19	Bacteria	33	10	6	8
20	Actinomycetes	1			

NSS = non-surface sterilized; SS = surface sterilized; A = sulphuric acid treatment.

effective in reducing the seed microflora substantially and increasing the percent germinability of the seeds. Emerging seedlings in these treatments were found very healthy and showed any sign of seedling infection from storage fungi.

Seed health test by agar plate method, where extracted seeds were used, revealed association of field fungi like *Botryodiplodia theobromae* Pat., *Fusarium moniliforme* J. Sheld., *Curvularia lunata* (Wakker) Boedijn, *Phoma* sp., *Colletotrichum gloeosporioides* (Penz. & Sacc.) C., etc. in all the three seed lots tested. Of these, many are capable of causing seedling rot and foliage infection. The high level of infestation of the teak seeds by these field fungi clearly indicates the possibility of infection by the pathogen during the early developmental phase of the fruits.

#### Dalbergia sissoides

A rich microflora comprising of 16 fungal genera, together with mycelia sterilia, bacteria and actinomycetes were detected on seeds of *D. sissoides* collected from different localities (Table 5).

Table 5. Spermoplane microorganisms detected on seeds of *D. sissoides* (Chinnar 1998 seedlot).

SI. No.	Microorganism		Blotter me	thod, % inc	idence		Agar plate
	Ç	NSS	SS	HW	CW	Α	method % incidence
1	Alternaria sp.						8
2	Aspergillus sp.	12					
3	Aspergillus niger	16	10	6	8	10	18
4	Chaetomium sp.	3					
5	Curvularia sp.						8
6	Fusarium sp.	3	6				14
7	Paecilomyces sp.	1					
8	Penicillium sp.	6					
9	Trichoderma sp.	3	2	4	8		8
10	<i>Verticillium</i> sp.	1					
11	Sterile mycelium (black)			14			14
12	Bacteria	9	16	8	8	8	22
13	Actinomycetes				6	4	4

NSS = non-surface sterilized; SS = surface sterilized; HW = hot water treatment; CW = cold water treatment; A = sulphuric acid treatment.

Most microorganisms were encountered on non-surface sterilized seeds in blotter tests. Among the seedlots, those from Wayanad and Dhoni harboured more number of spermoplane microbes than the seeds from Chinnar. The spermoplane microbes detected include the common storage fungi, field fungi, bacteria and actinomycetes. Among the storage fungi, *Aspergillus* spp., *Penicilium* spp. and *Chaetomium* sp. were the predominant ones. The incidence of these storage fungi ranged from 38 to 69%. However, the occurrence of field fungi like *Alternaria* sp., *Curvularia* sp., *Drechslera* sp., *Bipolaris* sp., *Fusarium* spp., *Pestalotia* sp., *Phoma* sp., etc. in all the three lots ranged from 4–41%. Seeds from Wayanad and Dhoni were found infested with large number of field fungi and their percentage was 41 and 34 respectively. While seeds from Chinnar recorded only a few field fungi and their per cent incidence was less than five (Table 5). As expected, the fungal genera, their frequency of occurrence as well as intensity of infestation were more in nonsurface sterilized seeds from all the three localities. Surface sterilization of the seeds with 0.01% mercuric chloride considerably reduced the occurrence of the fungal genera to 1–3 and also the percent incidence to 6–10. Even though, bacteria were detected on seeds from all the three localities, their incidence was substantially reduced by surface sterilization and also by other seed pre-

treatments. Cold water, hot water, and acid treatments carried out to enhance the seed germinability, were also effective in excluding most of the spermoplane microflora. Seed health test employing agar plate method could detect a large number of field fungi like *C. gloeosporioides, Curvularia* sp., *Fusarium pallidoroseum* (Cooke) Sacc., *Phoma* sp., *Pestalotia* sp., etc. Of these *C. gloeosporioides* was encountered in high frequency on seeds from Wayanad (25%) and Dhoni (10%). The percent incidence of *Fusarium* (mostly *F. pallidorosum*) was also ranged from 6–18 in all the seedlots. Many of these field fungi are potential pathogens of *Dalbergia sissoides* seedlings in nurseries. The results indicate that at least a few of these fungi which are possibly seed-borne may play a role in deterioration of seeds in storage as well as incidence of seedling diseases in nurseries.

#### Albizia lebbeck

A rich microflora comprising of 19 fungal genera, together with unidentified mycelia sterilia, bacteria and actinomycetes were encountered on seeds of *A. lebbeck* collected from the three different agroclimatic sub-zones of the State (Table 6). Seed health test by blotter method revealed

Table 6. Microorganisms detected on seeds of A. lebbeck (Palakkad 1998 seedlot).

SI. No.	Microorganisms	Blo	otter method	, % incidenc	е	
	<u> </u>	NSS	SS	HW	Α	
1	Aspergillus spp.	10.0	5.0	13.0	7.0	
2	Aspergillus niger	0.5				
3	Beltrania sp.	8.0	8.0	12.0		
4	Chaetomium sp.	2.0	4.0	1.0	3.0	
5	Colletotrichum gloeosporioides	0.5				
6	Fusarium sp.	1.0	4.0			
7	Paecilomyces sp.	0.8		1.0		
8	Penicillium sp.	16.0	3.0	6.0		
9	Rhizopus sp.	2.3	2.0			
10	<i>Trichoderma</i> sp.		2.0	2.5	3.0	
11	Sterile mycelium (black)	0.5	1.5	1.0		
12	Sterile mycelium (white)	1.0	0.5		2.0	
13	Bacteria	17.0	11.0	32.0	4.0	
14	Actinomycetes	5.5	1.0		3.0	

NSS = non-surface sterilized; SS = surface sterilized; HW = hot water treatment; A = sulphuric acid treatment.

a large number of spermoplane microbes on non-surface sterilized seeds. Among the seedlots tested, those from Palakkad recorded more number of spermoplane microbes which include common storage moulds, field fungi, bacteria and actinomycetes. Among the storage moulds, Aspergillus spp., Chaetomium sp., Rhizopus sp., Penicillium sp., etc. were the predominant fungi. Their frequency of occurrence ranged from 21–48%. Among the field fungi recorded on seeds, Beltrania sp., C. gloeosporioides, Fusarium sp. and Phoma sp. are the important ones and their per cent incidence in the seed samples from the three localities ranged from 7 to 21. Seeds from Chinnar recorded the least number and per cent incidence of field fungi, while the seeds from Palakkad yielded more number of field fungi as well as their per cent incidence. Incidence of bacteria in seeds ranged from 7 to 19% in non-surface sterilized seed samples. Bacteria were found mostly associated with the discoloured and deformed seeds and such seeds become completely rotten with heavy bacterial ooze. Though surface sterilization with 0.01% mercuric chloride reduced the per cent incidence of the bacteria, both hot water and acid treatment increased the per cent incidence. A high per cent (32%) incidence of bacteria was recorded in hot water treatment of seedlots from Chinnar and Kuzhalmannam. However, in general, hot water and acid

treatments to break the seed dormancy and to enhance the seed germination, also reduced the number of spermoplane microflora and their intensity.

### **Growing-on test**

*T. grandis*: Seedling emergence started four to six days after sowing in the sterile perlite medium and continued up to 21 days. However, a large number of them emerged within 8 to 12 days of sowing. Percentage germination was slightly higher (51%) than that obtained in blotters. Seedling infection, damping-off, collar rot and cotyledon rot were observed on the emergents and isolations made from the diseased specimens yielded *F. moniliforme*, *F. oxysporum* Schlect. and *C. lunata*.

*D. sissoides*: Seedling emergence started 5 to 7 days after sowing in the sterile perlite medium and continued up to 16 days. Most seedlings emerged within 10 to 12 days of sowing. Percentage germination (56%) was found lower than that obtained by blotter method. Seedling infections, collar rot caused by *Fusarium* sp. and leaf spot caused by *Colletotrichum gloeosporioides* were recorded.

A. lebbeck: Seeds from Palakkad were used for the growing-on test. Emergence of seedlings started 4 to 5 days after sowing in sterile perlite medium and continued up to 9 days. Most seedlings emerged within 5 to 7 days of sowing. Percentage germination (73%) was found lower than that obtained by blotter method. Seedling infection, cotyledon infection (bacterial), rot of radicle and plumule (bacterial) and leaf spot caused by Fusarium sp. and Colletotrichum gloeosporioides were observed.

#### Seed pre-treatment

T. grandis: The percent germination of seeds from different localities, ranged from 36 to 48 in blotter test. Soaking the seeds in concentrated sulphuric acid for 20 min gave higher percent germination (Table 7); highest germination of 78% was obtained for seed lot from Nilambur, followed by seed lot from Chinnar (74%).

Table 7. Effect of seed pre-treatments on percent germination of *T. grandis* seeds.

Percent seed germination						
Locality	Zone	Sub zone	NSS	SS	Α	
Wayanad	KL3	С	46	40	64	
Chinnar	KL2	b	48	34	74	
Nilambur	KL3	С	39	36	78	

 $NSS = non \ surface \ sterilized; \ SS = surface \ sterilized; \ A = sulphuric \ acid \ treatment.$ 

*D. sissoides:* Pre-treatments of seeds, soaking in cold water for 24 hr dipping in hot water and then soaking in cold water for 24 hr were equally effective in increasing the germinability of seeds of *D. sissoides* (Table 8). Highest per cent germination of 96 was observed in cold water treatment of seeds from Dhoni.

Table 8. Effect of various seed pre-treatments on percent germination of *D. sissoides* seeds.

Locality	Zone	Subzone		Per cent g	ermination	
			NSS	SS	HW	CW
Dhoni	KL3	b	38	47	82	96
Chinnar	KL2	b	4	14	20	38
Wayanad	KL3	С	54	64	94	80

NSS = non-surface sterilized; SS = surface sterilized; HW = hot water treatment; CW = cold water treatment.

A. lebbeck: Seed pre-treatments, hot water soaking and conc. sulphuric acid treatment (5 min) were equally effective (Table 9). In hotwater treatment, seedlot from Palakkad gave higher per cent germination (92), while seedlot from Kuzhalmannam recorded only 60% germination. Seedlots from all the three localities gave a high percent germination which ranged from 94–95%. The results indicate that acid treatment has to be carried out to get a maximum per cent germination.

Table 9. Effect of seed pre-treatments on germination of seeds of A. lebbeck.

Locality	Zone	Sub zone	NSS	Α		
Kuzhalmannam	KL3	Palakkad	7.5	SS 34.5	60.0	94.0
Palakkad Chinnar	KL3 KL2	Palakkad Munnar	60.0 26.5	51.5 63.0	92.0 80.0	95.0 94.0

NSS = non-surface sterilized; SS = surface sterilized; HW = hot water treatment; A = acid treatment.

## **Discussion**

Most of the tropical trees exhibit irregular phenology depending on the local climatic conditions. Due to high variation in period of seed/fruit maturity in a particular tree species in different localities, collection of seeds from trees becomes difficult. Hence, seeds are usually collected from the forest floor after the seed fall which often get infected by decay organisms. In general, tropical seeds harbour rich microflora and thereby cause seed decay and affect the seed germination. Earlier, Mohanan and Sharma (1991) reviewed the status of seed pathology of 63 tropical and 13 temperate forest tree species in India. The present study shows that apart from common storage moulds, teak fruits harbour a few potential fungi like *Phoma* sp., *C. gloeosporioides* and *Fusarium* sp., which are also known to be seed-borne in various crops. Earlier, Cercospora sp. and Fusarium sp. have been recorded as possible seed-borne fungi in teak (Sharma and Mohanan 1997). Although, enclosed in stony endocarp, teak seeds are found to be invaded by fungi and caused shriveling and decay of seeds in the locules. Teak fruits infested by insects which made extensive tunnels in mesocarp and endocarp were found colonized by both field and storage fungi. The role of these fungi in seed deterioration, seed abortion and locule emptiness cannot be ruled out. Growing-on test using extracted seeds also revealed infection on emerging seedlings by fungi like Fusarium spp. and C. lunata which were also recorded in blotter and agar tests. The poor germinability of teak seeds is usually attributed to seed dormancy due to stony impermeable endocarp (Unnikrishnan

and Rajeev 1990). The physiological conditions in the form of nutrient imbalance has also been reported as an important factor for low germinability (Gupta and Pattanath 1975). The present study confirms the results of Murty (1973) and Ghosh (1977) on seed emptiness as one of the major factors for the low germinabilty. However, the present study reveals that locule emptiness due to seed abortion, possibly be caused by seed-borne fungi, rather than the unknown sterility factor operating in Verbanaceae family, may be the leading factor for low germinability of teak seeds.

So far, no information is available on spermoplane microflora as well as on seed technology of *D. sissoides*. Recently, Chacko and Mohanan (2002) have generated data on seed health and seed technology of this indigenous species. The percent study also shows incidence of many field fungi on seeds and a few of these potential fungi may possibly play a role in deterioration of seeds in storage as well as incidence of seedling diseases in nurseries. To safe guard the seeds against spermoplane microflora, and also to check the seedling diseases in nursery, seed dressing with fungicide like thiride or captan 2g/kg of seeds is desirable.

From A. lebbeck seeds only a few species of Aspergillus, Penicillium, Fusarium and Rhizopus have earlier been reported (Mittal and Sharma 1979, Tiwari and Sharma 1981). In the present study, seed lots from different localities revealed many spermoplane organisms including field fungi like Drechslera sp., Phoma, sp., F. solani, C. gloeosporioides, etc. C. gloeosporioides and Fusarium sp. are suspected to be seed-borne as both the fungi caused seedling infection in nurseries. However, seed rot caused by bacteria (Pseudomonas sp.) was the major seed health problem encountered in most of the seed lots. The bacterial infection occurs on pods and spread to the developing seeds. Rainfall during the seed maturation period may possibly enhance the bacterial rot. As both hot water and acid treatments enhanced the per cent seed germination, any of the seed pre-treatment can be practiced. Since, A. lebbeck seeds are available in plenty, long-term seed storage is not required, however, seed dressing with fungicide like thiride or captan 2g/kg of seeds can reduce the spermoplane microflora and thereby the possible seed decay.

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