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EPIDEMIOLOGY AND CONTROL OF DISEASES OF EUCALYPTUS CAUSED BY CYLINDROCLADIUM SPP. IN KERALA

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Abstract

Extensive survey of nurseries and plantations throughout Kerala State revealed nine species of *Cylindrocladium* associated with diseases of *Eucalyptus* spp. Cylindrocladium leaf blight (CLB) was the major disease affecting all growth stages of eucalypts and *C. quinqueseptatum* was the dominant species. Other species in the order of importance were *C. ilicicola, C. theae, C. clavatum, C. camelliae, C. floridanum, C. parvum, C. curvatum* and *C. scoparium.* C. *quinqueseptatum* was present throughout Kerala, however, other species had discernibly restricted distribution; *c. theae* and C. ilicicola were localised in high elevation areas of the State.

in vitro germination of conidia of *C. quinqueseptatum* began after 4.5 h of incubation and attained about 95% within 8 h; germination was optimal at 25°C. Conidial germination on the intact leaves of 2-month-old *E, grandis* occurred after 3 h of incubation. Formation of appressorium over the epidermal cells was recorded first on the abaxial surface of leaves at 6 h and later on the adaxial surface. Formation of appressorium over the stomata was found only very rarely and most leaf penetrations occurred directly through epidermal cells.

Rainfall influenced considerably in increasing the severity of CLB which was further intensified when an intercrop (taungya) of tapioca (Manihot *utilissima* Phol.) was cultivated in a 2-yr-old plantation of *Eucalyptus tereticornis.* High severity of the disease was positively correlated with high rainfall; high relative humidity during drier months had little impact on disease severity.

Susceptibility of 36 provenances belonging to 16 species of *Eucalyptus* to CLB caused by *Cylindrocladium quinqueseptatum, C. clavatum* and *C. ilicicola* differed significantly in detached leaf inoculations. Various provenances of an eucalypt species also showed significant differences among themselves. Generally, susceptibility ratings of a provenance to different species of *Cylindrocladium* showed significant differences; there were only a few provenances which gave either equally resistant or susceptible reactions to all the three species. *C. clavatum* proved to be the most virulent species, *C. ilicicola* the least and C. *quinqueseptatum* intermediate.

Seventy stock cultures of C. *quinqueseptatum* (CQ) were distinguishable into 10 groups based on cultural characters; each group of cultures showed significant differences in cultural characters on nine growth media used. Though potato dextrose agar (PDA) and yeast malt agar (YMA) were the best media for excellent growth, sporulation and microsclerotia (MS) production, malt extract agar (MEA) and YHA were the best in showing distinct differences in cultural characteristics between the isolates. Growth rate of isolates was not very useful in discerning variability among the isolates. Utilisation of carbon (C) and nitrogen (N) sources by selected five CQ isolates varied considerably as evident from mycelial growth and MS production in liquid culture media. The differential behaviour of CQ isolates in culture indicated that possibly they are different strains.

A wide range of pathogenic variability was observed among the above five CQ isolates, which were confirmed as different physiologic strains on seven differential provenances of *Eucalyptus* belonging to *E. tesselaris, E. saligna, E. brassiana, E. urophylla* and *E, grandis.* Susceptibility ranking of these provenances to the five isolates also differed significantly indicating differential interaction between isolates and provenances. Analysis of variance showed that CLB severity of a provenance was mainly governed by the genetic differences of the isolates and also that the provenances had closer genetical relationship. The results provide the first evidence for the existence of physiologic strains in C. *quinqueseptatum.*

A total of 22 fungicides were evaluated *in vitro* for their efficacy against *C. quinquereptatua* (CQ), C. *ilicicola* (CI), C. *floridanum* (CF), *C. parvum* (CP), and C. *camelliae* (CC). Though there were a number of fungicides effective (ED_{100} i.e., cent percent inhibition in conidial germination/diameter growth) in conidial germination and poisoned food techniques, only carbendazim provided complete inhibition of CQ, CI and CC in soil-fungicide screening technique; carbendazim was also highly effective against CF and CP. On comparison of three fungicidal evaluation techniques it was concluded that for a pathogen producing microsclerotia, like soil-fungicide screening technique is the most appropriate for obtaining reliable results.

in vitro studies indicated that PCNB was the most effective fungicide in controlling *Rhizoctonia* damping-off, when applied as pre-emergence treatment. Since damping-off was significantly affected by seed rate and moisture regime, the study suggested that these two parameters need to be standardised for eucalypt nursery so as to keep incidence and severity of daaping-off under check.

Three-year nursery trials conducted at Chandanathode (Wynad) to test the efficacy of fungicides (singly or in combination), their dosage and time of application revealed that for controlling all the seedling diseases of eucalypts at least three applications of fungicides are required. First application of MEMC, mancozeb and carbendazia, given as pre-emergence seedbed drench, controlled daaping-off, web blight and seedling blight disease. It was followed by second and third applications 'of carbendazim, prior to pricking out into containers and planting out in the field respectively; these two treatments effectively controlled CLB in the container nursery and field.

Nursery practices influenced incidence and severity of diseases (viz. dasping-off caused by *Pythium* sp. ; *Rhizoctonia solani* and *C. quinqueseptatum;* web blight by *R. solani;* seedling blight by C. *quinqueseptatum,* and seedling wilt by *Sclerotium rolfsii)* and growth of seedlings of *E. grandis;* to a certain extent microclimatic conditions were also affected by nursery practices. Shading with coconut leaf thatch led to low light intensity (av. 1,463 lux), high soil water potential, low soil and ambient teeperatures, severe damping-off and web blight diseases and poor shoot : root ratio of seedlings. Seedbeds under coirmat had dispersed light (av. 22, 299 lux), high severity of seedling blight and shoot wilt, and good growth (Shoot:root ratio) of seedlings. In both types of shading, high soil moisture regime and high seed rate contributed to high disease severity as well as low shoot: root ratio of seedlings. Different seed rates (2.8, 5.6 and 7.0g m^{-2} affected significantly the seedling density as well as availability of prickable seedlings of *E. grandis* from the seedbed nursery. Percentage of prickable seedlings in respect of seedling density decreased as the seed rate increased, pattern of number of seedlings available at different time period being the same. Therefore, as a part of the sound nursery management practices the

seed rate for raising the nursery economically and ensuring healthy and disease-free nursery stock should be determined based on the seed viability.

All the fungicidal treatments controlled effectively the CLB in direct-sown and transplanted seedlings. After 21 months of planting there was no significant difference in height growth of direct-sown and transplanted seedlings. However, percentage survival was higher in transplanted seedlings as compared to direct-sown seedlings. Direct sowing technique (small containers) may be feasible in large-scale planting programme provided adequate protection is given to seedlings against weeds and cattle damage to circumvent low survival of seedlings.

1. General introduction

It is now more than two decades since the large-scale planting of eucalypts commenced primarily to meet the raw material demand of paper and pulp industries in Kerala. The area under eucalypts increased steadily since 1960s and at present the State has about 40,000 ha of eucalypt plantations raised by the Kerala Forest Department and Kerala Forest Development Corporation. *Eucalyptus grandis* Hill ex Maiden is grown mainly in high elevation areas while *E. tereticornis* Sm.is restricted to low elevations and plains. Except in a few localities, the performance of eucalypts in Kerala is far from satisfactory (Chand Basha, 1986). Diseases appear to have contributed substantially towards the low yield, especially in low elevation areas falling under high rainfall zones. Cylindrocladiua leaf blight (CLB) caused by C. *quinqueseptatum* Boedijn & Reitsma was the first serious disease to be recorded by Sehgal *et al.* (1969)which affected seedlings in nurseries and plantations. Subsequently, another serious disease namely pink disease caused by *Corticium salmonicolor* Berk. and Br., came to the forefront (Seth *et al.*, 1978).By the end of 1970s both the diseases had spread in epiphytotic scale throughout the State affecting eucalypts significantly; the disease pressure was especially high in high rainfall areas. The pink disease caused stem cankes in 2 to 4 years old trees of *E. tereticornis* resulting in significant loss in height growth. CLB affected the seedlings in the nursery and coppice shoots, foliage and young branches of *E. grandis* and *E. tereticornis* up to *3-5* years in plantations. Thus, CLB emerged as one of the most serious diseases of *Eucalyptus* in Kerala, required immediate attention as it affected both the species at all the growth stages.

When large-scale planting of eucalypts began during the early 1960s, apparently there was not much problem posed by CLB. However, within a few years CLB became a serious problem in raising healthy nurseries, accounting for up to almost 100 percent seedling mortality in seedbeds and containers in high rainfall areas during the monsoon (June - September). C. *quinqueseptatum,* causing foliar diseases of *Eugenia caryophyllata* (Sprengel) Bullock et Harrison, *Anacardium occidentale* L., *Acacia auriculiformis* A. Cunn. ex. Benth., *Hevea*

brasiliensis Mull. Arg, and *Terminalia paniculata* Roth. (Sarma and Nambiar, 1978; Sharma *et al.,* 1979, Nair and Jaysree, 1986; Sharma and flohanan, 1982; Mohanan and Sharma, 1982, 1984, 1985a, b, 1986, 1988) in Kerala, adopted the susceptible eucalypts and within a few years caused epiphytotic of CLB after the initial inoculum buildup. This way, C. *quinqueseptatum* which is a pathogen of minor importance in Australia (Bolland *et al.,* 1985) has become the major pathogen posing serious threat to eucalypt plantation programme in Kerala.

Besides CLB, there were other diseses too which caused extensive seedling mortality, and a clear picture of a disease complex affecting eucalypt seedlings in the nursery has emerged recently from the studies of Sharma *et al.* (1985). They found that *Cylindrocladium* spp. together with *Rhizoctonia solani* Kuhn. state of *Thanatephorus cucumeris* (Frank.) Donk, *Pythium* spp. and *Sclerotium rolfsii* sacc. cause a disease complex at different growth stages of seedlings. As these diseases relate to a particular growth phase of seedlings, they appear in a chronological succession causing mortality at every stage of seedling growth; the extent of damage caused by these diseases usually depend upon the prevailing microclimatic conditions and the nursery management practices. These pathogens, which may not be as serious as *Cylindrocladium,* have the potential to cause considerable mortality of seedlings in the nursery. Hence, in any disease control strategy, these nursery pathogens, which are part of the disease complex, cannot be ignored as controlling *Cylindrocladium* alone may not have a positive effect in the nursery management . Uith this in view, along with *Cylindrocladium* all other pathogens of the disease complex were also included in this study though not part of the original project proposal.

Considering the magnitude of CLB, its control is necessary to provide healthy seedlings for the afforestation programmes in the State. But there is a large gap in information on various aspects of CLB and unless this gap is filled any attempt to control CLB will be futile. The purpose of this project was to generate information on host x pathogen x environment for Eucalyptus - Cylindrocladium system which will be directly useful in controlling the CLB successfully. For adopting strategies for the control of CLB in nursery and plantations a clear understanding of the epidemiology of the disease is a

prerequisite. Except for some preliminary epidemiological studies conducted on CLB of *E.microcorys* F. Mull. in Australia by Bolland *et. al.* (1985), no detailed information is available on this aspect. Varied types of symptoms of CLB observed in seedlings, saplings and mature trees of different eucalypt species in various parts of Kerala possibly indicate the association of more than one species of *Cylindrocladium.* In this situation knowledge is necessary not only on various species of *Cylindrocladiurn* involved but also on their geographic distribution.

The most common method of controlling fungal diseases like CLB in forest nursery is by chemicals. There are numerous examples to show that fungal diseases can be effectively and economically controlled by fungicides. For a chemical control strategy to be successful, especially in a forest nursery where the seedlings are intensively managed, behaviour of the pathogen on the host - the infection process, the factors responsible for infection and subsequently its spread and variation in virulence should be clearly understood. This helps in applying the suitable chemicals at appropriate time to gain the maximum benefit from the chemicals. To be more effective, the strategy of chemical control should form a part of nursery management practices. Since nursery management practices have a direct bearing on the heaith of seedlings, occurrence of diseases and subsequent damage caused to the seedlings reflect to a great extent how good or bad are the nursery practices. In view of the fact that nursery practices, especially the seed rate, watering schedule, etc. for raising eucalypt seedlings are found to vary greatly and large-scale mortality of seedling has been recorded, there is a need to standardize the nursery practices to suit different climatic zones (high and low rainfall regions) in Kerala.

In plantations, where CLB causes extensive premature defoliation and die-back of shoots during the initial 4-5 years of establishment the most appropriate disease control strategy has to be of introducing disease resistance by way of planting eucalypt provenances/species resistant to CLB rather than chemical control which will be not only impractical but also prohibitive. This approach requires the knowledge of degree of resistance available in various species/provenances, which can be exploited against CLB through selection or breeding.

Since, earlier studies of Sharma *et al.* (1987)have shown occurrence of resistance in eucalypts for the pink disease, there are possibilities of resistance to various *Cylindrocladium* spp. too. For this a large number of eucalypt provenances need to be screened against the existing pathogen papulation of *Cylindrocladium* spp., which may possess genetical variability as being composed of even physiologic races. Besides the host resistance, appropriate cultural practices to be followed during the establishment of a plantation need to be investigated to provide significant protection against CLB.

In this report materials and methods, results and related discussion pertaining to studies on the above aspects of Cylindrocladium leaf blight are presented in separate chapters. At the end, general discussion analyses the results on the prospects of bringing about control of CLB in order to improve productivity in eucalypt plantations in Kerala.

2. Cylindrocladium spp. Associated with Various Diseases of Eucalyptus in Kerala

A preliminary survey conducted during 1979 indicated that Cylindrocladium leaf blight was responsible for serious losses in eucalypt nurseries in Kerala, Since *Cylindrocladium* was found to be associated with a variety of diseases affecting different plant parts in eucalypts of varying maturity, occurrence of more than one species was suspected. To ascertain this, an extensive survey in 70 nurseries and 30 plantations of *E. grandis* (Hills) Maid. and *E. tereticornis* Sm. and various research plots of *E. alba* Blum., and *E. globulus* Lbill was carried out during 1979-1982.

MATERIALS AND METHODS

A total of 70 *Eucalyptus* nurseries of *E. grandis* (Eg), *E. tereticornis* (Et) and *E. globufus* (Eg) raised by the Forest Department in various localities of Kerala were visited between December and Hay during 1979-1982 and symptoms and damage caused by *CyIindrocladium* infection at different stages of growth were recorded.

Thirty plantations of three species of *Eucalyptus (E .grandis, E. teretfcornis* and *E. globulus),* selected in different geographical and climatic areas of the State, were surveyed for *Cylindrocladium* infection during dry (December-April) and wet (June, October) seasons and symptoms of diseases recorded; research plots of *E. alba, E. citriodora, E. camaldulensis,* and *E. torelliana* located in Vazhachal and Kottappara were also surveyed. Disease specimens collected from various nurseries and plantations were transported to laboratory in polythene bags for isolating the pathogen. All the isolations were carried out on potato dextrose agar (PDA) medium and identification of species attempted from cultural and morphological characters. For authentic identification, the type cultures were referred to CAB International Mycological Institute, England.

Post-eaergence daaping-off

Typical damping-off of young seedlings 17 to 20 days old) was the first disease to appear which caused considerable damage in many nurseries surveyed. The disease usually occurred roughly in circular patches and spread rapidly under high soil moisture due to excessive watering of seedbeds.

Seedling blight

Seedlings, 1 to 2 month old, of ***E. grandis*** and ***E. tereticornis*** were equally susceptible to blight disease; the disease caused upto 70% mortality of seedlings. Infection of stem near the ground level by ***Cylindrocladium*** usually resulted in typical seedling blight. Profuse mycelial and conidial growth were frequently observed on the dead tissues of the seedlings. More than one species of ***Cylindrocladium*** were found to be associated with this disease.

Seedling stem infection

It was observed frequently during March-Nay in seedbeds as well as in container seedlings; the disease was usually associated with excess watering and dark-thick shade over the seedlings. The infection, occurred at any part on the lower half of the stem, was characterised by white powdery mass of conidia, The affected seedlings, which primarily showed physiological wilting, eventually died.

Die-back of twigs and branch

The twig infection was observed in coppice shoots, and branches of young and mature trees during the peak of monsoon (July/August). The disease, found to be very severe in high ranges (munnar, Vallakadavu and Pamba areas), killed upto 75% of the twigs, including the main shoot. Within a month new shoots developed from the live tissues. The infection appeared somewhere on the twig and caused a canker characterised by a slight depression on the stem, where ***Cylindrocladium*** was frequently found to produce profuse mycelium and

conidial mass during high humid periods. The portion of twig above the infection was killed outrightly. Occasionally, tip blight where the young growing bud and some immature leaves near the apex got infected, was also responsible for causing die-back of shoots.

Cylindrocladium leaf blight

This was the most serious disease prevalent both in nurseries and plantations (coppice shoots as well as young trees, 1-to 5-year-old) affecting growth of plants. Severe infection of *E. tereticornis* was recorded at Taliparamba, Tamarassery, Nelliampathy, Kottappara, Vazhachal, Kothamangalam, Punalur and Thenmala while of *E. grandis* at Vazhal, Munnar, Idukki, Val lakadavu, Uppupara, Pamba and Attappara. The disease caused extensive to complete premature defoliation accompanied by die-back of tender shoots during the peak period of monsoon (July/August), Defoliated twigs generally developed new shoots within one month. The initial symptom was appearance of minute greyish-black water-soaked lesions on the leaves of any maturity. Later, these lesions coalesced to form larger necrotic areas, which on drying turned brown giving typical blighted appearance. In high humid areas, the initial symptoms observed on leaves of *E. grandis* and *E. tereticornis* were large greyish-black irregular spots, sometimes covering the entire leaf. Such heavy foliage infection caused premature defoliation.

Cylindrocladium species

A total of nine species of *Cylindrocladiun;* were identified from 92 isolations of which 49 were those of C. *quinqueseptatum,* 12 of *C. ilicicola,* including *Calonectria ilicicola,* 11 of *Cal. theae,* seven of *C. clavatum* five of *C. camelliae,* four of C. *parvum,* three of *C. floridanum* including *Cal.floridana,* and one each of *C. curvatum* and *C. scoparium.* In a number of instances, more than one species was recorded from the same specimen. C. *quinqueseptatum* was isolated from the specimens collected throughout Kerala, irrespective of host species of eucalypts or geographical location. However, other species had discernible spatial distribution with narrow host range. C.

ilicicola and *C.theae* were localised only in high ranger of Wynad and Pamba with the exception of Idukki and Munnar. The more frequent association of C. *ilicicola* and *C. theae* with *E. grandis* was possibly due to cultivation of this species in high elevation areas where these two *Cylindrocladium* species occur.

Nine species of *Cylindrocladium* were associated with various diseases of different eucalypt species as given below.

1. *C. quinquereptatum* Boidijn & Reitssa

   Hosts: *E. alba, E. citrfodora, E . camaldulensis, E. grandis, E. globulus, E. rostrata, E. tereticornis, E. torelliana*

   Diseases: Damping-off, seedling blight, root rot, stem infection, leaf and shoot blights, tip blight, die-back of twigs and branches.

   Distribution: widespread throughout Kerala.

2. *Calonectria ilicicola* and *Cylindrocladium ilicicola* (Hawley) Boedijn & Reitsaa

   Hosts : E. *teretfcornis, E. grandis*

   Diseases: Damping-off, seedling blight, leaf and shoot blights, stem canker, die-back of twigs and branches

   Distribution: Uidespread in high ranges of Kerala.

3. *Calonectria floridana* Sobers (including *Cylindrocladium floridanum* Sobers & Seymour)

   Hosts: *E. tereticornis* and *E. grandis.*

   Diseases: Damping-off, seedling blight, root infection.

   Distribution: Spatial; Peechi, Cheenkanipally, Chandanathode.

4. *Calonectria theae* Loos and *Cylindrocladium theae* (Petch) Alf & Sob,

   Host : *E. grandis*

   Diseases: Leaf blight,stem canker, die-back.

   Distribution: Uidespread in high ranges of Kerala.

5. *Cylindrocladium clavatum* Hodges & nay

   Hosts: *E. grandis* and *E. tereticornfs.*

   Diseases: Seedling blight, leaf blight, seedling stem infection, die-back of shoots.

   Distribution: Spatial; Kothamangalam, Pattikkad, Yadakkenchery, Pezhad

6, *Cylindrocladium camelliae* Venkataramani & Venkata Ram

Hosts: *E. grandis* and *E. tereticornis*

Diseases: Seedling blight, leaf spot, root rot.

Distribution: Spatial; Kunnathur (Thaliparamba), Chandanathode, Munnar.

7. *C. parvaum* Andreson

Hosts: *E. tereticornis, E. grandis* and *Eucalyptus* hybrids *(E. tereticornis X E. grandis* FR1/4, FRI/5.

Diseases: Daaping-off, seedling blight.

Distribution: Spatial; Tellicherry, Thaliparamba (Kunnathur), Uynad, Peechi.

8. C. *curvatum* Boedijn & Reitsaa

Host: E. *tereticornis*

Disease : Root rot

Distribution: Spatial; Cheenkanipalli.

9. *C. scoparium* Morgan

Hosts: *E. grandis* and *E. tereticornis.*

Diseases: Seedling blight, leaf blight.

Distribution: Spatial ; Peechi.

DISCUSSION

A total of nine species of *Cylindrocladium* were found associated with various eucalypt diseases in Kerala. Besides, Nair and Jayasree (1986) have reported one more species, C. *coihouni* Peerally from Kerala. The occurrence of C. *floridanm* (and its perfect state *Calonectria fioridanam), C. clavaturn, C. parvunt* and *C. theae* and its perfect state *Calonectria theae* on *Eucalyptus* are new records from India. Earlier, Sobers and Seymour (1967)have reported *C. floridanum* to cause leaf spots of *E. tereticornis* in U.S.A. The finding of C. *ilicicola* and its perfect stage *Calonectria ilicicola* is new to *E. tereticornis* and *E. grandis* in Kerala, although they have been reported to cause leaf spots of *E. globulus* Linn.in Karnataka (Reddy, 1973), die-back (Figueiredo and Cruz, 1963) and leaf spots (Alfenas *et al.,* 1979) of *Eucalyptus* spp. in Brazil. *C. quinqueseptatum,* reported earlier only on *E. grandis* and *E. tereticornis* (Bakshi *et al.,* 1972) was found to infect five more species of *Eucalyptus* viz. E. *alba, E.*

camaldulensis, E. citriodora, E. globulus and *E. torelliana.* C. *scoparium,* though isolated only once, is a new record for the State since it was recorded earlier only from Goa and Dehra Dun (Bakshi *et al.,* 1972). *C. camelliae* has earlier been reported to cause root rot of *Camellia sinensis* (L.) 0.Kuntze Venkataramani & Venkata Ram, 1961), *Myristica fragrans* Howtt (Rahman *et al.,* 1981) and leaf spots of *Visteria sinesis* (Sins) SU. (Reddy, 1975) in South India. C. *clavatum* causes root disease of *E. saligna* Sm., *Araucaria angustifolia* (Bert). *0.* Kuntze and several species of pines in Brazil (Hodges and May, 1972). *Calonectria theae* and its anamorph, *C. theae* are also the first report from India on *E. grandis.* The anamorph was initially described as *Cercosporella theae* Petch (Petch, 1917) and changed to *Cylindrocladium theae* by Alfieri *et al.,* (1972). Even though the teleomorph, *Calonectria theae* was reported earlier on dead tea leaves by Gaad (1929) as well as on artificial culture media by Subba Rao (1942), the full description of the fungus was only given by Loos (1949).

The occurrence of many *Cylindrocladium* species, some localised in a particular geographical area and their causing various diseases of eucalypts at all growth stages is suggestive of complex problems associated with control measures.

3. *In vitro* and *in vivo* Conidial Gemination of *Cylindrocladium quinqueseptatum*

For planning chemical control strategy of Cylindrocladium leaf blight (CLB)a, clear understanding of its epidemiology is essential. Except for some preliminary epidemiological studies on CLB of *E. microcorys F.* Muell. in Australia by Bol land *et a l.*(1985), no detailed information is available on conidial germination and infection process, Hence, detailed investigations were undertaken to study the *in vitro* and *i n vivo* conidial germination of C. *quinqueseptatum* in relation to some environmental and host factors.

MATERIALS AND METHODS

In vitro conidial germination

For obtaining optimum conidial germination two techniques viz. hanging drop and cavity slide were compared. Conidial suspension was prepared by pouring 10 ml of sterile water, containing one drop of Tween-20, on to a 12-day-old culture of C. *quinqueseptatum-947* in a 90 mm Petri dish, swirling around vigorously to dislodge mature conidia. This suspension was diluted further with sterile water to obtain three conidial concentrations viz. 1.5×10^{3} ml^{-1}, 2.0×10^{3} ml^{-1} and 3.0×10^{3}ml. The suspension was shaken gently before drawing it for drop placement. In the hanging drop technique, three drops, each 0.1 ml of conidial suspension, were placed on a clean and dry glass slide which was inverted upside down and placed over a v-shaped glass tube in a petri dish, with conidiai suspension drops in hanging position. The bottom and lid of the Petri dish were fitted with two wet filter papers to provide the high humidity required for conidial germination and for preventing the drops from drying up. In the cavity slide technique, 0.1 ml of conidial suspension was placed in both the cavities of a slide and the cavities covered with a large cover glass, the edges of which were sealed with petroleum jelly. For the hanging drop technique. there were two replicate Petri dishes while for the cavity slide technique there were three replicate slides for each concentration-temperature combination. Both the set-ups were kept at

20°C and 25°C separately in BOD incubators. These two temperatures were chosen in view of the fact that this represented the range of average minimum temperatures encountered in high ranges and plains respectively during the monsoon in Kerala, when high CLB infection was recorded.

A conidum was considered germinated when the length of germ tube was more than its width. For assessing conidial germination, 25 observations were recored from each set-up in both the techniques at 10 x magnification and mean calculated. For statistical analysis the data were transformed to angular transformation. A three-factor ANOVA was performed followed by cluster analysis (Calinski and Corsten, 1985) to determine the best temperature (T) x concentration (C) x germination technique (GT) for maximum conidial germination.

After having established in the above experiment that the hanging drop technique and 1.5 X $10^3 ml^{-1}$ conidial concentration were the best for assessing conidial germination of C. *quinqueseptatum* another experiment was conducted where effect of six different temperatures viz., 10, 15, 20, 25, 30, and 35°C on conidial germination was studied at incubation periods of 5, 6, 7, and 8 h. The slides were observed every 30 minutes but observation recorded only when the germination percentage reached ca. 20. From six replicate drops of each temperature x incubation period 30 observations were recorded at random at 10 x magnification and the mean calculated. After angular transformation the data were subjected to two-way ANOVA followed by cluster analysis to determine the best temperature x incubation period for optimum conidial germination. Data for 5 h were omitted in the analysis as the conidial germination was < 20%.

In vivo conidial germination and infection

Two-month-old seedlings of *E. grandis* of similar height and number of leaf pairs were utilised in the experiment. The leaves of the test seedlings were washed twice with sterile tap water to remove dust particles. After the leaf surface had dried, the seedlings were sprayed with sterile distilled water using a fine nozzle atomizer and the seedlings transferred to a humidity chamber maintained at 95% r.h. at 25 ± 2°C. After 12 h, these seedlings were inoculated with the

conidial suspension on adaxial and abaxial surfaces till run-off, using a fine spray atomizer. Conidial suspension was prepared as described earlier with the conidal concentration adjusted to 1.5 x $10^3 ml^{-1}$. *In vitro* viability of conidia was tested by the hanging drop method in the humidity chamber. Four young and mature leaves, two each for adaxial and abaxial surfaces, were collected from the inoculated seedlings after 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9 h of incubation, Leaves obtained from 4 to 6 h of incubation period were processed for scanning electron microscopy by freeze drying and gold coating under vacuum. These were examined using Hitachi S-540 scanning electron microscope for the formation of infection structures and penetration. Leaves obtained from 6 to 9 h of incubation were cleared in pyridine solution; two minutes for younger leaves and five minutes for mature leaves were found sufficient for clearing. The cleared leaves were stained and mounted in lactophenol cotton blue. Observations on germ tube growth, development of infection structures such as appressorium and penetration through stomata were recorded using Leitz Dialux - 20 microscope and photomicrographs taken with Leitz Orthomat Photomicrographic attachments.

RESULTS

In vitro conidial germination

Conidia usually produce a single germ tube each end cell but development of germ tubes from intercalary cells was also not uncommon and upto five germ tubes were recorded from a single conidium (Fig.3.1A,B,C,E). However, the growth of the terminal germ tubes was more rapid than those produced from the intercalary cells. If only one germ tube was produced either from the terminal ends or from the intercalary cells, the growth of the germ tube remained restricted. Within 30-45 minutes of germination, a thick walled septum was formed adjacent to the conidial wall. Initially, the germ tubes were composed of smaller rectangular cells with a pointed terminal cell but cells produced after 6 h were elongated. Elongation of the germ tube was rapid after 6h and branching occurred within an hour.

Statistical analysis showed that both the conidial germination technique (GT) gave similar result. Interaction T x GT was found to be highly significant ($P < 0.011$, possibly because of T which was highly significant in influencing the germination. Among the three conidial concentrations, germination differed significantly ($P < 0.01$), being the highest at the lowest concentration of 1.5×10^3 ml^{-1}. Cluster analysis indicated that the hanging drop method using the lowest conidial concentration at 25°C gave maximum conidial germination. Conversely, in the cavity slide technique maximum conidial germination was obtained at 20°C and not at 25°C (Table 3.1).

Table 3.1 Comparison of conidial germination of C. quinqueseptatum in hanging drop (HD) and cavity slide (CS) techniques at two tcmperatures (mean of 25 observations)

Conidia1 concentration ml^{-1}	% germination			
	20°C		25°C	
	HD	CS	HD	CS
1.5×10^3	90.16[a]*	94.54[a]	94.56a	92 10[a]
$2,0 \times 10^3$	84.39[b]	92.92[b]	92.13[b]	82.58[b]
3.0×10^3	68.75[c]	73.13[b]	84.25[b]	79.81[c]

* Values with different superscript in a column are statistically different

Temperature influenced the germination greatly as the conidia germinated only at 20, 25 and 30°C; and was not at, 10, 15 and 35°C (Table. 3.21. With increasing incubation period the percentage germination also increased; the percent conidial germination at 20, 25 and 30°C gradually increased from 5 h onwards and it was maximum at 8 h. Though germination percentage at 5 h incubation at 20°C was initially lower than at 25°C it soon increased rapidly and at 6 h percentage germination at both the temperatures was identical. However, at 8 h the percentage germination was significantly higher (P

<0.01) in 25°C as compared to 20°C. At 30°C the conidial germination after 8 h of incubation was only 21.26%.

Table 3.2 Conidial germination of *C. quinqueseptatum* in hanging drop technique at different incubation period at three temperatures[a] (mean of 30 observations)

Incubation period (h)	% germination[b]		
	20°C	25°C	30°C
5	2.14	14.21	1.58
6	69.94	51.23	9.12
7	82.51	82.59	19.28
8	86.50	94.69	21.26

[a] There was no conidiai germination at 10,15, and 35°C.

[b] Significantly affected by incubation period at $p < 0.05$ and temperatures $P < 0.01$.

In vivo conidial germination

Conidial germination details were similar to that for *in vitro* studies except that *in vivo* germination of conidia began after 3 h of incubation and branching of germ tubes occurred during 4-5 h of incubation. Occassionally, fusion of germ tubes was observed on the leaf surface (Fig. 3.1 D,E). Two germ tubes arising either from different conidia or the same conidiurn fused and gave rise to a common germ tube; fusion of terminal cells of two germ tubes as well as fusion of a terminal cell with any intercalary cell of another germ tube were also observed.

Growth of the germ tube was faster on younger leaves than on mature (Table 3.3). Also, the germ tube length was significantly greater on the adaxial surface than on the abaxial surface of young leaves; in mature leaves there was no significant difference. In

general, conidia near the leaf tips of both the surfaces had longer germ tubes than on other parts of the lamina.

Table 3.3 Germ tube growth on adaxial and abaxial surfaces of young and mature leaves of 60-day-old seedlings of *Eucalyptus grandis*

Time of incubation (h)	Germ tube length in (μm) and (S.E.)			
	Young leaves		Wature leaves	
	Adaxial	Abaxial	Adaxial	Abaxial
4	109.13 (± 9.4)	89.25 (± 5.7)	101.25 (± 5.1)	91.0 (± 7.0)
4.5	190.47 (± 11.9)	114.13 (± 8.4)	130.2 (± 10.3)	154.0 (± 17.6)
5	246.92 (± 19.81	169.57 (± 12.0)	142.52 (± 11.5)	158.65 (± 14.5)
6	351.75 (± 25.8)	260.47 (± 37.61	209.37 (± 16.5)	194.25 (± 11.9)

Appressorium formation by germ tubes occurred after 4 h of incubation (Fig. 3.2A). Stomatal penetration was rarely observed, most germ tubes bypassing them or passing over them on abaxial surface (Fig. 3.2 E,F). Germ tubes developed appressoria only after growing for certain distance from the conidium, and only once was an appressorium observed on a germ tube close to the conidium (Fig. 3.2A). A growing germ tube could be distinguished from the one forming an appressorium; the terminal portion of the latter developed dense cytoplasmic contents, a slight bulging and ceased to grow further while in the former no such changes were noticed. Around the inflated tip of the germ tube the wax platelets of leaf were found dissolved (Fig. 3.2B) and some mucilaginous thread like structures arising from the tip, adhered firmly to the leaf surface (Fig. 3.2C). In the case of stomatal penetration, the appressorium was formed over the stomata covering the entire stomatal opening, while for direct

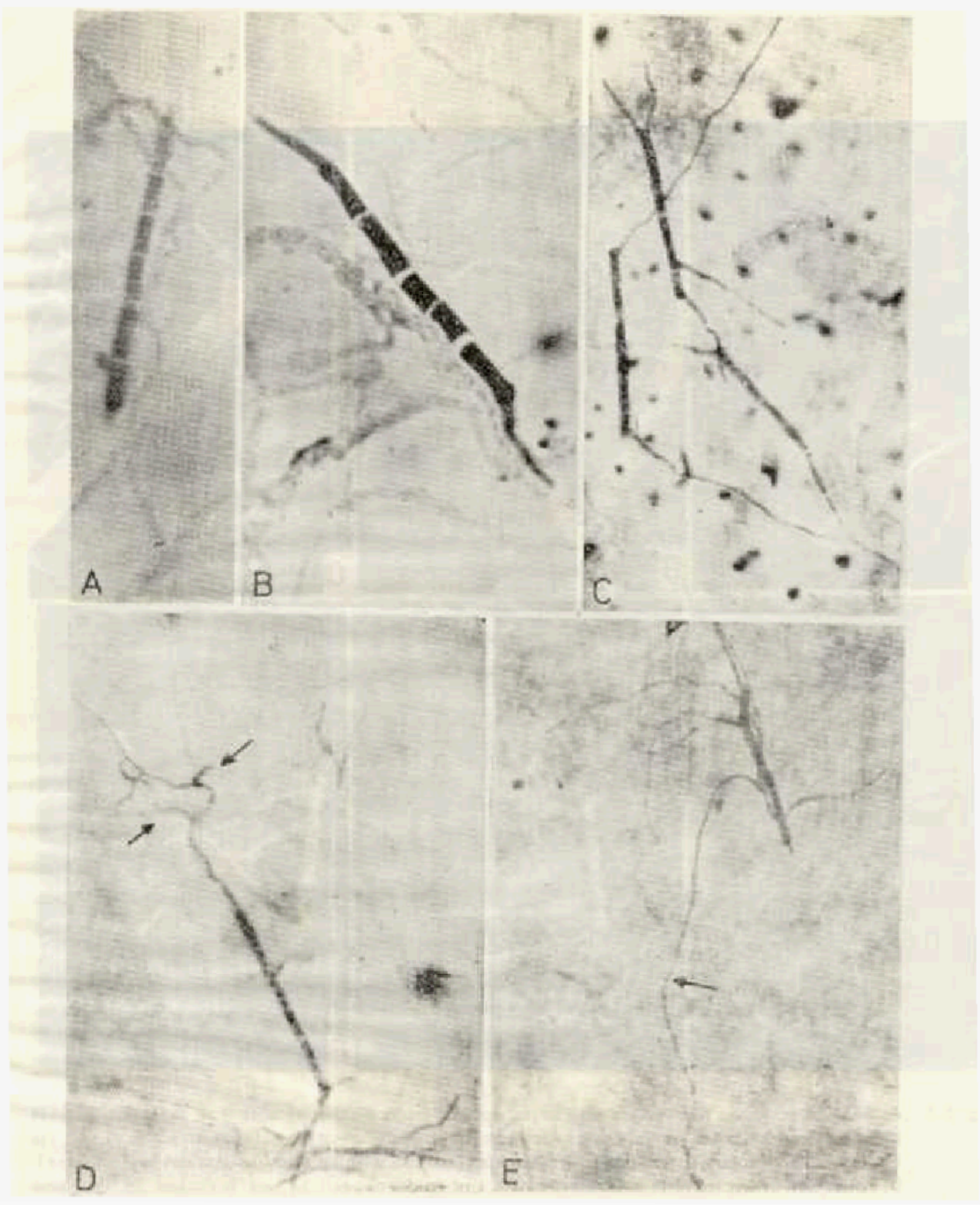


Fig. 3. 1. A: In vitro germination of conidium after 4.5 h of incubation. B: Conidium with two young germ tubes at both the terminal ends. C: In vivo germination of conidia on adaxial surface of leaf of E. grandis showing long germ tubes after 4 h of incubation. D: Fusion of branches of germ tubes (marked with arrows) arising from different conidia on the adaxial surface of leaf of E. grandis. E: A conidium with five germ tubes on adaxial leaf surface. Note fusion of one of the germ tubes (marked with an arrow) with germ tube from another conidium.

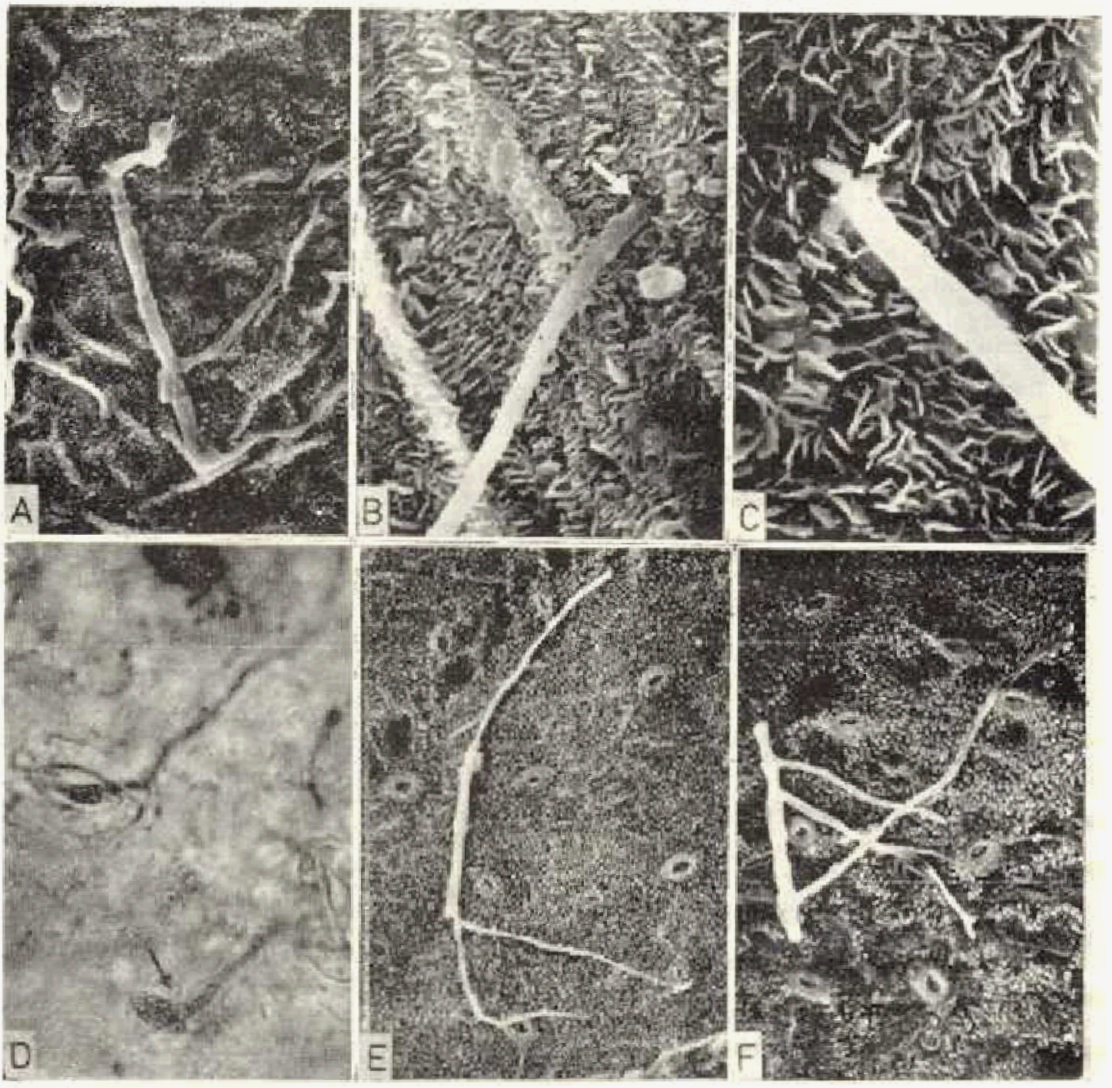


Fig. 3.2. A: SEM showing the germ tubes with round appressorium on the abaxial leaf surface. B: SEM showing dissolution of wax platelets around the tip of the germ tube (abaxial surface). C: Tip of germ tube with some mucilagenous strands (marked with an arrow) (adaxial leaf surface). D: Formation of appressoria over the stomata and epidermal cell by two branches of the same germ tubes (abaxial leaf surface). E, F: SEM of the abaxial surface to show that germ tubes do not show any affinity for stomatal penetration.

penetration, appressoria, oval to angular in outline, were formed at any place over the epidermal cell. In one tnstance, germ tube branches from the same conidium had developed appressoria over the epidermal cells as well as over the stomata (Fig,3,2D).

In the mature leaves appressoria formation occurred as early as 4.5 h of incubation while in young leaves at 6 h. Necrotic lesions developed within 12 h of inoculation and appeared earlier in young leaves as compared to mature leaves.

DISCUSSION

In *in vitro* studies, conidial germination was ca. 95% whereas in *in vivo* it was almost 100 percent. Similarly, the conidial germination was initiated only after 4 h of incubation in the former while in the latter within this period appressoria had developed, Higher germination and rapid development of appressoria in the latter case could be due to stimulus either from the leaf surface or leaf leachates. The conidial germination of C. *quinqueseptatum* occurred only between 20-30°C with maximum at 25°C. The optimum temperature of 25°C fits 'well into Togashi's (1949) 'average' optimum for plant pathogenic fungi and results confirm observations of Bolland *et al.* (1985). The temperature range for optimum germination is similar to that encountered during the monsoon when a high incidence and severity of leaf blight is observed in Kerala. Greater conidial concentration also reduced germination percentage. It is not known whether this is due to production of self inhibitory substance (Allen, 1955) by conidia or due to some other physical factor such as nonavailability of adequate O_2 in water.

The effiiciency of the pathogen is also evident from the results obtained in this study where each conidium produces 2-4 germ tubes which subsequently branch further thus bringing about multiple infections though one conidium. Fusion of germ tubes originating from the same conidium or different conidia may possibly explain the pathogenic variability in different isolates of C. *quinqueseptatum.* Formation of longer germ tube on the adaxial leaf surface than on the abaxial could be due to the delay in formation of appressorium and subsequently penetration which occurs first on the abaxial surface and

later on the adaxial. This difference could be due to differences in leaf surface characteristics such as the ornamentation of wax platelets. Though no observations were made on the mode of penetration by appressorium, it is possibly through an infection peg arising from the lower surface of appressorium. Bolland *et al.* (1985) have reported a direct penetration by the germ tube.

The germinating conidia showed no affinity for stomatal penetration. This observation is at variance to earlier report by Bolland *et al.* (1985) who found the penetration of leaves of *E. microcorys* is secured only through stomata. Furthermore, they observed that if the germ tube did not encounter a stomatal opening it branched profusely and its growth ceased. However, no such profuse branching of germ tubes was recorded even on the adaxial surface where the frequency of stomata is either very low or absent altogether. These differences may be due to the host species or the aggressiveness of the strain of C, *quinqueseptatum* employed in thesestudies.

The formation of appressorium and process of stomatal penetration were similar to that described by Bolland *et al.* (1985), except for the presence of muciiagenous threads and the dissolution of the surface wax platelets around the appressorium. Though, penetration occurred first in mature leaves after 3.5 h of incubation as compared, to 6 h in young leaves, necrosis around the site of infection developed first in the latter and also spread more rapidly. Anahasur *et al.* (1976) have reported *in vitro* toxin production by C. *quinqueseptatum* which would account for necrosis, and young tissues may be more susceptible to this toxin.

4. Severity of Cylindrocladium Leaf Blight in Eucalypt Plantation in Relation to intercropping with Tapioca and Rainfall

Cylindrocladium leaf blight (CLB) of *Eucalyptus tereticornis* caused by *C. quinqueseptatom* usually attains an epidemic status in high rainfall areas of Kerala during the monsoon (June-September) resulting in large-scale mortality of young seedlings in nurseries and extensive defoliation of young trees 1- to 2-year-old) and young coppice shoots in plantations (Sharma and Mohanan 1982; Sharma *et al.*, 1984, 1985). Initially, CLB begins to appear on leaves of lower branches near the ground and spreads upwards to higher branches. However, in seedlings and young trees the infection may initiate at any part. For planning a chemical control strategy, a clear understanding of the epidemiology of CLB is essential. Since no information is available on the influence of climatic conditions and cultural practices followed during the establishment stages of eucalypt plantations such as cultivation of tapioca *(Manihot utilissima* Pohl.) as a taungya crop on the severity of CLB, these studies were undertaken.

MATERIALS AND METHODS

A young (2-yr-old) E. *tereticornis* plantation at Thalakode, Kothamangalam Forest Division (1980 plantation, 9.5 ha), known to have had high incidence of CLB in previous years, was selectd during 1982. Because of the high mortality of outplanted seedlings (<50%) due to CLB infection, this plantation was restocked during 1981. Taungya crops of ginger *(Zingiber officinale* Rose) and tapioca *(Manihot utilissima)* were raised respectively during 1981-1982 and 1982-1983. The severity of CLB infection was assessed on 25 trees each, selected at random in 4 planting rows at monthly intervals during 1982 and 1983; observations during October-December 1983 were recorded from trees in 5 planting rows. Planting and harvesting dates of tapioca, approximate height of tapioca and that of eucalypt plants were also recorded. The CLB severity was rated on a scale as follows and mean severity calculated as described earlier by Sharma *et al.*

Symptoms	Severity	Severity rating
Absent	Nil	0
Upto 114 of lower crown of tree infected; no premature defoliation	Very low	1
Upto 1/2 of lower crown of tree infected; 10-25% crown defoliated;	Low	2
Upto 3/4 of lower crown of tree infected; 25-50% of crown defoliated	Medium	3
Upto 314 of lower crown of tree infected; 50-75% of crown defoliated die-back of shoots present	High	4
Whole tree infected; 75% of crown defoliated; extensive die-back of shoots present	Severe	5

RESULTS

High CLB severity coincided with the rainfall months whereas high relative humidity alone during dry months did not seem to favour infection appreciately (Fig. 4.1). During January-May of 1982 though the CLB severity remained very low, it showed an upward trend especially after showers during March-May. After heavy rainfall during June, the severity increased rapidly and it was high during July and August. Subsequently, as the rainfall declined, the CLB severity also declined gradually to low level by December 1982. Though there was no rainfall between November 1982 and March 1983, the CLB severity did not decline to the extent recorded during dry months, January to March 1982. Instead, from February, the severity started to increase which after the rains began in March, attained severe status in October. However, during November and December 1983 the severity declined abruptly though occasional rains continued till December, unlike during 1982 when no rains occurred in November and December. The pattern of disease development was also different as compared to 1982.

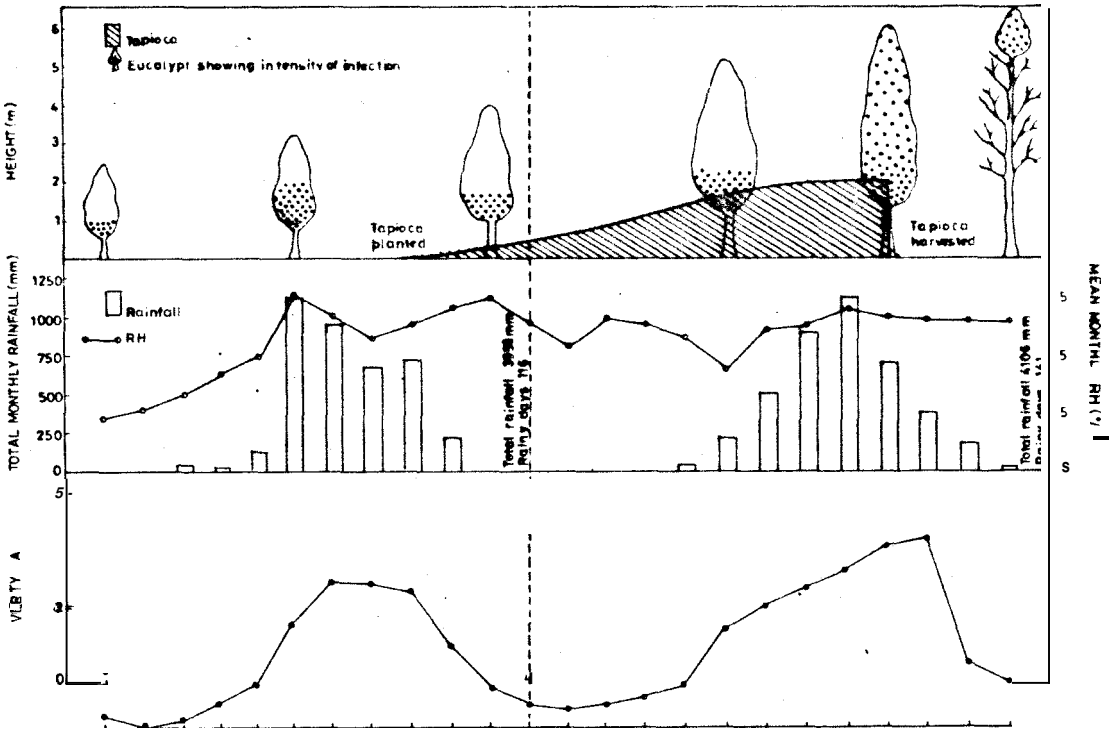


Fig. 4.1. Severity of Cylindrocladium leaf blight (CLB) in relation to microclimatic conditions and tapioca cultivation during 1982-83.

When tapioca was planted in August 1982, the CLB severity had already attained a high status due to heavy rainfall during June and July. The tapioca plants grew rapidly and covered within 6 months about one-fourth of the crown of eucalypt trees. All the leaves of shoots in this part had severe CLB infection and defoliated. Also,the shoots exhibited die-back symptoms due to multiple twig infections of branches. The CLB infection gradually spread upwards above the tapioca canopy level and caused extensive defoliation. By the time the tapioca was harvested in September more than three-fourth of the crown had been defoliated. During October the CLB progressed further up and infected the remaining leaves at the top of the crown. During November and December a new flush of foliage startd to appear and the trees looked healthy but a low degree of fresh infection appeared on new foliage possibly spread from the remaining older, infected leaves at the top.

DISCUSSION

The cultivation of tapioca as a taungya crop in the eucalypt plantation contributed to severe. The overall high CLB severity during

1983 was possibly due to the congenial conditions such as high rainfall and green house conditions by tapioca which helped to build up of high inoculum potential for disease development. The rapid build up of CLB during 1983 and its sudden decline after the harvest of tapioca shows the role of the agroforestry crop as a predisposing factor in the manifestation and spread of this disease. Usually the tapioca cultivated in eucalypt plantations is a tall variety which reaches a height of 2-2.3 m enclosing the foliage of lower branches of eucalypt, thus rendering them prone to infection. Cultivation of a dwarf variety of tapioca is likely to reduce the severity of CLB.

In aerobiological studies conducted using Burkard spore trap at Thalakode during 1980-81 only a very few conidia of *Cylindracladium* could be observed even during the peak of leaf blight infection. Unsuccessful trapping of *CyIindrocladium* conidis could be due to the mucilagenous sheath which makes them sticky forming clumps, hence heavy and not airbone worthy. Mucilagenous sheath is possibly also the reason for these conidia to require free water for germination (Bolland *et al.,* 1985. This points to the important role of rain or dew drops in infection. Severe infection was observed only when rain occurred. Though in some part of the dry period high humidity persisted, there was no increase in disease severity. Claton (1942) observed that even high humidity cannot germinate conidia of some species which require free water. Reitsma and Sloof (1950) also found severe leaf infection of clove caused by *C. quinqueseptatum* only during the wet period whereas during the dry period the development of disease was abruptly arrested and spread of infection within the host greatly retarded.

Positive correlation of CLB severity with the high rainfall pattern appears to have some management implications. *E. tereticornis,* which is highly susceptible to CLB and also to the pink disease caused by *Corticum salmonicolor* B. & Br., may not be the suitable species for high rainfall areas of Kerala. However, till the time a suitable speices of *Eucalyptus* or other fast growing hardwood suitable for use as pulpwood (Seth *et al.,* 1978) is identified for high rainfall areas of Kerala, it is advisable to manipulate the cultural practices so as to minimize the disease hazards.

5. Relative Susceptibility of Eucalypt Provenances to Cylindrocladium leaf blight

Though Cylindrocladium leaf blight (CLB) can be controlled effectively and economically in nurseries using prophylatic fungicidal treatment, chemical control of CLB in plantations will be prohibitive and impractical. The long-term solution for managing CLB is possibly to raise resistant provenances/species of eucalypts (Sharma, 1986). To find out the potential of introducing resistances as a strategy of CLB management artificial inoculation tests were carried out to assess the relative susceptibility of different eucalypt provenances to three predominant species of *Cylindrocladium* i. e., *C. quinqueseptatum,* C. *ilicicola* and *C. clavatum.*

MATERIALS AND METHODS

Eucalypt provenances

Seeds of 46 provenances belonging to 16 species of *Eucalyptus* were obtained from the Commonwealth Scientific and Industrial Research Organization (CSIRO), Canberra, Australia, while those of local *E. grandis* and *E. tereticornis* from the Silviculturist, Tamil Nadu Forest Department, Coimbatore, Tamil Nadu. Seedlings were raised in small metallic trays (75 cm x 75 cm x 15 cm) filled with steam sterilised fine-sieved forest soil. Two-month-old seedlings were transplanted in polythene containers filled with fine-sieved soil. The seedlings were kept under a shed provided with a transparent plastic roofing to protect young seedlings from rain water, which helps to promote CLB through water dispersed conidia.

Cylindrocladium spp.

Cultures of C. *clavatum* (IMI 2701851, *C. ilicicola* (IMI 250216) and C. *quinqueseptatum* (1M1 2807421, isolated respectively from *E. tereticornis, E. grandis* and *E. tereticornis* were raised on potato dsxtrose agar medium in 90 mm Petri plates at 25+2^{o}C. Ten-day-old cultures were utilised for preparing the conidial suspension (containing 2 x 10^{5} conidia ml^{-1}) in sterile water for inoculation.

During the experiment the germinability of conidia of *Cylindrocladium* spp. in hanging drops ranged between 94-96%.

Inoculation procedure

For inoculation, sixth leaf from the apical bud was detached from 9-month-old seedlings of identical height. The leaves were placed immediately in clean polythene bags, the inner side of which was sprayed with sterile water. Leaves of each provenance were kept separately with proper labelling. All the provenances could not be tested to three species of *Cylindrocladium* due to nonavailability of seedlings of the same height. Number of provenances tested against *C. clavatum* (CC), *C.* illicicola (C1) and *C. quinqueseptatum (CQ)* were respectively 47, 40 and 49.

Homogeneous conidial suspension of each *Cylindrocladium* sp. was sprayed with an atomizer separately on the abaxial surface of six replicate leaves each of different provenances, mounted on sterile moist filter paper. The atomizer, with fine nozzle to give droplets of uniform size, was connected to a pressure pump at 0.5 kg cm^{-2}. It was operated each time for 30 sec to give uniform conidial deposition over the leaf surface. The conidial suspension was swirled well to make it homogeneous before each spray. The inoculated leaves were lifted gently with two forceps and placed abaxial surface facing up over the filter paper in large Petri plates. The filter paper had been moistened with 5 ppm of gibberellic acid solution. For *CC* and *CQ*, the leaves were incubated at 30 + 2^0, while for C1, at *25* + 2°C.

Evaluation of host response

Observations on type of lesions (spreading or restricted), their size, shape and colour, total number of lesions per leaf, hypersensitive reaction, if any, were recorded. Later, each leaf was marked separately, dried in between filter papers and its area determined using Licor-1300 (USA) leaf area meter. Three observations were recorded for the same leaf and mean area calculated which was used for calculating lesions cm^{-2} from the total number of lesions on each leaf. Depending upon the lesion density the provenances were

rated on the following scale for susceptibility to CLB. The scale was based on field observations as well as numerous laboratory inoculation experiments.

Lesions Cm^{-2}	Susceptibility rating
1 - 20	Resistant (R)
>20 - 40	Susceptible (S)
>40 - 60 and above	Highly susceptible (HS)

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and then the provenances ranked for their relative susceptibility using Waller Duncan's multiple range test (DMRT) (Steel and Torrie, 1980).

RESULTS

The ANOVA demonstrates significant differences in susceptibility of various eucalypt provenances to three *Cylindrocladium* spp. i. e., CC, CI and CQ (Table 5.1).

Table 5.1. Analysis of variance of susceptibility reactions of various eucalypt provenances to *C. quinqueseptatm, C. clavatum C. illicicola* in detatched leaf inoculations

Source	*Cylindrocladium*		*Cylindrocladium* spp. *C. clavatum*		*C. ilicicola*	
	d.f.	Variance	d.f.	Variance	d.f.	Variance
Treatment	47	12.82*	46	20.68*	39	7.83*
Residual	238		231		200	
Total	285		277		239	

* Significant at P<0.001

Among the three species, the variance for CI was the least followed by that of CQ and CC. This indicates a closer relationship between the susceptibility level of the provenances to *C. ilicicola* than the other two species. The percentage of provenances giving resistant reaction was highest (60) to CI, lowest to CC (19.14) and intermediate to CQ (35.41). A reverse trend was observed for the provenances giving highly susceptible and susceptible reactions, the figures for three *Cylindrocladium* spp. being 4.0%, 30.0% (CI), 31.9%, 48.08% (CC) and 29.16%, 35.4% (CQ). It possibly implies that CC is the most virulent species and CI, the least.

In general, there appeared to be no correlation between level of susceptibility and a Subgenus/Section of the genus *Eucalyptus* as the response of different provenances varied significantly from resistant to susceptible within a Subgenus/Section (Table 5.2–5.6).The relative susceptibility of different provenances of a eucalypt species also varied considerably to three *Cylindrocladium* spp. This is clearly evident from the responses of provenances of *E. grandis* and *E. tereticornis*. Of the eight provenances of *E.grandis,* including Local TN, three gave resistant (R) reaction, two susceptible (S) and three highly susceptible (HS) to CQ. Similar varying responses were observed for CC and Cl, the respective figures for R, MS and S reactions being 1, 3, 2 and 2, 1, 1. The susceptibility reactions of provenances of *E. tereticornis* also varied greatly depending upon the *Cylindrocladiun* sp. However, there were three provenances of *E. tereticornis* (12944, 13277, 13319) which gave identical resistant reactions to three *Cylindrocladium* spp. Besides, *E. tessellaris* 12967 (R), *E. cloeziana* 13278 (HS), *E. urophylla* 12896 (S), and *E. camaldulensis* 12964 (S) is also gave identical reactions to the three *Cylindrocladium* spp. However, there were 15 provenances which gave similar (either resistant or susceptible) reactions to atleast two *Cylindrocladium spp.*

Besides the differences in the level of susceptibility among the provenances of an eucalypt species, some provenances also gave varying host reactions in respect of different *Cylindrocladium* spp. Usually, the lesions produced by *Cylindrocladium* sp. were angular, light to dark grey in colour; the lesions spread and coalesced with prolonged

Table 5.2. Relative susceptibility of provenances of *E. tessellaris*, *E. citriodora*, *E. cloeziana* and *E. pilularis* to *Cylindrocladium* spp. in detached leaf inoculations.

Subgenus and *Eucalyptus spp.*	Seed lot no. ex CSIRO Australia	Origin/Locality	*Cylindrociadium spp.* C. quinqueseptatum Mean lesions cm^{-2}	C. quinqueseptatum Susceptibility rating	*C. clavatum* Wean lesions cm^{-2}	*C. clavatum* Susceptibility rating	C. ilicicola Wean lesions cm^{-2}	C. ilicicola Susceptibility rating
Subgenus *BLANKELLA*								
1. *E. tessellaris*	12967	NW of Hareeba Qld	6.0[d]	R	14.1[e]	R	2.2[b]	R[3]
Subgenus *CORYMBIA*								
2. *E.* citriodora	12379	Herberton-IrvinebanK Qld	64.5[a]	HS	31.2[cd]	S[2]	-	-
Subgenus *IDIOGENES*								
3. *E. eloziana*	10691	Veteran NE Gympie Qld	65.9[a]	HS	39.1[bc]	S	-	
4. "	11641	Fairview Station Qld	-	-				
5. "	12201	16.6 Km Eungella Old	17.1[a]	R[1]	27.3[d]	S	35.5[a]	S
6. "	12435	34 Kms of Theodore Qld	-	-	46.5[ab]	HS	-	
7. "	12945	6 Kms of Helenvale Old	22.1'	S	42.0[bc]	HS	4.1[b]	R[3]
B. "	13278	Cardwell Old	44.3[b]	HS	53.2"	HS	40.7[a]	HS
Subgenus MONOCALYPTUS								
9. E. pilularis	12803	Fraser island Old	76.9[a]	HS		-		

1. Values with the same letterls) in vertical do not differ significantly at P 0.01 Late flecking and green island reaction;
2. Restricted minute lesions;
3. Late flecking and restricted minute lesions

period of incubation. However, there were a few provenances where the necrotic lesions were minute, measuring <1 mm in dia and remained restricted. In some others they were either purple or brown in colour. In some provenances such as *E. cloeziana* 12201 (R), *E. grandis* 12409 (S) and *E.propinqua* 12800 (HS) restricted lesions were accompanied by green island reaction and late flecking to CQ. Late flecking was also observed in *E.* grandis 13022 and *E.tereticornis*

Table 5.3. Relative susceptibility of provenances of *E. deglupta*, *E. saligna*, *E. pellita*, *E. resinifera* and *E. propinqua* to *Cylindrocladium* spp. in detached leaf inoculations

Subgenus and *Eucalyptus* sp.	Seed lot no. ex CSIRO Australia	Origin/Locality	*Cylindrocladium* spp. C. *quinqueseptatum* Mean lesions cm^{-2}	Susceptibility rating	C. *clavatum* Mean lesions cm^{-2}	Susceptibility rating	C. ilicicola Mean lesions cm^{-2}	Susceptibility rating
Subgenus *SYMPHYOMYRTUS*								
Section *Equatoria*								
1. *E. deglupta*	12322	Kervat New Guinea	4.3^{f*}	R	27.4^{def}	S^1	6.0^{de}	R^3
2. "	12976	Monkayo area Philippines	65.5^{a}	HS	90.2^{a}	HS	-	-
3. "	12977	New Battan Philippines	49.8^{b}	HS	76.5^{b}	HS	-	
4. "	-	Unknown Philippines	34.5^{cd}	S	-	-	1.1^{e}	R
Section *Transveraaria*								
5, *E. saligna*	13027	Blackdown TLand SF5 Qld	21.4^{de}	S	36.4^{cd}	S	11.7^{cde}	R
6. "	13334	Barrington Tops NSU	16.0^{ef}	R_1	41.4^{c}	HS	16.3^{bcd}	R
7. *E. pellita*	11947	Near Kuranda Qld	20.0^{cde}	S_1	19.7^{f}	R^1	12.5^{cde}	R
8. "	12013	5 Km S of Helenvale Qld	15.8^{ef}	R^1	29.1^{def}	S^1	21.6^{abc}	MS
9. "	13165	Julatten Qld	19.3^{e}	R	20.1^{f}	S	30.1^{a}	MS
10, *E. resinifera*	13166	Mt Levis Timb Res 66 Old	34.6^{c}	MS^1	24.4^{ef}	S^1	-	-
11. "	13318	NF of Kendall	58.3^{ab}	HS^1	31.6^{cde}	S^1	28.2^{ab}	S^1
12, *E. propinqua*	12800	19 Km ME of Gympie Qld	46.2^{b}	HS^2	36.4^{cd}	S	11.9^{cde}	R^1

* Values with the same letter(s) in vertical columns do not differ significantly at

1. Late flecking and green island reaction;
2. Restricted minute lesions;
3. Late flecking and restricted minute lesions

13319. Additionally, some provenances within a species also showed significant differences in the colour of the lesions on the adaxial surface. This was observed only in infection by CC and C1. Instead of normal greyish-black lesions, ***E. propinqua* 12800, *E. grandis* 13020, *E. pellita* 13165,** and ***E. saligna* 13334** gave rise to purple

lesions and *E. grandis* 13022, 13025 developed brownish lesions to CC; *E. urophylla* 12896. *E. microcarpa* 12795, *E. pellita* 11947 and *E. brassiana* 13415 developed brownish lesions with Ci.

Table 5.4. Relative susceptibility of provenances of *E. grandis* (Subgenus Symphyomyrtus Section Transversaaria) to *Cylindrocladium* spp. in detached leaf inoculations

Seed lot no. ex CSIRO Australia	Origin/Locality	Cylindrocladium spp. C. quinqueseptatum Wean lesions cm^{-2}	C. quinqueseptatum Susceptibility rating	C. clavatum Wean lesions cm^{-2}	C. clavatum Susceptibility rating	C. ilicicola Wean lesions cm^{-2}	C. ilicicola Susceptibility rating
1. 12409	14.5 Km S Ravenshoe Old	26.6^{cd*}	S	59.9^{ab}	HS[4]	22.5^{b}	S
2. 13020	NNU Coffs Harbour NSW	30.9^{bc}	S[2]	44.2^{cd}ef	HS	16.5^{ab}	R
3. 3022	NW Caboolture NSW	57.6^{a}	HS[3]	35.1^{cd}f	S	10.4^{cd}	R[4]
4. 13203	20 Km E of Gympie Old	11.9^{a}	R	63.0^{a}	HS	35.6^{a}	S
5. 13025	V of Paluma Qld	9.5^{e}	R	39.5^{cd}ef	S	5.4^{cd}	R[4]
6. 12970	SF 194 Herberton Range QId	45.0^{a}	HS	57.6^{abcd}	HS	4.2^{d}	R[4]
7. 13283	Mount Lewis. T, Res 66 QId	18.1^{de}	R	45.5^{c}de	HS	42.0^{a}	HS
8. -	Local Tamil Nadu India	41.5^{ab}	HS	47.3^{cd}e	HS	11.5^{bcd}	R

* Values with the same letters(s) in vertical columns do not differ significantly at

1. Late flecking and green island reaction;
2. Early flecking;
3. Late flecking;
4. Restricted iinute lesions

DISCUSSION

Quantitative assessment of relative susceptibility of eucalypt provenances to three *Cylindrocladium* spp. causing Cylindrocladium leaf blight (CLB) under identical experimental conditions shows a great deal of variation. Highly significant variance in ANOVA in respect of all the species of *Cylindrocladium* indicates the suitability of

Table 5.5. Relative susceptibiolity of different provenances of *E. tereticornis* (Subgenus Symphyomyrtus; Section - Exsertaria) to *cylindrocladium* spp. in detached leaf inoculations

Seed lot no. ex CSIRO Australia	Origin/Locality	*Cylindrocladium* spp. C. quinqueseptatur Mean lesions cm^{-2}	Susceptibility rating	C. clavatum Mean lesions cm^{-2}	Susceptibility rating	C. ilicicola Mean lesions cm^{-2}	Susceptibility rating
1. 13398	E of Kupiano	13.1 b*	R	10.9 cd	R	20.5 b	S
2. 13410	Sirinumu Sogeri Flot FNG	36.8 a	S	27.8 a	S	49.4 a	S
3. 13399	Oro Bay to EMO FNG	13.3 b	R	26.5 ab	S	6.1 c	R
4. 12944	S of Helenvale Qld	10.2 b	R[1]	7.0 bc	R[1]	6.2 c	R[1]
5. 13277	Cardwell Qld	16.2 b	R[1]	10.8 cd	R	4.3 c	R[1]
6. 13319	N of Yoolgoolga NSW	20.0 b	R[2]	0.0 d	R[1]	7.6 c	R[1]
7. -	Local Tamil Nadu, India	37,1 a	S	28.5 a	S	16.2 c	R

* Values with the sane Ietter(S) in vertical columns do not differ significantly at P<0.01%.

1. Restricted minute lesions:

2, late flecking

detached leaf inoculation technique in discerning the level of susceptibility in different eucalypt provenances. The results provide first evidence of differential susceptibility in different provenances of eucalypt species to three CLB pathogens. Earlier, Bolland et al. (1985) screened ten eucalypt species to CQ and reported varying degree of suceptibility among the various species. However, Sobers (1968) did not find any difference in the susceptibility of *E. camaldulensis* Dehnh., *E. rudis* Endl., *E. saligna* Sm. and *E. tereticornis* to C. *pteridis* Wolf.

Only a few of the provenances show similar level of susceptibility to three *Cylindrocladius* spp. Of the three species, CC proves to be the most virulent as a large number of provenances are susceptible to this species. The converse is true for the provenances giving resistant reactions. Filer (1970) has also reported differences in virulence of *C.fioridanum* Sobers & Seymour to yellow poplar

Table 5.6. Relative susceptibility of various provenances of *E. urophylla*, *E. camaldulensis*, *E. brassiana*, *E. exserta* and *E. microcorys* to *Cylindrocladium* spp. in detached leaf inoculations

Subgenus and Eucalyptus sp.	Seed lot no. ex CSIRO Australia	Origin/Locality	Cyiindrocladium spp. C. quinqueseptatum Hean lesions cm^{-2}	Suscepti-bility rating	C. clavatum Hean lesions cm^{-2}	Suscepti-bility rating	C. iliciicola Hean lesions cm^{-2}	Suscepti-bility rating
Subgenus SYMPHYOMRTUS								
Section Exsertaria								
1. *E.* urophylla	3	12895 Mt. Handiri Indonesia	18.9^{cd*}	R	63.5^{b}	HS	18.7^{bcdefg}	R
2. "	4	12096 Mt. Lewotobi Indonesia	28.6^{bc}	S	22.6^{de}	S	20.9^{bcdef}	S
3, "	5	13357 Mt. Egon Indonesia	21.1^{cd}	S	11.9^{f}	R^{2}	18.1^{cdefghi}	R
4. E. camaldulensisl		12181 3.5 Km S of Katherine WP	65.8^{a}	HS	10.9^{f}	R	11.0^{efghij}	R^{2}
5. "	2	12964 Emu Creek Petford Old	20.5^{cd}	S	22.1^{de}	S	23.1^{bcd}	S
6. E. brassiana	30	13397 rot to Mipim PWG	26.2^{c}	S^{1}	12.9^{ef}	R^{2}	21.0^{bcde}	R^{2}
7. "	31	13395 West of Morehead PNG	34.2^{b}	S	76.9^{a}	HS	28.1^{bc}	S^{2}
8. "	32	13415 8.8 Km WE Bamaga Qld	32.5^{b}	S^{2}	26.8^{cd}	S^{2}	8.3^{ghij}	R^{2}
9. "	33	13404 Cooktown Old	38.1^{b}	S	24.2^{d}	S	44.8^{a}	HS
10. "	34	13410 44 Km W Coin Qld	34.0^{b}	S	76.0^{a}	HS	16.6^{cdefgh}	R
11. "	35	13412 65 Km W Winlock R Qld	12.8^{d}	R^{2}	21.1^{def}	S	4.5^{j}	R^{2}
12. *E, exerta*		11020 I1Iiott R Bundaberg Qld	9.5^{d}	R	26.5^{cd}	S2	-	-
Section Sebaria								
13. E. microcorys	9	12795 Gallangowan Qld	13.9^{a}	HS	27.8^{c}	S	30.7^{b}	
14. "	-	12804 Fraser Island Qld	40.2^{b}	HS	36.0^{c}	S	-	-

* Values with the same letter(s) in vertical columns do not differ significantly at

1. Late flecking
2. Restricted minute lesions;

Liriodendron tulipifera) seedlings; the former was the most virulent while the latter the least.

There appeared to be no definite trend for susceptibility of eucalypt provenances belonging to a particular Subgenus or Section. This is expected as the provenances, even within a species, show

tremendous variation in susceptibility. Of the five Subgenera, four are represented by only one species/provenance while the remaining Subgenus Symphyomyrtus has 12 species, coming under six Sections. Even within these 12 species, no pattern in the level of susceptibility is seen. However, Bolland et al. (1985) reported that there was a correlation between the eucalypt species belonging to Subgenus. This may be coincidental as they used only different species and not different provenances of various species as used here. However, they further pointed out that since only three of the five Subgenera were represented by two or more species in their studies, testing of additional species from all Subgenera will be .required to conclude their observations. In an earlier study Bertus (1976) has tested the pathogenicity of 62 species of *Eucalyptus* belonging to Subgenera Blakella, Corymbia, Endesmia, Gaubaea, Monocalyptus and Symphyomyrtus to *C. scoparium.* He found that all the species developed infection but he did not give any other details related to differences in susceptibility either among the species or Subgenera.

Among the two eucalypt species widely grown in Kerala i.e., *E. grandis* and *E. tereticornis,* the former appears to have only one promising provenance (*E. grandis* 13025), which is resistant to CQ and CI and susceptible to CC. Susceptibility of this provenance to CC may not be of any serious consequence as it does not occur in high ranges where *E. grandis* is grown. On the other hand *E. tereticornis* has three provenances (12944, 13277 and 13319) resistant to all the three *Cylindrocladium* species, and another one (13398) resistant to CQ and CC, but susceptible to CI. Since, CI does not occur in lower elevations where *E. tereticornis* is being raised, susceptibility to this species will not pose any problem and all the four provenances may prove to be promising in the field.

Eucalypt provenances not only differ in their level of susceptibility of CLB caused by different *Cylindrocladium* spp. but also in their host reactions. A total of 23 provenances developed minute restricted necrotic lesions to one or more *Cylindrocladium* species. Of these only four provenances (*E. resinifera* 13318, *E. tereticornis* 12944, *E. brassiana* 13397, 13415) gave such reaction, to all the three *Cylindrocladium* species. Even with prolonged incubation these minute lesions did not spread or coalesce as usually happens

with normal lesions. Besides, in a few provenances such as *E. brassiana* 13397 to CQ and *E. deglupta* 12322, *E. tessellaris* 12967, *E. cloeziana* 12945 to C1 the flecking of necrotic lesions was also delayed. Production of restricted minute necrotic lesions could possibly be the host's hypersensitive reaction which is indicative of vertical resistance (Sensu Van der Plank, 1968). Additionally, late flecking of necrotic lesions is also indicative of vertical resistance in eucalypt provenances to *CyIindrociadium* spp. However, for confirming the presence of any vertical resistance in eucalypt provenances monoconidial isolates of *Cylindrocladium* have to be used instead of field isolates as done here. Since for assessing the relative susceptibility of provenances only the number of lesions were taken into account, provenances even with apparent hypersensitive necrotic lesions were graded along with the others. The reason for doing this was that, even leaves with extensive necrotic lesions are likely to defoliate prematurely. However, a total of 17 provenances with fewer restricted necrotic lesions per unit area may be the resistant provenances in practical sense as compared to those with fewer but large Iesions. These are *E. tessellaris* 12967, *E. cloeziana* 12945, *E. deglupta* 12322, *E.* grandis 13022, 13025, 12970 and *E. camaldulensis* 12181 to Cl; *E. citriodora* 12379, *E. grandis* 12409 and *E. urophylla* 13357 to CC; *E.* propinqua 12800 to CQ; *E.* tereticornis 13277 and *E. brassiana* 13412 to CQ and CI; *E. tereticornis* 13319 and *E.* brassiana 13397 to CC and CI; and *E. tereticornis* 12944 and *E. brassiana* 13415 to CQ, CC and CI. These provenances may prove to be superior in the field to other resistant provenances.

Detached leaf inoculation method used for quantitative assessment of relative susceptibility of eucalypts to various *Cylindrocladium* spp. appears to be the appropriate method for intensive preliminary selection of resistant provenances under identical conditions. The other advantage of th's method is that a large number of provenances can be screened simultaneously within a short period.

Control of CLB of eucalypts in future will be based on raising provenance/species with durable field resistance. As a first step in this direction this study has identified provenances with relatively resistant reaction which might be a good indicator of field tolerance

to CLB infection. However, due to great variation observed in susceptibility of eucalypt provenances to three species of *Cylindrocladium,* it will be essential to screen them against all the *Cylindrociadium* spp. known to cause leaf blight in a particular geographical area of Kerala before arriving at any conclusion on their field resistance.

6. Cultural Variation in *Cylindrocladium quinqueseptatum* Isolates

During routine isolation of C. *quinqueseptatum* (CQ) from diseased *Eucalyptus* material, collected from different parts of Kerala, a great deal of cultural variation was observed in the isolates. This together with differences recorded in leaf blight reactions on various eucalypt species to field isolates gave an indication to the existance of physiological strains in CQ. Since sources of resistance in *Eucalyptus* to CLB are not clearly understood, for an effective and viable tree selection programme it is essential to assess the variation in pathogenicity and virulence of the CQ population. Hence, the objective of the present investigation was to study the variability in cultural characters, growth, utilization of carbon and nitrogen sources, and virulence of various CQ isolates with a view to ascertain the existence of physioIogica1 specialisation in C. *quinqueseptatum.*

MATERIALS AND METHODS

Cylindrocladium quinqueseptatum isolates

Seventy CQ stock cultures, isolated from eucalypts growing in various localities in Kerala, were designated in ten groups, based on their cultural characteristics on potato dextrose agar medium (PDA). From each of these groups one isolate was selected; Ten-day-old monoconidial cultures, derived from each of these ten isolates viz. 755, 897, 947, 961, 963, 968, 1071, 1075, 1078 and 1080 were used in this study; a11 the cultures were grown on PDA at 25 + 2^{0}C.

Cultural characters and diameter growth of *CQ* isolates on different media

Cultural characters and diameter growth of ten CQ isolates were studied on nine different media viz. Ctapek dox agar (CDA), glucose asparagine agar (GAA), glucose tyrosine agar (GTA), glucose yeast extract agar (GYEA), glucose lima bean agar (GLBA), malt extract agar (MEA), potato dextrose agar (PDA), Vegetable agar (V-8) and yeast

Table 6.1. Origin of ten isolates of *Cylindrocladium quinqueseptatum* and their conidial morphology

C, quinque septatum isolate	Origin			Conidial morphology recorded on PDA	
	Eucalyptus sp. and type of infection	Locality	Altitude (m above msl)	Dimensions (µm)	Septation
CQ-755	*E.* grandis - seedling stem infection	Chandanathodu	810	73–93.5 x 6.6–7.1	4 - 5 (mostly 5)
CQ-897	*E.* citriodora - leaf infection 4-yr-old tree	Peechi	50.0	60–82 I 4.9–6.5	3 - 5
CQ-947	E. grandis - leaf infection 2-yr-old	Vattapoil (Periya)	750	90–114.4 X 6.5–7.8	4 - 5
CQ-961	*E.* grandis - seedling stem infection	Chandanathodu	810	86.5–109 x 6.1–8.8	5 - 7
CQ-963	*E.* grandis - seedling ster infection	Chandanathodu	810	71–99 x 6.5–7.2	3 - 5
CQ-968	E. grandis - leaf infection 3-yr-old trees	Chandanathodu	810	79–108 x 6.8–8.8	5 - 6
CQ-1071	*E.* camaIduIensis leaf infection 5-yr-old trees	Vazhachal	400	66–88 x 6.4–8.8	3 - 5
CQ-1075	*E.* grandis - leaf infection 3-yr-old trees	Uppupara (Panba)	950	61–90 x 6.6–7.1	3 - 5
CQ-1078	*E.* grandis - leaf infection 2-yr-old trees	Kulanavu	850	60–88 x 5.5–6.8	4 - 6
CQ-1080	*E.* grandis - leaf infection 3-yr-oid trees	Uppupara	950	86–107.6 x 6.6–7.8	5

malt agar (YMA) supplied by Himedia, Bombay. For each isolate and medium, there were three replicates of flat bottom assay petri dishes (11 cm dial, containing 15 ml of the medium. From each replicate dish, inoculated in the centre with a mycelial disk taken from the margin of 10-day-old culture of CQ isolate and incubated at 25 + 2°C. From each replicate dish, diameter growth was recorded at two places

of the colony on the fourth, sixth, eighth and fourteenth day of incubation. Other cultural characters such as colony colour, type of mycelium, sporulation and microsclerotial **(MS)** production were recorded after ten days of incubation. For scoring sporulation, six random observations were taken under 10X objective and an average number of conidiophores per microscopic field was obtained and their intensity converted to a numerical rating as follows: 0, absent; 1, poor (1-15 conidiophores); 2, moderate (16-50); 3, abundant (51-85); 4, profuse (>86). The development o MS was rated according to relative abundance and density of MS in the agar medium: 0 = absent; 1, poor (widely scattered); 2, numerous (moderate); 3, good abundant; 4, excellent (closely compacted). All the replicate dishes of LBA, GTA and V-8 in respect of isolate 947 and GAA in respect of 1075 were discarded as they became contaminated. Hence, observations for these isolate/medium combination could not be recorded. Besides, one replicate dish each of isolates 755, 897, 1078 and 1080 on GTA, isolate 1080 on V-8, isolates 755, 963 on PDA, isolate 968 on GYEA and isolate 1078 on MEA also became contaminated during the incubation and, hence, discarded.

For statistical analysis the growth data of each isolate on a medium were transformed to log and subjected to regression analysis to calculate B-value, from the equation log Y = a-B/x, where y is mean of three replicates and x days of incubation; B-value is the growth rate. B-values, with significant F-values, were then subjected to ANOVA to test whether the difference between the growth rates of isolates in a particular medium is significant. This was followed by cluster analysis (Calinski and Corsten, 1985) to separate various isolates with significantly different growth rates. besides, the growth data for all the isolates on one medium were also subjected to completely randomized design **(CRD)** overtime analysis (Gomez and Gomez, 1984) to ascertain whether their growth pattern was similar or different as they grew from fourth to fourteenth day of incubation. For this, the growth data of all isolates on a given medium were analysed separately for each incubation period.

Effect of various carbon (C) and nitrogen (N) sources on growth and MS production

Five CQ isolates vir. 897, 947, 961, 963 and 1080 having very distinct cultural and morphological characters were selected for this study. The effect of 11 C sources (Table 6.5) and 13 N sources (Table 6.4) on growth and MS production of five CQ isolates were compared in 25 ml of synthetic liquid media in stationary 250 ml Erlenmeyer flasks. Each C source was added to a basal medium with $NaNO_3$ as a N source at the rate equivalent to log dextrose per litre. The basal synthetic medium consisted of KNO_3, 2.0 g; $MgSO_4$. $7H_20$, 0.5g; KH_2PO_4, 1.0 g; KCI, 0.5 g and double distilled water, 1000 ml, One millilitre of a stock solution of micronutrients, containing Mn^{+2}, 0.05 mg; Zn^{+2}, 0.2 mg; Fe^{+3}, 0.1 mg was added to 11 of the basal medium. The initial pH of the medium was adjusted to 6.5 with 5N HCl before sterilisation. For studying the effect of various N sources on growth, dextrose was taken as a C source in the basal medium. The nitrogen compounds were added in quantity necessary to provide 430 mg of nitrogen per litre. The pH of the media was adjusted to 6.5 with 0.3M K_2HP0_4 before sterilization. The media were sterilized through a Millipore filter (pore site 0.45 um) and each flask was seeded with a single 4 mm disk of mycelium cut from the advancing margin of 8-day-old culture of a CQ isolate growing on PDA at 25 + 2°C. The cultures were incubated at 25 + 2°C for 16 days after which the MS production was scored on a similar scale as given above. The mycelial weights were determined after filtering the medium through Uhatman filter paper no. 41 disks (90.0 mm dia), washing with distilled water and drying at 60^0C for 24 h. The mycelial growth (weight) of the isolates was rated as follows: Poor - upto 25 mg; Fair->25-50 mg; good - >50-75 mg; very good - >75-100 mg; excellent - >100 mg. The mycelial weight data were analysed using subjecting them two-way ANOVA and cluster analysis.

RESULTS

Cultural charactsrirtics of *CQ* isolates on various growth media

All the growth media showed significant differences in cultural characteristics of various isolates. In general, among the media

which supported fast growth of most of the isolates, **PDA** and **YMA** were the best with abundant to profuse sporulation and **MS** production. In **MEA** and **GLBA**, though sporulation was abundant to profuse, **MS** production was either poor (former medium) or absent (latter medium). On the other hand, **GAA** and **CDA** gave poor sporulation and **MS** production varying from poor and moderate (latter) to abundant (former). Among the media which provided slow growth of **CQ** isolates, **GTA** was the best with sporulation and **MS** production varying from abundant to profuse. Poor to moderate sporulation and **MS** production were observed in V-8, while in **GYEA** sporulation was poor to moderate and **MS** production, absent to poor.

Most of the isolates differed in some or the other cultural characters on a given medium. The common differences encountered were in colony colour, mycelial characters, and intensity and pattern of sporulation and **MS** production. **MEA** was the best medium in exhibiting distinct characters for as many as six isolates (755, 897, 947, 1071, 1075, 1078) . It was followed by **YMA** (isolate nos. 947, 961, 963, 1075, 10781, **GTA** (isolates 968, 1071, 1075, 10801, **CDA** (isolates 755, 961, **9631, GAA** (966, 1078, 1080), **LEA** (isoiates961, **963,** 1075), **GYEA** (isolates 947, 755), **FDA** (isolates 897, 1075) and V- 8 (isolates 1071, 1075). Isolate 1075, slow grown on most of the media showed distinct characters on five media **(GTA, GLBA, PDA, YMA,** V-8), followed by isorates755 (**CDA, GYEA, MEA**), 961 (**CDA, LBA, YMA**), 963 (**CDA, GLBA, YMA**), 947 (**GYEA, MEA, YMA**), 1071 (**GTA, MEA,** V-8), 1078 (**GAA, YMA, MEA**) on three media; isolates 968 (**GAA, GTA**), 897 (**MEA PDA**) and 1080 (**GAA, GTA**) could be distinguished only on two media.

Based on intensity and/or pattern of sporulation and MS production alone, a number of isolates could be easily discerned from each other on different media except 961 isolate (Table **6.2).** These were isolates 755 and 963 on **CDA**, isolate 968 on **GAA**, isolates 1075 and 1080 on GTA, isolate 755 on **GYEA**, isolate 1075 on **GLBA**, isolates 755, 897, 947 and 1078 on **MEA**, isolates 897 and 1075 on **PDA**, and isolates 947 and 1075 on **YMA.**

Table 6.2. Intensity of sporulation and microsclerotia production by isolates different growth media

Cylindrocladium quinqueseptatum isolates	sporulation (S) and microsclerotia (MS) production on different growth media																			
	CDA		GAA		GTA		GYA		LBA		HEA		PDA		V-8		YHA	
	S	MS	S	MS	S	MS	S	MS	S	MS	S	MS	S	MS	S	MS	S	MS	S	MS
CQ-755	2[a]	3	1	2	3	3	2	1	2	0	3	1	3	4	1	1	4	3		
CQ-894	1	1	1	3	4	3	1	1	3	0	3	2	3	2	2	2	4	3		
CQ-947	1	3	2	2	b	b	2	0	b	b	4	4	4	4	-	-	3	2		
CQ-961	1	3	2	3	4	3	2	0	3	0	4	1	4	3	2	2	3	3		
CQ-963	1	2	1	3	3	3	2	0	3	0	4	1	4	3	2	2	4	2		
CQ-968	1	1	2	4	4	3	1	0	3	0	4	1	4	3	2	2	4	2		
CQ-1071	1	0	1	2	2	4	1	1	1	1	2	2	1	1	1	1	2	3		
CQ-1075	2	1	2	3	4	3	1	0	3	0	4	3	3	4	1	1	4	4		
CQ-1060	2	1	1	2	4	2	1	0	3	0	4	1	3	4	1	1	4	3		

a 0, Absent; poor; 2, moderate; 3, abundant: 4, profuse

b Observations could not be recorded due to contamination.

Diameter growth of *CQ* isolates on different media

Diameter growth of different CQ isolates varied significantly on a given medium. For convenience, growth data only for the fourth and fourteenth day of incubation are presented here (Table 6.3). Overtime analysis indicated that on all media except GYEA the CQ isolates showed significant differences in diameter growth at different periods of incubation. It means that the diameter growth of atleast some of the CQ isolates, on a particular medium, differed from each others they grew from the fourth day, to fourteenth day of incubation. For example, on the fourth day CQ isolates growing on MEA could be

Table 6.3. Diameter growth of CQ isolates on different growth media at fourth and fourteenth day of incubation in ascending order according to their significance in CRD overtime analysis

Diameter growth (mm) of CQ isolates on different media at fourteenth day of incubation																	
CDA		GAA		GTA		GYEA**		GLBA		MEA		YMA		V-8		PDA	
4	14	4	14	4	14	4	14	4	14	4	14	4	14	4	14	4	14
968^{a}	961^{a*}	1080^{a}	1080^{a}	1075	1075	1075^{a}	1075^{a}	1075^{a}	1075^{a}	1075^{a}	1075^{a}	1075^{a}	1078^{a}	1075^{a}	961^{a}	1075^{a}	1075^{a}
(8.5)	(36.6)	(19.1)	(52.1)	(20.1)	(27.6)	(8.1)	(31.5)	(32.6)	(51.5)	(5.5)	(39.5)	(31.6)	(45.3)	(30.6)	(38.1)	(10.5)	(39.8)
961^{a}	968^{b}	1078^{a}	1078^{a}	961^{a}	1078^{b}	1071	1071	955^{a}	968^{b}	1078^{b}	1078^{a}	1080^{a}	1075^{a}	961^{a}	1071^{a}	897^{a}	1078^{b}
(13.5)	(45.1)	(25.5)	(54.8)	(22.1)	(48.5)	(19.1)	(34.0)	(30.3)	(72.8)	(17.5)	(50.2)	(32.5)	(53.6)	(30.6)	(39.1)	(25.6)	(61.5)
897^{a}	947^{b}	897^{a}	897^{a}	1071^{a}	961^{b}	755	755	961^{b}	1078^{b}	947^{b}	947^{b}	1078^{a}	1080^{a}	1078^{a}	897^{a}	755^{b}	1080^{c}
(13.5)	(50.5)	(29.1)	(68.6)	(28.5)	(50.1)	(19.1)	(34.6)	(34.8)	(73.0)	(17.8)	(61.0)	(33.0)	(57.8)	(31.8)	(39.8)	(28.5)	(73.6)
1078^{a}	897^{b}	755^{b}	961^{b}	963^{b}	897^{b}	1078	963	897^{b}	1071^{b}	897^{b}	961^{b}	1071^{a}	1071^{a}	755^{a}	1078^{a}	1071^{b}	1071^{c}
(14.3)	(54.3)	(41.6)	(71.0)	(29.6)	(52.0)	(20.1)	(34.7)	(35.2)	(76.2)	(18.1)	(65.5)	(37.6)	(59.3)	(31.5)	(40.1)	(28.6)	(75.1)
947^{b}	1080^{b}	961^{b}	968^{b}	1078^{a}	755^{b}	963	897	1071^{b}	1080^{b}	1071^{b}	1071^{b}	897^{a}	897^{b}	1071^{a}	1075^{a}	1071^{b}	1071^{c}
(18.1)	(56.1)	(43.6)	(74.5)	(30.0)	(54.7)	(20.5)	(36.0)	(37.0)	(76.0)	(18.8)	(65.6)	(42.0)	(76.0)	(35.0)	(42.6)	(28.8)	(75..5)
1080^{b}	1078^{c}	968^{b}	1071^{b}	968^{a}	963^{b}	897	947	1078^{b}	755^{b}	755^{b}	968^{c}	947^{a}	755^{b}	897^{a}	755^{a}	947^{b}	947^{c}
(18.1)	(59.0)	(47.8)	(74.8)	(33.0)	(56.5)	(20.6)	(40.0)	(38.1)	(77.5)	(21.1)	(72.0)	(44.3)	(76.6)	(37.1)	(45.8)	(30.6)	(75.8)
1075^{b}	755^{c}	1071^{b}	755^{b}	897^{a}	1071^{b}	947	968	963^{c}	961^{b}	961^{b}	897^{c}	755^{a}	961^{b}	963^{a}	1080^{a}	963^{b}	755^{c}
(18.1)	(61.0)	(48.1)	(75.1)	(34.6)	(62.6)	(20.6)	(40.0)	(43.1)	(77.5)	(22.1)	(74.0)	(44.8)	(76.8)	(39.8)	(46.0)	(31.6)	(76.5)
1071^{b}	1071^{c}	947^{c}	947^{b}	755^{a}	1080^{b}	961	961	968^{c}	963^{c}	963^{b}	755^{c}	961^{a}	947^{b}	1080^{a}	968^{a}	961^{b}	897^{c}
(22.5)	(61.8)	(54.5)	(75.3)	(34.9)	(64.2)	(24.5)	(43.1)	(44.5)	(82.6)	(22.5)	(77.5)	(46.0)	(77.6)	(40.0)	(46.3)	(31.8)	(77.7)
755^{b}	1075^{c}	963^{c}	963^{b}	1080^{a}	968^{b}	1080	1080	1080^{c}	897^{c}	968^{b}	963^{c}	968^{a}	968^{b}	968^{a}	963^{a}	1080^{c}	963^{c}
(27.0)	(63.0)	(65.3)	(82.0)	(40.5)	(71.8)	(26.1)	(43.8)	(44.6)	(84.5)	(26.0)	(79.1)	(47.3)	(80.0)	(41.0)	(48.1)	(38.3)	(78.5)
963^{c}	963^{c}					968	1078			1080^{b}	1080^{c}	963^{a}	963^{b}			968^{c}	968^{c}
(40.8)	(66.5)	-	-	-	-	(30.2)	(44.6)	-	-	(27.8)	(80.0)	(50.1)	(83.6)	-	-	(37.0)	(81.0)

* Isolate numbers with the same script in a column do not differ significantly at P < 0.001%

** Means arranged in ascending order; CRD over time analysis not performed as ANOVA was not significant.

separated into two groups, i.e., isolate 1075 was significantly different from the rest. However, on the fourteenth day the isolates with similar growth fell in three groups viz. (i) isolates 1075, 1078, (ii) isolates 947, 961, 1071 and (iii) isolates 755, 897, 963, 968, 1080. Similarly, the growth pattern of isolates also changed on other media. Isolate 1075 was the only one which differed significantly from the other isolates at different days of incubation (in brackets) on PDA (4,6,8,14), MEA (4,6,8), LBA (6,8,14) and YMA (14). This isolate differed from others mainly because of slow growth on these media. Certain other isolates eg. 963 on CDA and YMA, 968 on GYEA, and PDA and 1080 on MEA showed the fastest growth as compared to others.

Growth rate of *CQ* isolates on different media

Growth rate of various CQ isolates differed significantly only on four growth media viz. GYEA, MEA, LBA and PDA; on other media the growth rate did not differ significantly. In cluster analysis, isolate 1075 was found to be significantly different from the rest on GYEA, MEA and PDA. However, GLBA differentiated the isolates into three separate groups i.e., (i) isolates 755 and 897 (ii) isolates 961, 965, 1071 and 1078, and (iii) isolates 968 and 1080.

Effect of C and N sources on growth and MS production

As compared to control (without N source), growth of all the five isolates was better in media with N sources (Table 6.4). Generally, organic N sources, especially glutamic acid and L- leucine supported better growth of all the isolates; isolate 897 grew equally well on inorganic N sources like ammonium sulphate, ammonium nitrate and potassium nitrate and isolate 1080 on sodium nitrate.

The most vigorous isolate was 947 which could utilise most of the N sources well showing excellent growth on peptone, caseine hydrolysate, L- glutamic acid, and very good growth on the rest except both the ammonium compounds and L-leucine. It was closely followed by isolate 961 which showed excellent growth on L-alanine, L-glutamic acid and L-leucine and very good on rest of the N sources except

Table 6.4. Dry mycelial weight (mean of two replicates) and density of microsclerotia (MS) production (in two replicates of five isolates of *C. quinqueseptatum* grown on 13 nitrogen sources with glucose as the carbon source

C. quinque-septatum	Growth para-meters	NH_4No_3	$(NH4)_2SO_4$	KNO_3	$NaNo_3$	Urea	Pep-tone	Casine hydro lysate	L-ala-nine	L-argi-nine	L-aspar-agine	L-gluta-mic acid	L-leu-cine	L-phenyl-alanine	Control (without nitrogen source)
CQ-897	Mycelial wt (mg)	78.0*	91.0^{c}	73.5^{c}	75.5^{c}	69.0^{b}	68.0^{b}	74.5^{c}	73.5^{c}	71.0^{b}	66.5^{b}	80.5^{c}	89.0^{c}	82.0^{c}	19.0^{a}
	Density of MS	0,0**	0,0	3.3	3,3	3,3	1,1	4.4	1.1	1.2	3.3	2.2	2.2	1.1	0,0
CQ-947	Mycelial wt. (mg.)	50.5^{2}	73.5^{c}	81.5^{c}	88.0^{c}	86.0^{c}	111.5^{d}	108.5^{d}	83.5^{c}	82.5^{c}	91.0^{c}	107.5^{d}	103.0^{d}	97.5^{c}	28.0^{a}
	Density MS	0,0	0,0	3,3	4,4	3,3	4,4	5.5	3.3	4.4	4.4	4.4	4.4	5.5	2.2
CQ-961	Mycelial wt.(mg)	67.0^{b}	80.5^{c}	710^{b}	70.5^{b}	78.0^{c}	92.0^{c}	96.0^{c}	113.5^{d}	92.5^{c}	92.0^{b}	104.5^{d}	115.0^{d}	86.0^{c}	17.0^{a}
	Density of MS	0,0	0,0	2,2	3,3	2.2	3,2	4,4	3,3	3,3	3,3	4,4	4,4	4,4	1,1
CQ-963	Mycelial Wt.(mg)	60.0^{b}	73.0^{c}	70.5^{b}	61.5^{b}	51.5^{b}	65.0^{b}	93.5^{c}	51.0^{b}	57.5^{b}	53.0^{b}	94.5^{c}	78.0^{c}	77.5^{c}	11.5^{a}
	Density MS	0,0	0,0	2,2	3,3	2,2	1,1	4,4	2,2	2,2	2,2	4,4	2,2	4,4	0,0
CQ-1080	Mycelial wt.(mg)	62.0^{b}	72.0^{b}	75.0^{c}	81.0^{c}	76.0^{c}	108.0^{d}	86.0^{c}	63.0^{b}	63.5^{b}	55.5^{c}	90.0^{c}	74.0^{c}	90.5^{c}	19.0^{c}
	Density MS	0,0	0,0	3,3	3,3	3,3	3,3	4,4	2,2	2,2	2,2	4,4	2,2	4,4	0,0

*Values with the same superscript are not significnatly different at 1%

**Numerical rating of microsclerotia production. For details see Materials and Methods.

sodium nitrate. Isolates 897, 947 and 1080 differed significantly from others as they could utilise nitrates effectively. Isolate 897 did not show excellent growth on any of the N source, but very good growth was observed on potassium nitrate, ammonium nitrate, ammonium sulphate, L-glutamic acid, L-leucine, and L-phenylalanine. For isolate 1080 excellent growth was recorded on peptone and very good growth on sodium nitrate, caseine hydrolysate, L-glutamic acid and L-phenylalanine. Isolate 963 was the slowest having moderate growth on most of the N sources, except on caseine hydrolysate, L-glutamic acid, L-leucine and L-phenylalanine, where it was very good.

Results of two-way ANOVA indicated significant differences in isolates, N sources and their interaction. This demonstrated that CQ isolates differed in their capacity to utilise various N sources. Hence, their maximum mycelial growth also varied depending upon the N. source.

From the cluster analysis it was possible to distinguish the difference among the CQ isolates on some of the N sources (Table 6.4). Isolate 947 differed significantly from the others in its utilisation of caseine hydrolysate while isolate 897 differed from the rest on ammonium nitrate. Similarly, isolates 1080 and 961 differed from the other isolates in the utilisation of ammonium sulphate, and peptone and L-alanine, respectively. Isolate 963 could not be differentiated from rest of the isolates on any of the N sources studied. Since in other N sources two to three isolates shoved similar utilisation 'no individual isolate could be differentiated from the rest. L-phenylalanine was the only N source where all the isolates behaved similarly giving no differential interaction.

Microsclerotial production also showed a great deal of differences among the isolates grown on various nitrogen sources (Table 6.4). There was no MS production in ammonium salts (ammonium nitrate and ammonium sulphatel ; in control only isolates 947 and 961 produced MS which were poor in growth. Caseine hydrolysate was the best N source for excellent MS production in all the isolates; L-glutamic acid was equally good for isolates 1080 and 961, and L-phenylalanine for isolates 1080, 961 and 947. Isolate 961 was the only one to produce excellent MS on L-leucine.

All the C sources supported better growth of CQ isolates than the control without C source (Table 6.5). Among the monosaccharides, hexose sugars were utilised better by all the isolates as the growth varied from good to very good depending upon the isolate than the pentoses; of the two pentose sugars tested D-arabinose alone supported good growth of only one isolate i.e. 947. In disaccharides, only cellobiose and D-sucrose supported good to excellent growth while on maltose it varied from poor to fair. Trisaccharide, D-raffinose supported good and very good growth of isolates 963 and 947 respectively; in other isolates growth was only fair. Polysaccharide starch was very well utilised only by isolate 963 as the mycelial growth was very good whereas in others the growth varied from poor to fair.

Two-way ANOVA clearly demonstrated significant differences in the utilisation of various C sources by CQ isolates. Isolate 963 utilised most of the C sources with excellent growth on sucrose, very good on D-galactose, D-glucose, D-cellobiose, and poor on D-ribose. This was the only isolate which could utilise starch with good mycelial growth. None of the other isolates showed excellent growth on any of the C sources tested. Isolates 961 showed very good growht on D-fructose, D-mannose, D-sucrose, good on D-galactose, D-glucose, D-cellobiose and poor on arabinose and starch. Isolate 947 had very good growth on D-raffinose. lts growth was good on D-arabinose, 0-glucose, D-mannose, D-sucrose and poor on D-fructose. The remaining two isolates did not utilise the C sources well as none of them supported even very good growth; isolate 897 had good growth on D-galactose, D-glucose, D-mannose, D-cellobiose, D-sucrose and poor growth on starch while isolate 1080 showed good growth on D-fructose, D-glucose, D-mannose, D-sucrose and poor growth on D-arabinose.

From the cluster analysis it is evident that solate 963 behaved differently in the utilisation of D-galactose and starch in comparison with the other isolates (Table **6.5)**. Similarly, isolate 947 showed significant differences in D-fructose and D-arabinose while isolate 1080 in D-galactose D-cellobiose and D-maltose. In the utilisation of other C sources there were **no** clear indications of differences in five CQ isolates as two to four of them utilised a particular C source equally wel1.

Table 8.5. Dry mycelial weight (mean of two replicates) and density of microsclerotial (MS) production (in two replicates) in respect of five isolates of *C. quinqueseptatum* grown in 11 carbon source with KNO_3 as the nitrogen source

C. quinque-septatum isolates	Growth Parameters	D-Glucose	D-mannose	D-galactose	D-fructose	D-ribose	D-arabinose	D-cellobiose	D-maltose	D-raffinose	sucrose	starch	control (without carbon (source)
CQ-897	Mycelial wt (mg)	59.5*	56.5^{b}	59.5^{b}	44.5^{b}	40.0^{b}	27.5^{a}	59.5^{b}	39.0^{b}	49.5^{b}	62.0^{b}	17.5^{a}	13.5^{a}
	Density of MS	4,4**	3,3	4,2	2,2	0,0	0,0	2,2	1,1	2,2	3,3	0,0	0,0
CQ-947	Mycelial wt (mg)	63.5^{b}	72.0^{c}	41.0^{b}	19.5^{a}	40.5^{b}	62.5^{b}	43.5^{b}	41.0^{b}	86.0^{c}	57.0^{b}	33.5^{a}	6.5^{a}
	Density of MS	3,3	4,4	2,2	2,2	1,1	1,1	2,2	2,2	3,3	4,3	0,0	0,0
CQ-961	Mycelial wt (mg)	62.0^{b}	83.0^{c}	64.0^{b}	84.5^{c}	33.5^{a}	17.0^{a}	73.0^{c}	43.0^{b}	52.5^{b}	80.0^{c}	21.0^{a}	4.5^{a}
	Density of MS	4,3	4,4	4,4	1,1	0,0	0,0	4,4	1,1	4,4	4,4	0,0	0,0
CQ-963	Mycelial wt (mg)	89.5^{c}	73.0^{c}	89.5^{c}	72.5^{c}	22.5^{a}	24.5^{a}	96.0^{c}	49.5^{b}	75.5^{c}	103.5^{c}	72.0^{c}	4.5^{a}
	Density MS	4,4	4,4	4,4	3,3	0,0	0,0	4,4	3,3	2,2	4,4	0,0	0,0
CQ-1080	Mycelial wt (mg)	66.0^{c}	60.5^{b}	30.5^{a}	63.5^{b}	48.0^{b}	20.0^{a}	32.0^{a}	29.0^{a}	49.0^{b}	59.0^{c}	26.0^{a}	3.5^{a}
	Density MS	4,4	4,4	1,1	1,1	0,0	1,1	2,2	1,1	3,3	3,3	0,0	0,0

* Values with the same superscript are significantly different 1%

** Numerical rating of microsclerotia production. For details see Materials and Methods

In general, MS production by five CQ isolates on various C sources showed some significant differences (Table 6.5). There was no MS production in control as well as on starch by all the isolates; D-arabinose and D-ribose also did not support any MS production by isolates 897, 961, 963 and 1080 (only D-ribose). The best C source for excellent MS production by most of the isolates (947, 961, 963 and 1080) was D-mannose followed by D-glucose (isolates 897, 961, 963, 1080), D-sucrose (isolates 947, 961, 963), D-cellobiose (Isolates 961,9631, D-galactose (Isolate 897, 961,963) and D-raffinose (isolate 961). In rest of the C sources, the response of the isolates varied from poor to very good. On the basis of poor MS production by isolates 947 and 1080 on D-ribose and D-galactose respectively, only these isolates could be differentiated from the others.

DISCUSSION

Most of the ten isolates show some variability in conidial dimension and septation; length of sterile hyphae and shape of vesicle, however, did not vary much. Conidia of isolates 947 were the largest and those of isolates 897 and 1078, smallest, Based on **conidiai** dimensions rest of the isolates couid be placed into three groups viz. (i) isolates 961, 968, 1080, (ii) isolates 755, **963** and (iii) isolates 1071, 1075. Though these differences in conidial morphology alone may not be reliable in distinguishing the **CQ** isolates into different strains, certainly it gives an indication of some variability among them.

From the results presented it is quite apparent that cultural characters such as radial growth, colony characters, sporulation and microsclerotia (MS) production form an important criterion for ascertaining differences among the CQ isolates as they differ considerably from medium to medium. Though PDA and YMA were the best media for growth, sporulation and MS production, MEA was the best medium as it could discern a maximum number of six isolates as distinct from the others. This means that not all the media are equally suitable in discerning the differences among the isolates. This could be due to differences in nutrional requirements of the isolates. While the other isolates could be differentiated only on

two or three media. Isolate 1075, which was slow growing on most of the media, stands out on five media indicating that it is a distinct strain. Among the cultural characters, sporulation and MS production are possibly important and more reliable in differentiating isolates due to their subtle variation in presence/absence, intensity and pattern. Nevertheless, it is clear from the above that when studies involve differentiation in pathogenic isolates it is better to use as many media as possible. Possibly because of using only one medium (glycerol peptone agar) Sobers (1988) could not find any differences in isolates of ***Cylindrocladium pteridis*** Wolf. Furthermore, considering a great deal of differences encountered in CQ isolates generalisation of cultural characteristics, based on a few isolates may not hold good. This is further confirmed as Hunter & Barnett (1976) reported that GLBA is good for growth, sporulation and **MS** production and GAA for growth and sporulation. On the contrary, our studies indicate that on GLBA, sporulation varied from abundant to profuse with MS completely absent (except isolate 1075 with MS production) and on **GAA** sporulation varied from poor to moderate; **YMA** and PDA were the best for growth, sporulation and **MS** production.

Growth rate **(GR)**, which is possibly closely related to the ability of an isolate to utilise nutrients in a particular medium, appears to be of limited use in discerning the isolate differences. This is due to the fact that GR of CQ isolates is statistically significant only on four media (viz. GYEA, MEA, PDA and GLBA) and the former three media were helpful to differentiate isolates into two groups whereas isolate 1075 is significantly different from the rest. However, on GLBA all the ten CQ isolates are separated into three distinct groups, indicating that this medium is better for discerning isolates on the basis of their GR. Some differences in GR of four isolates of ***C. scoparium*** also been reported by Bertus (1976). He found that one isolate each differed significantly at 15°C and 25°C in GR from the others.

Overall growth of five CQ isolates is found to be better on C sources than on N sources but both appear to be dependable characters in differentiating the isolates into strains, Though, statistically significant differences are found in the utilisation of C and N sources the latter is better as it helps in distinguishing four

isolates (897, 947, 961, 1080) as compared to three (947, 963, 1080) in the former. Better growth of most of the isolates is recorded in organic N sources, especially the peptide and protein than inorganic sources, though isolates 897, 947 and 1080 utilise inorganic sources equally well. This confirms earlier observation of Hunter & Barnett (1976) on 58 isolates of several species of ***Cylindrocladium***. Conversely Weaver (1974) reported that ***C. floridanum*** and ***C. scoparium*** utilise organic and inorganic N sources equally well, with a few important differences. Monosaccharide and disaccharide sugars seem to be preferred by most of the isolates, except isolates 947 and 963 which utilised respectively the trisaccharide, D-raffinose and starch equally well. Some of the C sources are utilised more rapidly than others by all the isolates. This is contrary to the report by Hunter & Barnett (1976) that ***Cylindrocladium*** isolates utilised all the C sources with remarkable similarity. Nevertheless, in general our results confirm observations of Hunter & Barnett (1976) that most of the ***Cylindrocladium*** spp. utilise well D-glucose, D-fructose, D-mannose, D-galactose, D-ribose, D-xylose, glycerol, D-sucrose and D-raffinose.

For the production of MS, caseine hydrolysate is found to be the best N source for all the isolates as has also been reported earlier by Hunter & Barnett (1976); D-glucose, D-mannose and D-sucrose, are the best C sources for good MS production by all the isolates.

From the foregoing discussion it is clearly evident that eucalypt isolates of ***C. quinqueseptatum*** show remarkable differences in their cultural characters on a given medium and in their capacity to utilise C and N sources. This indicates that they could be different strains which may also vary in virulence.

7. Pathogenic variation in *Cylindrocladium quinqueseptatum*

Results presented in previous chapters have shown that various ***Eucalyptus*** provenances differ significantly in their level of susceptibility to C. ***clavatum***, *C.* ***ilicicola*** and ***C. quinqueseptatum***, the three major species associated with cylindrocladium leaf blight (CLB) in Kerala. Furthermore, significant differences were also found in cultural characters and growth rate on different culture media, and carbon and nitrogen requirements of various isolates of ***C. quinqueseptatum*** (CQ). Whether this variability among CQ isolates is also reflected in their virulence and pathogenicity on ***Eucalyptus*** is not known. Since sources of resistance in ***Eucalyptus*** to CLB are not understood, for an effective and viable tree selection for disease resistance it is essential to assess the variation in pathogenicity of the population of CQ and also to know whether different CQ isolates possess general or specific virulence (Hadley et al., 1979). To achieve the above objectives five monoconidial isolates of CQ were tested on a set of differential provenances of ***Eucalyptus*** selected on the basis of their susceptibility to CLB.

MATERIALS AND METHODS

Eucalypt differential provenances

A total of eleven provenances of ***Eucalyptus*** viz. ***E. tessellaris*** 12967 **(6.0** mean lesions cm^{-2}), ***E. brassiana*** 13412 112.81, ***E. tereticornis*** 13398 (13.11, ***E. urophylla*** 12895 (18.91, ***E. saligna*** 13027 (21.41, ***E. brassiana*** 13415 (32.5) ***E. grandis*** TN Local (41.51, and ***E. propinqua*** 12800 (46.2) were selected based on their susceptibility to CQ as found in the previous study. The seeds of these provenances were obtained from the Commonwealth Scientific and Industrial Research Organization (CSIRO), Canberra, Australia, except those of E. grandis TN Local which were procured from the Silviculturist, Tamil Nadu (TN) Forest Department, Coimbatore, Tamil Nadu (India). The seedlings were raised as described earlier in Chapter 5.

Selection of *CQ* isolates and inoculum preparation

Five CQ isolates viz. Nos. 755, 897, 947, 968, 1080 varying in morphological and cultural characteristics, growth rate and their carbon and nitrogen requirements were selected. Ten-day-old cultures were utiiised for the preparation **of** inoculum, containing of 2 x 10^5 conidia ml^{-1} of sterile water. During the experiements the germinability of conidia of different isolates was recorded in hanging drop cultures; the percentage germination ranged between 95-97.

Inoculation procedures and evaluation of host response

The procedures for inoculation and evaluation of host response were the same as described earlier in Chapter 5, except that the concentration of conidia in the suspension was adjusted to 1.4-1.6 x 10^5 ml^{-1}.

Susceptibility rating and frequency *of* virulence

A cluster analysis (Calinski and Corsten, 1985) was done for the mean leaf lesions cm^{-2} for various eucalypt differential provenance **(P)** and isolate (I) combinations to distinguish and separate resistant (R), susceptible **(S)** and highly susceptible **(HS)** reactions. A separation point for R and S, and HS was calculated by taking the average of two clusteres and adding the standard error of the means (Eyal et a l . ,1985; Kari and Sharp, 1986). The average standard error of the mean was calculated by taking the square root of the error mean square and dividing it by the square root of replicates **(SE** = error **MS/r)**. Based on multiple comparison of means of various I and P combinations, it was found that if SE is added to the average value instead of subtracting, the separation points between Rand S, and S and **HS** are more realistic, The final separation value (lesions cm^{-2}) between R and S was 15.25 (12.50 + 2.75) which was rounded off to 16. Simiiariy, the separation value between S and HS was 39.230 (32.57 + 6.65) which was rounded to 40. This way, provenances having mean lesions cm^{-2} upto 16 were considered as R,>16 to 40 as S and >40 were considered as HS. The frequency of virulence of each isolate was

calculated by dividing the number of provenances with S reaction (including HS reaction) by the total number of 11 eucalypt differential provenances (Kari and Sharp, 1986).

Statistical analysis

Data for each isolate were subjected separately to one-way ANOVA and eucalypt differential provenances ranked using Duncan's multiple range test (DMRT). To find out any significant provenance x isolate interation the data were also subjected to two-way ANOVA. Multiple comparison of mens was done to find out differences among'the isolates in their reactions on a differential provenance.

RESULTS

In one-way ANOVA, Eucalyptus differential provenances showed significant differences in CLB susceptibility to all the five CQ isolates (Table 7.1). Susceptibility ranking of different provenances to five isolates also differed significantly indicating differential interaction which is clearly evident by the two-way ANOVA (Tables 7.2, 7.3). The isolates and provenances differed significantly at p = 0.001 in virulence and susceptibility respectively and the interaction between them was also significant at p = 0.001. This showed that relative CLB susceptibility between provenances depended on the CQ isolates. Similarly, the relative virulence between isolates depended upon the provenances. Since mean square of isolates was greater than that of provenances, it possibly indicated that disease severity is mainly governed by the genetically different isolates and also that the provenances have a closer genetical relationship.

Cluster analysis of mean ieaf lesions (cm^{-2}) of 55 combinations of isolates and differential provenances showed three statistically significant clusters with mean lesions (cm^{-2}) of 7.66, 24.25 and 58.11 representing respectively R, S and HS combinations (Table 7.4). In R cluster, there were as many as 37 combinations involving all 11 provenances and five isolates while S cluster had only 10 combinations all excepting one provenance i.e. *E.* propinqua 12800 and only three isolates (755, 897 and 1080).In **HS** cluster, there were only 4

Table 7.1. Mean lesions (cm^{-2}) of Cylindrocladium leaf blight produced by five isolates of C. quinqueseptatum on detached leaves of *Eucalyptus* differential provenances

S. No.	Eucalyptus differential provenance	Leaf lesions (cm^{-2}) caused by different isolates of C. *quinqueseptatum* 755	897	947	968	1080
1.	*E.* tessellaris 12967	17.62^{b*}	9.24^{def}	14.52^{f}	0.78^{d}	6.32.
2.	E. brassiana 13412	24.56^{a}	16.89^{b}	70.08^{a}	8.49^{a}	7.21^{f}
3.	*E. tereticornis* 13398	2.25^{e}	6.78^{f}	29.68^{cde}	1.32^{d}	5.57^{f}
4.	E. *urophylla* 12895	5.69^{e}	32.44^{a}	37.11^{bcdef}	5.84^{abc}	18.38^{a}
5.	E. *saligna* 13027	6.18^{e}	11.06^{def}	33.08^{bcde}	4.58^{bcd}	11.93^{bcd}
6.	*E.* brassiana 13397	17.25^{bcd}	10.13^{def}	56.67^{ab}	1.91^{d}	7.40^{f}
7.	E. *urophylla* 12896	17.42^{bc}	14.25^{bc}	15.17^{cde}	7.92^{ab}	7.43^{f}
8.	*E.* grandis 13020	6.95^{e}	17.46^{bc}	53.47^{abc}	4.23^{bcd}	12.71^{bc}
9.	*E,* brassiana 13415	3.85^{e}	12.82^{cde}	39.89^{bcde}	5.87^{abc}	8.55^{ef}
10	*E.* grandis TN Local	4.25^{e}	18.06^{bc}	31.77^{cde}	4.21^{bcd}	11.01^{bcde}
11	*E.* propinqua 12800	13.32^{bcd}	8.33^{ef}	52.25^{abcd}	2.29^{cd}	13.04^{b}

Values in each column with same superscript(s) do not differ significantly at p = 0.01.

combinations involving isolate 947 and *E.* ***bassiana*** 13412, 13397, *E.* grandis 13020 and *E.* propinqua 12800. The Latter clearly indicated that isolate 947 is the most virulent of five. This was further confirmed from the data presented in Table 7.5 which shows that isolate 947 is virulent on all the provenances, except *E.* ***tessellaris*** 12967 and *E.* ***urophylla*** 12896. These two provenances may be closely related genetically as they gave identical susceptibility reactions to all the five CQ isolates, isolate 1080 was virulent only on *E.* urophylla 12895, whereas isolate 968 did not give virulent reaction on any of the differential provenances. The remaining two isolates 897

Table 7.2. Combined one-way ANOVA of mean leaf lesions (cm^{-2}) of Cylindrocladium leaf blight produced by five isolates of C. *quingueseptatum* on *Eucalyptus* differential provenances

C. *quinqueseptatum* isolates	Mean square	V-ratio
775	332.47	9.98**
897	313.00	10.57**
947	1804.14	4. 14**
960	39.96'	3.91**
1080	88. 27	10.48**

** Significant at P <0.01.

Table 7.3. Two-way ANOVA of mean leaf lesions (cm^{-2}) of Cylindrocladium leaf blight produced by five isolates of C. *quinqueseptatum* on *Eucalyptus* differential provenances

Source	SS	DF	MSS	V-ratio
Frovenance (P)	6968.22	10	696.82	7.1**
lsolate (1)	49534.78	4	12383.67	126. 19**
PX I	18816.78	40	470.41	4.79**
Error	26986.09	275	98.13	
Total	102305.80	329		

** Significant at P = 0.001.

and 755 gave virulent reactions respectively on 4 and 5 provenances of which only E.brassiana 13412 was common to both. The frequency of virulence of five isolates showed significant differences among them.

Isolate 947 had the highest percentage of virulence of 81.81, and isolate 968, the least (0) as it gave virulent reaction on all the provenances; the respective figures for isolates 1080, 897 and 755 were 9.09%, 36.36% and 45.45%.

Table 7.4. Cluster analysis of mean leaf lesions (cm^{-2}) produced by five isolates (1) of *C. quinqueseptatum* on *Eucalyptus* differential provenances (P)

Clusture No.	No. of P X I combinations (Provenances cluster[a])	Frequency of P X I combination in cluster (%)	Isolate No.	Mean leaf lesions[b] cm^{-2}	Disease rating[c]
1	37 (1-11)[a]	67.27	all 5 isolates	7.66	R
2	10 (1-10)	18.18	755, 897, 1080	24.25	S
3	4 (2,6,8,11)	7.27	*947*	58.11	HS

a: Number refer to serial numbers in Table 7.1.
b: Mean leaf Iessions based on six replicate leaves.
c: R, Resistant; S, Susceptible; HS, Highly susceptible.

The most susceptible provenance on which atleast three isolates gave S to HS reactions was *E. **brassiana*** 13412, while the least susceptible or resistant were ***E. tessellaris*** 12967, ***E. urophylla*** 12896, ***E. tereticornis*** 13398, ***E. saligna*** 13027, and ***E. brassiana*** 13415 on which only one isolate gave susceptible reaction (Table 7.5).

DISCUSSION

Disease management today is based mainly on expensive fungicides and modified cultural practices, which provide only partial protection. Therefore, effective disease control strategies also include introduction of resistant cultivars as a major component. This

Table 7,5. Cylindrocladium leaf blight reactions on detached leaves of differential provenances of *Eucalyptus* to five isolates of C. *quinqueseptatum*

Sl. No.	Differential provenances	Susceptibility reaction[a] of provenance to isolates of *C. quinqueseptatum*				
		755	897	947	968	1080
1.	*E. **tessellaris*** 12967[b]	**S**	**R**	R	**R**	**R**
2.	*E. **brassiana*** 13412	***S***	***S***	**HS**	**R**	R
3.	*E. **tereticornis*** 13398[c]	**R**	**R**	***S***	**R**	R
4.	*E. **urophylla*** 12895	**R**	***S***	***S***	**R**	***S***
5.	*E. **saligna*** 13027c	**R**	**R**	**S**	**R**	R
6.	*E. **brassiana*** 13397	***S***	**R**	**HS**	**R**	R
7.	*E. **urophylla*** 12096[b]	***S***	**R**	**R**	**R**	R
8.	*E. **grandis*** 13020	R	***S***	**HS**	**R**	**R**
9.	*E. **brassiana*** **13415**[c]	R	R	S	R	R
10.	*E. **grandis*** TN Local	R	***S***	***S***	R	**R**
11.	*E. **propinqua*** 12800	***S***	R	**HS**	**R**	**R**

is more relevant to tree crops, having long rotation. For the identification of provenances with long-lasting resistance it is essential to assess variation in the virulence of the pathogen in the existing populations. it has been recommended to use a number of pathogen isolates in the selection programme that will maximise the genetic gains among the plant genotypes because it is a function of heritability which is in turn a function of the genetic variance (Falconer, 1981). In this context it is imperative to distinguish pathogenic strains in a given population of ***Cylindrocladium quinqueseptatum***

It is well known that presence of specific virulence can be detected only when isolates are evaluated at identical inoculum densities and environmental conditions as done in the present

experiment. Comparison of the five monoconidial isolates, taken from each of the parent isolates, showed that four (755, 897, 947, **1080)** have specific virulence or wide variability in their reactions which possibly means that the provenances may have in common same genes for resistance (Hadley et al., 1979, Black and Beute, 1984). One isolate (968) possibly possesses general or uniform virulence within the sampled population as it gave identical reactions to all the eucalypt genotypes. It is evident from the results that the dynamics of virulence in the population of CQ is much more complex than excepted.

High statistically significant isolate x provenance (I x P) interaction clearly shows the specificity in horizonta1 resistance (HR) in various *Eucalyptus* differential provenances. It could result in positive selection pressure by the chosen provenances on the pupulation of the **CQ** strains. Thus, following the establishment of monoculture plantings from the same seed source it could result in epidemic as has been observed in Kerala. Also, the isolate-specific reactions of the provenances to CQ may possibly show long periods of co-evolution of the pathogen and Eucalyptus in Austraiia. As regards the interaction methoc for the identification of specific resistance in host, several criticisms have been raised including the possible confounding of I **x** P effects with 1 x P x environment effects (Jenns and Leonard, 1984) and spurious I x P effects due to lack of a proper scale for measuring disease severity (Winer, 1984). Another serious problem with the ANOVA approach for detecting specificity in HR is that such effects usually account for only small fractions of the total variability even with complex specificity (Parlevliet and Zadoks, 1977; Fleming and Person, 1982). However, in our experiments the inoculations were done with uniform inoculum under identical environmental conditions and also instead of a disease measuring scale the GLE severity was measured quantitatively as lesions cm^{-2}. Furthermore, in ANOVA of ***CP-Eucalyptus*** system the mean squares **(MS)** for the main effects as well as for the I x P interactions are very high with a low variance. This means that conclusions drawn here wiii have less chances of error. It is interesting to note that the results do not seem to fit in any of the six models proposed for the host-pathogen interaction in apparently horizontal pathosystems (Carson, 1987). In all the six models evaluated by him the MS are high for the

cultivar but in ***CQ-Eucalyptus*** pathosystem the MS is high for the isolate.

Pathogenicity studies confirm a great deal of variability in virulence among the five monoconidial ***CQ*** isolates which are distinguishable in different strains on the basis of quantitatively distinct reactions on a set of differential provenances and their virulence. This is the first evidence for the existence of physiologic strains in ***Cylindrocladium quinqueseptatum.*** Because of the variability inherent in the pathogen, prevalence of its sexual stage ***(Calonectria quinqueseptatum)*** and its wide host range, development of physiologic strains will be but expected. As is typical of'many soil-borne pathogens together with the production of conidia of ***CQ*** in slimy masses, CLB spread among field sites will be restricted as compared to air-borne pathogens such as those causing rust diseases. Under these circumstances, it is expected that the population of ***CQ*** strains will be stabilized in their environment during ***Eucalyptus*** rotations. This is evident from the incidence of CLB which was very high initially but slowly and gradually showing a slight decline and appearing to be stabilized. Effects of monoculture plantings from the same seed source on virulence of CQ might also vary among plantations in high and low elevations due to different eucaiypt species grown in these areas and environmental interactions. Once the pathogen is changed by the introduction of resistant provenances the selection pressure should remain intact until further selection pressure is applied.

8. *In vitro* Evaluation of Fungicides Against Cylindrocladium spp.

Despite the economic importance of eucalypt seedling and leaf blight diseases no proper control measures have been worked out in a systematic manner. In laboratory studies, Anahosur *et al.* (1977) found Bavistin and Thiram as highly effective (ED 100) in inhibiting the growth of C. *quinqueseptatum* in poisoned- food technique. Since there are more than one species of *Cylindrocladium* associated with various diseases of eucalypts, and fungicides were not evaluated using soil-fungicide technique to confirm the inhibition of microsclerotia produced by the pathogen, these observations have little importance in controlling *Cylindrocladium* diseases in Kerala. With the objective of affording chemical control of *Cylindrocladium* diseases in nurseries, various fungicides were evaluated *in vitro* for their efficacy against two major species i.e., C. *quinqueseptatum* and *C. ilicicola.* Though not as harmful as the former, three other species viz. C. *camelliae, C. floridanum* and C. *parvum* were also included in some of the screening methods to find out fungicide(s), if any, equally effective against all the five species of *Cylindrocladium.*

MATERIALS AND METHODS

Cylindrocladium spp. were isolated from diseased eucalypt seedlings and their cultures maintained on potato dextrose agar medium (PDA). A total of 22 fungicides (Table 8.1)were evaluated against various *Cylindrocladium* spp. following conidial germination technique (CGT), poisoned-food technique (PFT) and soil-fungicide screening technique (SFST). The purpose of employing three techniques was to ascertain the efficacy of fungicides in inhibiting conidial germination and mycelial growth and rendering microsclerotia non-viable. For conidial germination, hanging drop method as described earlier in Chapter 3 was used. Conidial suspensions of C. *quinqueseptatum, C. ilicicola, C. floridanurn* and *C. parvum* were prepared from 7-day-old cultures in 0.05, 0.1, 0.2, 0.3 and 0.52 a.i. solutions of various fungicides and hanging drop set-ups incubated at 25 + 2°C. From six replicate hanging drops of each treatment

iconcentration of a fungicide) 25 observations were recorded on percent inhibition of conidial germination after 12 h of incubation. Similar observations were also recorded from the control set where conidia were germinated on sterile tap water. For each observation percent inhibition over control was calculated as follows and the mean determined.

$$I = \frac{T - C}{100 - C} \times 100$$

where, I = percent inhibition in conidial germination over control, T = percent inhibition in treatment, and C = percent inhibition in control.

For FFT and SFST only three fungicidal concentrations viz. 0.05, 0.1 and 0.2% (a.i.) were used and PDA was utilised as the growth medium. C. quinqueseptatum, C. *ilicicola, C. floridanum* and C. *parvum* were screened by PFT while C. *quinqueseptatum,* C. *ilicicola* and *C. camelliae* through SFST. In PFT, all the 22 fungicides were screened. The requisite quantity of the fungicide was added to autoclaved medium *of* 25°C and its equal quantity dispensed in assay Fetri dishes using a plunger. Mycelial discs, 4 mm in dia taken from the periphery of actively growing 7-day-old culture were inoculated at the centre of each Petri dish and the latter were incubated at 25+ 2°C. There were three replicates for each concentration and a control was maintained without fungicide. Three observations on colony diameter were recorded on the seventh and fifteenth day of incubation from each replicate. The percent inhibition of growth in each treatment was calculated by the following equation (Vincent, 1927) and the mean calculated.

$$I = \frac{100(C-T)}{C}$$

Where I = inhibition over control, C = growth in control and T = growth in treatment.

The soil-fungicide screening technique (SFST) described by Zentmeyer (1955) and Corden and Young (1962) was modified for evaluating the efficacy of fungicides in rendering the microsclerotia nonviable. The procedure is as follows. Air dried nursery soil was

sieved through a sieve having 5 mesh cm^{-2} and autoclaved for 45 min at 1 kg cm^{-2} pressure. After cooling 10 g of the soil was placed in sterile glass vial of 30 mm dia and 80 mm length. A culture disc (8 mm in dia) punched from an actively growing 10-day-old colony having abundant microsclerotia was transferred over the soil. Another 10 g of sterile soil was placed over the disc. About 7-9 ml of fungicide solution prepared in sterile water, was gently poured over the soil surface using a sterile pipette; each concentration had three such vials. In control vials only distilled sterile water was poured. The mouth of the vial was covered with aluminium foil. The vials were incubated for 24 h at 25+ 2°C. After incubation, the soil from the vials was emptied gently and the agar disc removed with sterile forceps. The disc was washed in three changes of sterile water to remove the adhering soil particles and transferred to a Petri dish containing PDA with the mycelial surface facing down. Observations on the diameter growth of the colony were recorded after seven days. Percent inhibition in growth in each treatment was calculated using the same equation as given above.

Diameter growth data of treatments and control in PFT and SFST were also analysed by two-way ANOVA. Due to high rainfall in Kerala ED_{100} was only considered as the effective dosage of a fungicide.

RESULTS AND DISCUSSION

Before attempting to control a disease in field, preliminary *in vitro* laboratory screening has its importance as it eliminates compounds that show little or no inhibition in conidial germination or colony growth. This objective is fully achieved from *in vitro* evaluation of fungicides against *Cylindrocladium* spp. as evident from the data presented in Tables 8.1 and 8.2. Results in respect of individual species of *Cylindrocladium* are discussed seperately.

Efficacy of fungicides against *Cylindrocladium* spp,

C. *quinqueseptatum*

There were a number of fungicides (Group 1) viz. chlorothalonil, captafol, mancozeb, zineb, copper oxychioride, guazatine, metiram,

Table 8.1. Evaluation of various fungicides against *Cylindrocladium quinqueseptatum* and *C. ilicicola* using three screening techniques

	Fungicide and concentration (% a.i.)		Percent inhibition over control[a]					
			C. quinqueseptatum			*C. ilicicola*		
			CGT	PFT	SFST	CGT	PFT	SFST
1.	Benomyl	0.05	38.48	100	3.87		100	6.95
	(Benlate)	0.2	72.14	100	13.23	100	100	6.85
		0.2	100	100	18.97	100	100	9.05
2.	Bordeaux mixture	0.05	95.55	51.20	0.37		78.86	4.52
		0.1	98.31	100	1.74	100	100	7.09
		0.2	100	100	6.61	100	100	7.71
3.	Captafol	0.05	100.00	35.34	10.48	100	65.02	6.73
	(Difolatan)	0.1	100	55.41	9.36	100	73.22	7.58
4.	Carbendazim	0.05	21.31	100	100	100	100	54.22
	(Bavistin)	0.1	56.13	100	100	100	100	69.03
		0.2	88.43	100	100	100	100	100.00
5.	Chlorothalonil	0.05	100	27.63	7.11	83.89	54.21	7.7 I
	(Daconil)	0.1	100	36.63	3,49	96.68	56,05	11.50
		0.2	100	47.00	4.86	100.00	59.51	20.19
6.	Copper oxychloride	0.05	100	71.12	6.61	85.09	100	2.32
	(Fytolan)	0.1	100	81.31	9.23	91.62	100	26.80
		0.2	100	84.36	11.23	97.54	100	30.59
7.	Dodine	0.05	100	62.66	-b	84.14	85.02	-b
	(Syllit 65)	0.1	100	73.16		86.51	100	-
		0.2	100	100		91.81	-	
8.	Etridiazole	0.05	94.55	86.93	7.61		100	6.12
	(Terrazole)	0.1	94.55	96.97	11.61	-	100	4.65
		0.2	100	100	18.21		100	8.44
9.	Etridiazole	0.05	85.93	68.94	2.24		83.85	13.63
	(Quintozene)	0.1	100	78.52	9.36		100	17.25
	Terrachlor Super-X	0.2	100	84.13	22.09		100	100
10.	Guazatine	0.05	100	88.80	2.87	93.65	100	0.0
	(Panolil)	0.1	100	100	6.61	96.79	100	0.0

contd. ...

11.	IBP	0.05	4.62	84.62	6.69	74.14	100	0.12
	(Kitazin)	0.1	5.49	87.39	8.36	93.52	100	6.11
		0.2	76.10	100	10.73	100	100	7.09
12.	Mancozeb	0.05	100	26.70	6.49	89.76	51.32	4.26
	(Dithane M-45)	0.1	100	41.46	5.24	95.15	64.90	7.71
		0.2	100	70.81	5.86	100	69.13	20.68
13.	Metiram	0.05	100	21.09	7.61		45.55	0.0
	(Polyram Combi)	0.1	100	22.02	8.73		63.36	0.0
		0.2	100	26.70	21.84		69.64	7.58
	Sodium azide	0.05		100	100		100	-
		0.1		100	100		100	
		0.2		100	100		100	
15.	TCNTB	0.05	95.85	100	-	55.85	100	
	(Busan-30)	0.1	96.73	100	-	96.73	100	
		0.2	97.45	100	-	97.45	100	
16.	Thiram	0.05	100	58.91	3.37	91.25	77.45	4.03
	(Thiride)	0.1	100	69.42	9.11	100	82.84	18.23
		0.2	100	79.70	12.73	100	85.40	21.66
17.	Triadimefon	0.05	26.03	47.59	-		56.95	
	(Bayleton)	0.1	29.79	48.87	-	100	58.11	
		0.2	69.04	55.17	-	100	65.92	-
18.	Tritorine	0.05	97.57	72.93	-	86.95	90.77	
	(Saprol)	0.1	100	86.23	-	100	100	
		0.2	100	100		100	100	
19.	Zineb	0.05	100	21.32	-	95.24	35,43	
	(Dithane-Z 78)	0.1	100	27.63	-	95.41	41.71	
		0.2	100	58.68	-		71.42	

[a]Uata for chloroneb, quintozene and tridemorph are not included since no dosage gave 100% inhibition.

[b]Dosage not attempted.

CGT = Conidal Gemination Technique; PFT = Poisoned-Food Technique;

SFSP Soi l Fungicide Screening Technique.

not directly comparable show that mancozeb (0.16%) was effective than benomyl (0.025%), captan (0.018%) and copper oxychloride see Bolland *et al.* 1985 paper for Cu oxychlorid% concentration; carbendazim was

dodine and thiram highly effective in bringing about cent percent conidial inhibition at 0.05% a.i. (Table 8.1). Certain other fungicides (Group 11) such as carbendazim, benomyl, Bordeaux mixture, busan-30, IBP (Kitazin), triforine, etridiazole + quintozene (Terrachlor Super-X), etridiazole (Terrazole) had E_{100} at 0.2 or 0.3% (a.i.) concentration. However, in PFT where ANOVA of fungicide (factor 1), concentration (factor 11) and their interaction was highly significant, the behaviour of some of the fungicides in Group 1 was at variance with conidial germination technique. None of the concentrations of chlorothalonil, captafol, mancozeb, zineb, copper oxychloride, metiram and thiram had ED_{100}, while in guazatine, dodine only nigher concentrations gave ED_{100}. Conversely, in Group I1 fungicides carbendazim, benomyl and busan-30 inhibited the growth at 0.05% (a.i). In SFST, where only factor 1 and 11 were significant and not their interaction, carbendazim was found effective with 0.05% a.i. at Ed_{100}; none of the concentrations of other fungicides, except tridemorph, had even ED_{50}. Besides, sodium azide, which was not evaluated in CGT, showed complete inhibition at all the concentrations in both the methods. Hence, it is clearly evident from the above results that carbendazim and possibly also sodium azide are the most promising fungicides against ***C. quinqueseptatum***.

Thoughthere are numerous reports for ***in vitro*** and ***in vivo*** screening of fungicides against ***Cylindrocladium*** spp., literature concerning ***C. quinqueseptatum*** is very meagre. In laboratory evaluation, Anahosur et al. (1977), reported that out of the ten fungicides tested in PFT only carbendazim and thiram had ED_{100}, respectively at 0.05% and 0.1% a.i. concentration; Hexaferb gave ED_{100} at 0.3% a.i. Our results of carbendazim, mancozeb, copper oxychloride (Fytoran) are in agreement with Anahosur et al. (1977), except for thiram and tridemorph. In our studies thiram gave maximum inhibition of 79% at 0.3% (a.i.) as against cent percent inhibition reported for 0.1%. Similarly, tridemorph gave complete inhibition at 0.2% a.i. as against for reported 70.59% at the same concentration. These differences in the efficacy of certain fungicides could be due to the agressive nature of the ***C. quingueseptatum*** strain (isolate 947) used in this study. Though results of Bolland *et.al.* (1985), who evaluated a few fungicides against ***C. quinqueseptatum*** in glass-house trial, are

not included in the trials. Since the results are presented in relative term it is not clear whether complete control of the disease was achieved by mancozeb. As regards the efficacy of sodium azid, which is not a well known fungicide, earlier Rowe et al. (1974) have demonstrated this fungicide to be the most effective against Cylindrocladium black rot of Arachis hypoges L. caused by C. crotalariae.

C. ilicicola

In CGT, carbendazim, captafol, benomyl, Bordeaux mixture and triadimefon were found to be equally effective in causing cent percent conidial inhibition at all the five concentrations (Group I); triforine, quintozene (PCNB) and thiram were effective only at 0.1% a.i. (Group 11) (Table **8.1)**. Tridemorph was least effective as it did not bring about 100% inhibition even at 0.5%. In PFT, of the Group I fungicides only carbendazim, benomyl and Bordeaux mixture were found most effective. In SFST, highest inhibition was obtained in carbendazim, **ED100** being 0.2%a.i.; other fungicides did not even have ED50. It is evident from the results that carbendazim is the only fungicide which can inhibit conidial germination and mycelial growth, and render microsclerotia non-viable. In ANOVA of PFT and SFT data, fungiceds, concentration and their interaction were highly significant. There is not literature available on the efficacy of any fungicide against this species.

C. floridanum

For this species, fungicides were evaluated only by CGT and PFT. In CGT almost all the 14 fungicides tested were found to be highly effective (Table 8.2). However, in PFT only carbendazim, benomyl, busan-30 and sodium azide gave cent percent inhibition of growth. Earlier, Horst and Hoistink (1968) have also reported that out of the 16 fungicides screened only carbendazim afforded control of Cylindrocladiurn blight caused by C. floridanurn and C. scoparium under field conditions. Potassium azide, a compound related to sodium azide, was found to be highly effective in controlling ***C. floridanum***

Table 8.2. Evaluation of fungicides against *Cylindrocladium floridanum*, *C. parvum* and *C. camelliae* using various screening techniques

Fungicides and concentration (% a.i)		Percent inhibition over control[a]			
		C. *floridanum*		C. parvum	C. *camelliae*
		CGT	PFT	PFT	SFST
1. Benomyl (Benlate)	0.05	100	100	100	.0.39
	0.1	100	100	100	2.75
	0.2	100	100	100	5.91
2. Bordeaux mixture	0.05		68.91	0.0	2.49
	0.1	96.47	76.27	0.0	2.89
	0.2	100.00	100	100	1.31
3. Captafol (Difolatan)	0.05		26.89	69.66	0.0
	0.1	91.66	35.79	69.40	0.0
	0.2	100	42.94	70.39	0.0
4. Carbendazis (Bavistin)	0.05	-	26.89	69.66	0.0
	0.1	95.89	100	100	100
	0.2	100	100	100	100
5. Chlorothalonil (Daconil)	0.05	[b]	21.46	50.06	[b]
	0.1	100	48.88	53.98	
	0.2	100	49.69	70.39	
6. Copper oxychloride (Fytolan)	0.05	-	0.0	0.88	0.0
	0.1	100	12.57	23.17	0.13
	0.2	100	27.81	30.26	0.0
7. Dodine (Syllit 65)	0.05		0.0	0.0	
	0.1	94.68	1.03	1.87	
	0.2	100	3.27	40.15	
8. Etridiazole (Terrazole)	0.05		70.17	68.67	
	0.1	-	82.82	83.85	
	0.2		100	100	
9. Etridiazole (Quintozene Terrachlor Super-x)	0.05		76.27	72.59	
	0.1		80.78	100	
	0.2		100	100	

contd....

10.	Guazatine	0.05		17.59	11.16	
	(Panoli I)	0.1	100	53.78	17.77	
		0.2	100	82.01	27.31	
11.	IBP	0.05		71.1%	84.58	
	(Kitazin)	0.1	100	75.06	100	
		0.2	100	82.01	100	
12.	Mancozeb	0.05		35.57	13.61	0.91
	(Dithane M-45)	0.1	100	49.49	12.88	0.0
		0.2	100	100	14.31	0.0
13.	Sodium azide	0.05		100	100	
		0.1	100	100	100	
		0.2	100	100	100	
14.	TCMTB	0.05		100	100	
	(Busan-30)	0.1	100	100	100	
		0.2	100	100	100	
15.	Thiraa	0.05		45.40	83.31	
	(Thiride)	0.1	100	47.24	100	
		0.2	100	54.61	100	
16.	Triadimefon	0.05		81.39	34.91	
	(Bayleton)	0.1		82.62	53.25	
		0.2	-	100	52.77	
17.	Triforine	0.05		83.43	10'	
	(Saprol)	G.1	100	88.48	100	-
		0.2	100	100	100	
18.	Zineb	0.05		9.81	0.39	
	(Dithane -Z	0.1	100	25.56	4.55	-
		0.2	100		8.72	-

[a]Data for chloroneb, metiram, quintozene and trideeorph are not included since no dosage gave 100% inhibition.

[b]Dosage not attempted.

CGT = Conidial Germination Technique; PFT = Poisioned-Food Technique; SFST = Soil Fungicide Screening Technique.

on peach seedlings (Weaver, 1971). In ANOVA, fungicides, concentration and their interaction were found to be highly significant.

C. parvum

Since the conidia of this species were minute and fungicidal particles obstructed in recording observations, conidial germination studies were not successful. In PFT, carbendazim, benomyl, busan-30, triforine and sodium azide were the most effective (ED_{100}) fungicides; IBP (kitazin), etridiazole + quintozene and thiram were effective only at 0.1%a.i. (Table 8.2). In ANOVA fungicide concentration and their interaction were found highly significant at 1%. There is no literature available on the fungicides effective against this species.

C. camelliae

Only SFST was used to evaluate the efficacy of six common fungicides. The only effective fungicide was carbendazim. In ANOVA. only fungicides were found to be significant and their concentrations did not appear to differ from each other in efficacy. This is the first report of an effective fungicide against ***C. camelliae.***

Comparison of efficacy of fungicides against various *Cylindrocladium* SPP.

It is evident from the results that there is a differential effect of many fungicides depending upon the species of ***Cylindrocladium.*** Similarly, the **ED_{100}** of a fungicide also varied depending upon the species. However, there were a few fungicides which were more or less equally effective against all the Cylindrocladium spp. tested using a particular screening technique. In conidial germination technique, of the 22 fungicides tested against ***C. quinqueseptatum*** **(CQ)**, 12 showed cent percent conidial inhibition at 0.1% a.i. For ***C. floridanum*** **(CF)** 10 out of 15 fungicides were highly effective. But for C. ilicicola **(CI)** which appears to be more toierant than other species only 6 out of 18 fungicides were found to be effective. There were certain fungicides equally effective against any two species but not against the third one.

In PFT, carbendazim, benomyl, busan-30 and sodium azide are equally effective against all the four species of Cylindrocladium **(CQ,**

Cl, CF and **CP**). However, copper oxychloride, Kitazin, guazatine Terrazole are highly effective against CI but not against CQ, CF and **CP**.

In **SFST**, carbendazim stood out as the only fungicide effective against all the three species of ***Cylindrocladium*** **(CQ**, CF and C1) screened; none of the others caused even 50% inhibition in growth.

On comparing the effective fungicides for various ***Cylindrocladium*** species it is amply clear that carbendazim is the only fungicide consistently found effective against all the five species of ***Cylindrocladium***. But surprisingly, in the CGT, carbendazim has ED100 only at 0.2% a.i. while in the other two screening methods ED_{100} is at 0.05% a.i. This anomaly in the behaviour of carbendazim is not clearly understood as repetitive tests gave similar results. In this situation, to control foliar infection caused by CQ, usually through conidia, only higher concentration (0.2%) of carbendazim will be effective. However, when applied to soil even the lower concentration (0.05%) will be effective in preventing infection which is usually through microsclerotia surviving in soil. Soil application of carbendazim, a broad spectrum systemic fungicide with apoplastic and symplastic movement in plant parts, has added advantage on its systemic distribution is effected more thoroughly by soil application (Peterson and Edgington, 1970; Anon., 1981). The period of persistency in the plant tissues is also long if it is applied through soil (Sole1 ***et al.***, 1973). Carbendazim has also been found effective against some other species of ***Cylindrocladium*** such as ***C. colhouni*** Peerally on ***Eucalyptus*** spp. (Nair and Jayasree, 1986) and C. ***scoparium*** Morg. on ***Azalia*** cuttings of (Horst and Hoistink 1968).

Comparison of fungicidal evaluation techniques

Generally, only one technique is employed for evaluating the efficacy of fungicides against a pathogen. However, in the present study fungicides were screened using three techniques and the results obtained are quite interesting. For all the species of ***Cylindrocladium*** tested almost a similar pattern of number of effective fungicides emerged. In CGT, a large number of fungicides were found highly effective (ED_{100}) while in PFT this number got reduced. In

SFST, the number of effective fungicides got further reduced leaving only one or two fungicides with ED_{100}. The possible explanation for this difference is that a large number of fungicides are effective in inhibiting the conidial germination or mycelial growth but not microsclerotia. In PFT, microsclerotia immersed in the agar disc are able to germinate and grow out while in SFST since the fungicide passes through the agar disc, mycelium and microsclerotia embedded therein get affected, thus bringing about total inhibition in growth, provided the fungicide is effective against the microsclerotia. Thus it is amply clear that for pathogen producing microsclerotia, the soil-fungicide screening technique (SFST) should be employed for finding out reliable and effective fungicides.

Though the results of field trials are always useful in judging the effectiveness of fungicides under various climatic/soil conditions, preliminary laboratory screening of fungicides that show little or no inhibition of conidial germination, mycelial growth or microsclerotia should be carried out to save time and efforts in conducting field trials. Unfortunately, many studies on chemical control are initiated in the field rather than in the laboratory, with the assumption that the effective fungicides already reported for a particular pathogen will also be effective against the disease in question caused by the same pathogen. In this process, the host origin of the pathogen and possibility of dealing with a different biotype of the pathogen are ignored resulting in partial success in the disease control,

9. Effect of Post-Sowing Pre-emergence Fungicidal Application on Damping-off of *Eucalyptus grandis* Caused by *Rhizoctonia solani*

Earlier investigations in eucalypt nurseries have shown that ***Cylindrocladium*** spp. and ***Rhizoctonia soiani*** Kuhn state of ***Thanatephorus cucumeris*** Frank & Donk are the major pathogens responsible for the pre-and post-emergence damping-off and other seedling diseases of ***Eucalyptus*** (Sharma et al., 1985). Among these ***R. solani*** causes heavy mortality of seedlings due to damping-off. Besides the inocuium potential, other important factors which may affect the severity and spread of damping-off are the soil moisture and seedling density. For standardising the nursery practices for eucalypts, the efficacy of a few fungicides was evaluated against ***R. soiani*** in ***in oivo*** studies in relation to inoculum concentration, soil moisture regime and seedling density.

MATERIALS AND METHODS

A culture of R. solani, obtained from damped-off seedlings of ***E. grandis,*** was grown on cornmeal-sand medium for two weeks at 25 + 2°C. Mycelial mats containing abundant sclerotia were harvested, air dried for 24h and powdered in a Waring blender. lnoculum was mixed separately with the sterilized soil in two proportions **(1:100** - 11; **1:1000** - 12; inoculum to soil on weight basis) in aluminum trays **(15** x 15 cm) as described earlier by Sharma and Sankaran (1987). Two levels of soil moistures were maintained by pouring water at the rate of 300 mi **(W1)** and 450 **ml (W2)** per tray. A total of 96 trays were thus prepared, covered with a paper and incubated at room temperature for five days. The trays were watered everyday as per the soil moisture regime requirements. Seeds of ***E. grandis*** were sown uniformly at two seed rates i.e., SR1 (320 mg/tray or 20 g/standard seed bed nf 12 x 1.2 m) and SR2 (560 mg/tray or 35 g/standard seed bed). Immediately after sowing one application of three fungicides viz. Captan, carboxin (Vitavax), and PCNB (Brassicol) was made separately at a concentration of 0.05% (a.i.) to each set of trays having different seed rates, inoculum concentration and water regime. A separate set of controls

having all the above treatments except fungicides was also maintained. All the treatment combinations had three replications.

Observations on the number of seedlings emerged, post-emergence damping-off, and other disease symptoms were recorded daily. Data on pre-emergence damping-off was generated from the average seedling density in control and number of seedlings emerged in treatments. The pre- and post-emergence data were analysed statistically using a four-factorial analysis and multiple comparison (DMRT) after appropriate transformations.

RESULTS

Pre-emergence damping-off

Of the three fungicides viz. carboxin, captan and PCNB applied as post-sowing treatment. PCNB was most effective in controlling the pre-emergence damping-off even though no complete control of the disease was brought about; the other two fungicides were less effective and behaved almost similarly. In control sets, where no fungicide was applied, severe pre-emergence damping-off was recorded (Table 9.1).

Significant difference in pre-emergence damping-off occurred at two levels of inoculum **(11** and 12) (Table 9.2); the disease incidence was higher in 12 than in 11. Though, high seed rate **(SR2)** had higher percentage of pre-emergence damping-off than low seed rate **(SRI)**, interactions between seed rates and other variables were non-significant; no significant difference was found in two water regimes (Wl and W2). Irrespective of seed rates the disease was generally higher in low inoculum (11) and low water regime (W1) combinations as compared to 12 x W2 This is confirmed in factorial analysis where there is a significant interaction between fungicide, inoculum and water regime (Table 9.2).

Post-emergence damping-off

All the three fungicides were highly effective in controlling the post-emergence damping-off; insignificant disease (0.13 - 8.41 %) was recorded only in **T1** of Captan treated trays. In control sets,

Table 9.1. Effect of post-sowing fungicidal treatment, inoculum concentration, water regime and sesd rate on incidence of damping

Fungicide	lnoculum	water regime	seed rate	Mean % pre-emergence damping-off	Mean % post-emergence damping-off
Captan	11	w1	SR1	19.56	0.13
			SR2	36.43	0.15
		w2	SR1	28.30	0.40
			SR2	36.46	0.33
	12	W1	SR1	20.96	-
			SR2	26.10	-
		w2	SR1	15.56	-
			SR2	13.30	-
Vitavax	I1	W1	SR1	24.46	-
			SR2	29.60	-
		w2	SR1	26.26	-
			SR2	39.83	-
	I2	w1	SR1	15.40	-
			SR2	17.30	-
		w2	SR1	14.60	-
			SR2	18.30	-
PCNB	I1	w1	SR1	5.23	-
			SR2	7.30	-
		w2	SR1	13.86	-
			SR2	18.76	-
	12	w1	SR1	9.06	-
			SR2	13.43	-
		w2	SR1	9.93	-
			SR2	8.04	-
	11	W1	SR1	13.33	33.16
			SR2	42.70	34.53

contd.....

		W2	SR 1	23.50	18.66
Control			SR2	42.30	23.66
	I2	w1	SR 1	36.60	27.73
			SR2	58.20	18.96
		w2	SR 1	13.30	64.66
			SR2	34.00	62.33

11, 1:100 (inoculum to soil); 12, 1:1000; SR1, 320mg/tray; SR2 = 560 mg/tray; W1 = 300 ml/tray; W2 = 450 ml/tray.

Table 9.2. Analysis of variance of data on pre-emergence damping-off

Source of variation	Mean square	F
Fungicides	17.177	9.341*
inoculum	10.322	5.614*
Water regime	0.086	0. 047^{ns}
Seed rate	16.978	9.003*
2-way interactions		
Fungicides X Inoculum	3.074	1. 672^{ns}
Fungicides X Water	1.538	0.836^{ns}
Fungicides X Seed rate	1.802	0.980^{ns}
lnoculum X Water	1.111	0.604^{ns}
Inoculum X Seed rate	0.636	0.346^{ns}
Water X Seed rate	0.028	0.015 ^{ns}
3-way interactions		
Fungicides X lnocuium X Water	5.883	3.199*
Fungicides X lnoculum X Seed rate	0,974	0.530^{ns}
Fungicides X Water X Seed rate	0.572	0.311^{ns}
lnoculum X Water X Seed rate	0.025	0. 015^{ns}
4-way interactions		
Fungicides X Inoculum X Water X Seed rate	0.377	0. 025^{ns}
Residual	1.839	

* significant at P = 0.01

ns not significant

depending upon the treatment combination, the percentage of disease ranged between 18-64. No significant difference was found in post-emergence damping-off in control either with different seed rates ISR1, SR2) and water regimes (Wl, W2) or inoculum concentration (11,121. However, interaction between fungicides, inoculurn and water regime was significant (Table 9.3).

Table 9.3. Analysis of variance of data on post-emergence damping-off

Source of variation	Mean square	F
Fungicide	316.183	87.675*
Inoculum	4.429	1.228^{ns}
Water regime	7.213	2.00^{ns}
Seed rate	0.002	0.001^{ns}
2-way interactions		
Fungicide X lnoculum	9.302	2.579^{ns}
Fungicide X Water	*6.038*	1.674^{ns}
Fungicide X Seed rate	0.001	0.000^{ns}
lnoculurn X Water regime	14.602	4.049^{ns}
lnoculum X Seed rate	2,242	0.622^{ns}
Water regimes X Seed rate	0.296	0.082^{ns}
3-way interactions		
Fungicide X Inaculum X Water regime	16.401	4.548^{ns}
Fungicide X lnoculum X Seed rate	2.292	0.635^{ns}
Fungicide X Water X regime X Seed rate	0.335	0.094^{ns}
Inoculum X Water regime X Seed rate	0.425	0.119^{ns}
4 - way interactions		
Fungicide X Inoculum X Water regime X Seed rate	0.380	0.105^{ns}
Kes ldual	3.606	

* significant at F = 0.01

ns not significant

DISCUSSION

Management practices and environmental conditions are known to influence damping-off caused by Rhizoctonia solani in forest nurseries so much so that great variability in the occurrence of disease both in the same nursery from season to season and in different nurseries during the same season has been encountered. There may also be great variation in different parts of the same nursery during the same season (Roth and Ricker, 1943).

Though Vitavax (Martin et,al., 1984) and Captan are known to afford good protection against R. solani, they were not so effective as PCNB in controlling pre-emergence damping-off. But the same fungicides provided almost complete control of post-emergence damping-otf. In earlier studies conducted by Sharma and Sankaran (1987) Captan and Terrazole (a PCNB preparation) were not effective but Vitavax controlled the web blight caused by R. solani. This clearly indicates that for controlling damping-off caused by R. solani a fungicide known to be effective against this pathogen should not be used unless its efficacy against a particular isolate of the pathogen causing the disease is ascertained. This is understandably due to the occurrence of anastomosis groups in R. solani. Success of a post-sowing fungicidal treatment is significant as it can be adopted as a standard nursery practice for the control of seedling diseases in eucalypt nurseries.

Of the two important variables, water regime and seed rate, the former appears to be more significant than the latter in influencing the damping-off though higher seed rates consistently had higher disease than the lower seed rate. From these findings it is clear that seed rate and water regime need to be standardized for eucalypt nursery so as to keep incidence and severity of damping-off under check.

10. Nursery Trials for Controlling Seedling Diseases of Eucalypts

In Kerala, a disease complex caused by atleast three pathogens viz. ***Cylindrocladium quinqueseptatum*** (leaf blight and damping-off), ***Rhizoctonia solani*** and ***Fythium*** is known to affect eucalypt seedlings in the nursery, bringing about considerable loss to the nursery stock (Sharma *et al.*, 1985). Under conducive microclimatic conditions, especially in high rainfall (>3500mm) areas, damping-off, web blight and leaf blight together may cause even 100% mortality of seedlings, thus posing practical problems to foresters in meeting the requirement of stock for raising a planned area of plantation. 'Therefore, chemical control of seedling diseases in the nursery, where their economic feasibility is justifiable, appears to be the only solution because it can be easily integrated with other nursery management practices. With this in view, the efficacy of the fungicides evaluated in the laboratory was further tested in the nursery trials conducted during 1981, 1982 and 1983 at Chandanathode, Wynad District, Kerala. Studies were also conducted for standardising nursery practices for eucalypts.

MATERIALS AND METHODS

Experimental site and preparation of nursery

The study was conducted in a nursery area at Chandanathode in Wynad District of northern Kerala, where high mortality (>80%) of seedlings of *E. **grandis*** due to Cylindrocladium leaf blight (CLB) was recorded in previous years. Chandanathode, app. 800 m above mean sea level, receives a high annual rainfall of 6000 mm or more. The area records very high relative humidity throughout the year with mean minimum and maximum daily temperatures 13°C and 32°C, respectively. The soil of the nursery area is well drained, loamy, medium deep and acidic in nature. During the past two consecutive years i.e., 1979 and 1980 the area was occupied by experimental eucalypt nurseries.

The soil of the area was thoroughly worked and experimental beds of 3 m x 1 m x 0.3 m were prepapred at an espacement of 60 cm; all the sides of the bed were provided with a protective covering of bamboo reeds to prevent washing away of the edges of seedbed due to heavy rains and watering.

Insecticide treatment

Each seedbed was drenched with Aldrex 30 EC at the rate of 15 ml in 30 l of water (0.015% a.i.) a week before sowing the seeds to protect seedlings from termite attack (Nair and Varma, 1981).

Fuaigation

Soil fumigation of seedbeds with methyl bromide (MB) (98% MB + 2% chloropicrin) and Di-trapex, attempted only during 1981 trials, was done as described below. Before application of fumigant the soil was moistened and repeatedly worked for three days to facilitate the germination of fungal spores/sclerotia/microsclerotia.

Methyl bromide: A total of six seedbeds were fumigated with MB at the rate of 100 g m^{-2} of soil. For effective penetration of the gas, about 35 pits, 15-20 cm deep, were dug per square meter of soil and the beds covered with thick polythene sheet and all sides sealed with mud except for a little space required for passing the gas through rubber tubes. After releasing the gas the tubes were removed and the gap was also sealed. After two days the sheets were partially opened from three sides to remove the excess gas, and the following day, the sheets were removed. The soil was turned over and mixed thoroughly twice a day for three days for releasing the residual gas caught in between soil particles. The beds were watered just before working of the soil.

Di-trapex : Twentyfive holes, 15-20 cm deep and 2 cm in dia were made per square meter of the bed to be treated with Di-trapex. Two millilitres of the solution was injected in each hole and soon covered with soil. Before covering the seedbeds with a polythene sheet as done with MB treated beds, 15 ml of Di-Trapex was sprinkled over the surface to give a total dosage of 55 ml m^{-2} of soil. Other procedures were similar to MB treatment.

Solar heat treatment

Solar treatment, attempted only during 1981 nursery trials, was given by mulching the seedbeds with thick black polythene sheets (220

gauge). Each bed was covered with pnlythene sheet, 2.50 x 4.50 m and edges sealed with mud on all sides. As far as possible the heds were kept moist constantly. Soil temperature was recorded by soil thermometer at five places before and after mulching and the mean calculated.

Shading

Shade was provided over the beds to protect the young seedlings from sun scorch. Conventional coconut leaf thatch (CLT)was used in **1981** trials, whereas coirmat (CM)of 7 mm mesh was used in **1981** and **1983** trials. After a month of emergence of seedlings, shade was removed partially and when the seedlings were 60-day-old it was removed completely.

Sowing

During the 1981 and 1982 trials one half of each seedbed (1.5 x 1.0 m) was sown with 15g of seeds of *E.* grandis and the other half with *E.* ***tereticornis,*** separately; in 1983 trials only *E. grandis* was used. The beds were borad-cast sown with a mixture of weighed quantity of seeds of Eucalyptus sp. and fine sieved soil **(1:4,** seed to soil weight basis) so as to distribute the seeds uniformly over the beds. The seeds were covered with a 2-3 mm thick layer of fine sieved soil to prevent them from dislodging during watering and to provide moisture during germination. The seeds of high viability (>98%) obtained from the Geneticist, Tamil Nadu Forest Department, Coimbatore, were used in the trials.

Watering schedules

Initially, during the first two weeks after sowing, seedbeds were watered very gently using fine spray rosecan to prevent dislodging and aggregation of seeds leading to subsequent over crowding of seedlings. At each watering 10 l of water was used for a bed.

1981 Nursery Trials: After sowing, each bed was watered three times daily till the time damping-off was recorded. Later watering was suspended for three days to check the spread of diseases. After the

1st fungicidal treatment, watering was gradually increased from one to three times daily till the seedlings were 80-day-old.

1982 Nursery trials:After sowing, each bed received water three to five times daily till emergence. Then the frequency was gradually reduced to three times a day up to 60 days after emergence. Later, the frequency was adjusted according to prevailing climatic conditions.

1983 Nursery trials:Initially, the seedbeds were watered four times daily with 18 l of water per bed at each watering. Later, 3 days after the emergence of seedlings the frequency was reduced to three times daily.

Fugicidal Treatment

The treatments were applied as a soil and foliar drench at the rate of 20 l of fungicidal solution per bed. Schedule of fungicidal treatments during 1981, 1982 and 1983 trials is given in Tables 10.1, 10.2, 10.3. There were three (1981 trials) or five (1982 and 1983 trials) replicate seedbeds for each treatment. A randomised block design was followed throughout the experiment.

Recording observations

Separate observations were recorded for *E. **grandis*** and *E. **tereticornis***. For convenience, various methods of recording observations were adopted. For damping-off, total number of active patches were counted and occurence of patches m^{-2} was calculated. To ascertain whether damping-off was controlled or still active after the treatment, a few bamboo splints, soaked in 0.1% Bavistin and air dried, were inserted at the perimeter of the patch. For seedling blight, a quantitative method was followed. A quadrate, 15 cm x 15 cm, was placed at nine predetermined positions in the bed and number of diseased seedlings counted. For calculating the percentage of diseased seedlings, seedling density was ascertained in three quadrats in each replicate bed and the mean calculated. Severity of Cylindrocladium leaf blight, Rhizoctonia root rot and Sclerotium shoot wilt was rated on a disease index scale (0–5).Since in all cases 100% seedling infection was recorded besides percentage of plants affected, more significance was given to percent of seedling foliage affected.

Table 10.1. Schedule of fungifidal treatments and their dosage used in 1981 *Eucalyptus* nursery trials at Chandanathode

Treatment No.	Fungicide(s)/Fumigant	Date of treatments and % concentration (a.i) of fungicides				
		25 March(12-day-old seedlings)	2 April(22 day-old seedlings)	25 April(45-day-old seedlings)	21 May(71-day-old seedlings)	5 June(65-day-old seedlings)
	Non-systemic fungicides					
T1	Bordeaux mixture	0.1	0.1	0.1	0.1	0.2
T2	Captafol (Difolatan)	0.2	0.1	0.1	0.1	0.2
T3	Chlorothalonil (Daconil-2787)	0.2	0.1	0.1	0.1	0.2
T4	Copper oxychloride (Fytolan)	0.2	0.1	0.1	0.1	0.2
T5	Dodine (Syllit-65)	0.05*	-	-	0.025	0.025
T6	Etridiazole (Terrazole)	0.1	0.1	0.1	0.1	0.1
T7	Etridiazole+Quintozene (Terrachlor Super-X)	0.1	0.05	0.05	0.05	0.1
T8	Mancozeb (Dithane M-45)	0.2	0.1	0.1	0.1	0.2
T9	Metiram (Polyram Combi)	0.2	0.1	0.1	0.1	0.2
T10	Quintozene (Brassicol)	0.1**	20g/m^2	25g/m^2	15g/m^2	20g/m^2
T11	Sodium azide	0.2*	-	-	0.025	0.025
T12	TCMTB (Busan-30)	0.2*	-	-	0.025	0.05
T13	Thiram (Thiride)	0.2	0.2	0.1	0.1	0.2
T14	Zineb (Dithane Z-78)	0.2	0.1	0.1	0.1	0.2
	Systemic fungicides					
T15	Benomyl (Benlate)	0.2	0.1	0.05	0.05	0.1
T16	Carbendazim (Bavistin)	0.2	0.1	0.1	0.1	0.1
T17	Chloroneb (Demosan)	0.25	0.25	0.125	0.1	0.2
T18	Tridemorph (Calixin)	0.5*	-	0.025	0.05	0.1
T19	Triforine (Saprol)	0.5*	-	-	0.025	0.05
	Fumigants					
T20	Methyl bromide (MB)	100 g/m^2 (25 Feb.)				
T21	Methyl isothiocyanate (Di-Trapex)	55 ml/m^2 (25 Feb.)				
	Combinations of fungicides/ fumigant and fungicides					
T22	Bordeaux mixture+benomyl	0.1+0.05	0.1+0.025	0.1+0.025	0.1+0.025	0.1+0.05
T23	Bordeaux mixture+tridemorph	0.1+0.1*	-	0.1+0.025	0.1+0.025	0.1+0.05
T24	Chlorothalonil+carbendazim	0.05+0.05	0.05+0.05	0.05+0.05	0.05+0.05	0.1+0.05
T25	Chlorothalonil+etridiazole	0.05+0.025	0.05+0.025	0.05+0.025	0.05+0.025	0.1+0.05
T26	Mancozeb+benomyl	0.05+0.05	0.05+0.05	0.05+0.025	0.05+0.025	0.1+0.05
T27	Mancozeb+chlorothalonil	0.05+0.05	0.05+0.05	0.05+0.05	0.05+0.05	0.1+0.1
T28	Copper oxychloride+benomyl	0.05+0.05	0.05+0.05	0.05+0.05	0.05+0.05	0.1+0.05
T29	Copper oxychloride+mancozeb	0.05+0.05	0.05+0.05	0.05+0.05	0.05+0.05	0.1+0.1
T30	Mythyl bromide+Bordeaux mixture+benomyl	-	-	-	-	-
	Non-chemical in means					
T31	Solar heating	-	-	-	-	-
	Untreated					
T32	Control	-	-	-	-	-

* Phytotoxic

** Copper oxychloride alone was applied in the 2nd application

Table 10.2. Schedule of fungicidal treatments and their dosage used in 1982 Nursery trials at Chadanathode

Treatment No.	Age of seedlings at which treatment given and dosage of fungicides (%a.i)						
	Pre-emergence treatment			Post-emergence treatment			
		E. grandis	*E. tereticornis*	*E. grandis*	*E. tereticornis*	*E. grandis*	*E. tereticornis*
	1 wk before sowing the seeds	2-day-old seedlings	4-day-old seedlings	26-day-old seedlings	28-day-old seedlings	73-day-old seedlings	75-day-old seedlings
T1	-	Carbendazim (0.05 I		Carbendazim (0.1)		Carbendazir (0.05)	
T2		Benomyl		Benomyl (0.1)		Benomyl (0.05)	
T3		Copper oxychloride (0.025) +Carbendazim (0.0251+Quintozene 30g/bed.		CarbendaziP (0.1) + Quintozene 45g/bed 115g/m) .		Carbendazim (0.05)	
T4		Bordeaux mixture (0.1)		Bordeaux mixturel0. 1)		Bordeaux mixture (o. 1)	
T5		Captafol (0.05)		Captafol (0.1)		Captafol (0.05)	
T6	Quintozene (Brassicol) 30g/bed	Copper oxychloride (0.025) +Carbendazim (0.05)		Copper oxychloride (0.05) +		Carbendazim. 05)	
T7		Copper owychloride (0.025 +Carbendazim (0.025)		Copper oxychioride (0.05) + Carbendazir (0.1)		Carbenaazim (0.05)	
T8		Captan (0.025) Carbendazim (0.025)		Captan (0.1)		Carbendazie (0.05)	
T9		CONTROL	-	-	-	-	-

Disease Index	Percent foliages affected
5	76 - 100
4	51 - 75
3	26 - 50
2	11 - 25
1	1 - 10
0	Nil

At each observation, diseased specimens were collected from various treatment for pathogen isolation and identification.

Table 10.3. Schedule of fungicidal treatments for controlling seedling diseases followed in 1983 Nursery trials at Chandanathode

Treatment No.	Number of replicate beds	Fungicide concentration (% a.i.)		
		Seedbeds		Container seedlings (transplanted1
		1st application just after sowing (48 days before erergence)	2nd application 54 days after erergence	3rd application 117 days after emergence and 57 days after
T1	5	Carboxin (0.05), Hancozeb (0.02) Carbendazim (0.021	Carboxin (0.05), Carbendazim (0.01)	Carbendazim (0.01)
T2	3	Carboxin (0.05), Carbendazir (0.02)	Carboxin (0.05), Carbendazim (0.01)	Carbendazim (0.01)
T3	5	Hethyl ethyl mercuric chloride (MEMC) (0.005), Mancozeb (0.02), Carbendazim (0.02 I	MEMC (0.01), Carbendazim (0.01)	Carbendazim (0.01)
T4	5	Rethyl ethyl mercuric chloride (MEMC) (0.005), Carbendazim (0.02)	MEMC 10.01 I, Carbendazir (0.01)	Carbendazir10.01)
T5	5	Captan (0.01), Carbendazim (0.02	Carbendazim (0.02)	Carbendazim (0.01)
T6	5	Mancozeb 10.02), Carbendazim (0.02)	Carbendazim (0.02	Carbendazim (0.01)
T7	5	Methyl ethyl mercuric chloride (MEMC) (0.0005), Bordeaux mixture10.1)	HEHC (0.01)	Carbendazim (0.01)
T8	3	Rethyl ethyl mercuric chloride (MEMC) (0.0051	MEMC (O.O1)	Carbendazim (0.01)
T9	2	Thiabendazol (TBZ) (0.02)	TBZ (0.02)	Carbendazo, 10.01)
T10	2	BSF (0.02)	BSF (0.02)	Carbendazim ((0.01)
T11	5	Untreated-Control	Untreated	Untreated

Statistical Analysis

All data was subjected to one-way or two-way ANOVA and whereever required multiple comparison of means and DMRT were also done.

RESULTS

Results of nursery trials conducted during 1981, 1982 and 1983 described below separately.

1981 **trials**

Seeds of ***E. tereticornis*** began germinating three days after sowing. Four seedling diseases viz. damping-off, web blight, seedling blight and cylindrocladium leaf blight were recorded respectively on nine, 24, 45 and 88 days after emergence of seeds. A total of five applications of fungicides were given on 15, 23, 72, 86 days of emergence of seedlings. Details in respect of control of these diseases are given below separately.

Damping-off

Besides ***Cylindrocladium guinqueseptatum,L*** damped-off seedlings aiso yielded ***Pythium, Rhizoctonia solani*** state of ***Thanatephorus cucumeris.*** However, the dominant pathogens were ***Pythium*** and ***Rhizoctonia*** accounting for *>75%* isolations. No damping-off was recorded in seedbeds fumigated with methyl bromide and Di-Trapex. Initially, the development of damping-off was very slow in beds treated with solar heat but later the disease progressed rapidly; the overall severity was less than in control (Table 10.4). The first application of various fungicides treatments given to 12-day-old seedlings did not control the disease hence another application of fungicides was given after 8 days. Triforine, sodium azide, tridemorph and TCMBT, which caused phytotoxicity of varying degree after the first treatment, were not appliea again. Observations recorded after a week indicated that the damping-off was controlled completely, except in etridiazole treatment. Low incidence of disease was also observed in ***E. grandis*** treated with Bordeaux + Benomyl and in ***E. tereticornis*** treated with carbendazim, triforine and chlorothalonil + Terrachlor Super-X; R. ***solani*** alone was isolated from damped-off seedlings of these treatments. Damping-off was more severe in ***E. tereticornis*** than in ***E. grandis.***

Web blight

The disease appeared when the seedlings were 24-day-old after two applications of fungicides had already been made. Third fungicidal application of most of the treatments controlled the disease completely except in beds of solar heating and mancozeb + benomyl treatments where it persisted till the seedlings became 120-day-old.

Seedling blight

Seedling blight appeared first in beds treated with etridiazole, methyl bromide (MB) and methyl isothiocynate (Di-Trapex) and spread rapidly; these treatments were the least effective as the disease severity was fairly close to untreated control beds. In due course the disease also appeared in other beds. Except in MB and Di-Trapex the disease was more severe in seedlings of ***E. grandis*** than those of ***E. tereticornis***. The most effective treatments were those of Copper oxychioride, benomyl, mancozeb, carbendazim, PCNB, Captafol, metiram, Bordeaux mixture and other combinations of fungicides (Table 10.5). In solar heated bed significantly less infection of seedling blight was recorded as compared to control. Two applications of fungicides given to 43- and 69-day-old seedlings controlled the seedling blight in most of the treatments. By the end of May when the seedlings were 70-day-old, seedling blight infection had disappeared completely.

Cylindrocladium leaf blight

Following the onset of monsoon in early June, the promising treatments for seedlingblight could not provide a total protection against Cylindrocladium leaf blight. Leaf spots appeared first, followed by stem canker, leaf blight and shoot blight Which became widespread by the middle of June. Both C. ***quinqueseptatum*** and ***C. ilicicola*** were found to be responsible for causing infection. In a number of instances both the species were isolated from the same specimen. ***E. tereticornis*** was found to be more susceptible to this disease than ***E. grandis***. In ***E. grandis*** captafol, benomyl, carbendazim, Bordeaux mixture + tridemorph and copper oxychloride + benomyl were highly effective while in *E.* ***tereticornis*** only carbendazim controlled the disease (Table 10.5).

Certain treatments such as T20 (methyl bromide) in ***E. grandis,*** and T20 (MB),T21 (Di-Trapex) and T30 (methyl bromide + Bordeux mixture + benomyl) in *E.* ***tereticornis*** had higher severity rating than in control. By the middle of July (120-day-old seedlings) only the treatments of benomyl, carbendazim and Captafol in *E.* ***grandis*** were free of disease whereas in ***E. tereticornis*** most of the treatments remained ineffective. Height growth and number of leaf pairs of seedlings upto 100-day of emergence did not show any significant

Table 10.4. Effect of various treatments in controlling damping-off *E. grandis* and *E. tereticornis* in 1981 nursery trials at Chandanathode

Sl.No	Treatment	Number of active damping-odd patches recorded in 1.5m^{-2} of seedbeds (mean of 3 replicates)					
		E. grandis			*E. tereticornis*		
		Before treatment (12-day-old seedlings)	After two treatments(14-and 22-day-old seedlings)	Mean % damping off (28-day-old seedlings)	Before treatment (12-day-old seedlings)	After two treatments (14-and 22-old seedlings)	Mean % damping off controlled (28-day-old seedlings)
	Non-systemic fungicides						
1.	Bordeaux mixture	3.67	0	100.00	5.67	0	100.00
2.	Captafol	4.67	0	100.00	8.0	0	100.00
3.	Chlorothalonil	5.0	0	100.00	3.33	0	100.00
4.	Copper oxychloride	8.07	0	100.00	11.00	0	100.00
5.	Dodine	6.33	0	100.00	5.67	0	100.00
6.	Etridiazole	5.67	3.33	42.27	7.00	4.33	38.15
7.	Etridiazole+Quintozene	5.67	0	100.00	7.67	0	100.00
8.	Mancozeb	6.33	0	100.00	6.33	0	100.00
9.	Metiram	6.67	0	100.00	4.00	0	100.00
10.	Quintozene	3.67	0	100.00	6.0	0	100.00
11.	Sodium azide	3.67	-	-	-	-	-
12.	TCMTB	6.0	0	100.00	7.67	0	100.00
13.	Thiram	5.0	0	100.00	9.00	0	100.00
14.	Zineb	3.33	0	100.00	8.0	0	100.00
	Systemic fungicides						
15.	Benomyl	3.67	0	100.00	2.33	0	100.00
16.	Carbendazim	1.67	0	100.00	2.0	0	100.00
17.	Chloroneb	4.33	0	100.00	10.00	0	100.00
18.	Tridemorph	7.00	-	-	5.0	-	-
19.	Triforine	5.0	0	100.00	3.67	1.67	454.96
	Fumigants						
20.	Methyl bromide	0	0	100.00	0	0	100.00
21.	Methyl isothiocyanate (Di-trapex)	0	0	100.00	0	0	100.00
	Combinations of fungicides/ fumigant and fungicides						
22.	Bordeaux mixture+benomyl	3.67	0.33	91.91	4.67	0	100.00
23.	Bordeaux mixture+ tridemorph	5.67	0	100.00	7.0	0	100.00
24.	Chlorothalonil+carbendazim	5.33	0	100.00	10.0	0	100.00
25.	Chlorothalonil+etridiazole	6.0	0	100.00	5.33	0.33	100.00
26.	Mancozeb+benomyl	7.33	0	100.00	5.33	0	100.00
27.	Mancozeb+chlorothalonil	7.0	0	100.00	6.67	0	100.00
28.	Copper oxychloride+benomyl	5.33	0	100.00	7.0	0	100.00
29.	Copper oxychloride+mancozeb	6.0	0	100.00	10.33	0	100.00
30.	Mythyl bromide+Bordeaux mixture+benomyl	0	0	100.00	0	0	100.00
	Non-chemical in means						
31.	Solar heating	3.66	3.0	18.04	3.33	2.33	30.03
	Untreated						
32.	Control	4.73	6.16	-	4.96	6.0	-

Table 10.5. Effect of various treatments of severity of seedling blight and Cylindrocladium leaf blight of *Eucalyptus* in 1981 nursery trials at Chandanathode

Sl. No.	Treatment	Seedling blight: Percentage of seedlings affected		Cylindrocladium leaf blight: Diseases rating (Mean of 3 replicate beds) (45-day-old) affected (15 cm^{-2})			
		E. grandis	*E. tereticornis*	*E. grandis*		*E. tereticornis*	
				68-day-old	86-day-old	68-day-old	86-day-old
	Non-systemic fungicides						
1.	Bordeaux mixture	0.04	0.0	0.67	2.0	1.0	2.67
2.	Captafol	0.0	0.0	0.0	0.0	0.0	0.67
3.	Chlorothalonil	0.41	3.0	0.0	1.0	1.33	2.33
4.	Copper oxychloride	0.0	0.0	0.0	0.67	1.33	2.33
5.	Dodine	1.04	0.41	0.67	2.0	1.0	3.33
6.	Etridiazole	15.77	12.11	2.0	3.0	2.67	4.0
7.	Etridiazole+quintozene	4.90	4.70	1.0	2.33	2.67	4.67
8.	Mancozeb	0.0	0.0	1.0	3.0	2.0	3.67
9.	Metiram	0.05	0.0	2.0	3.0	3.0	4.0
10.	Quintozene	0.0	5.0	0.67	2.0	3.0	4.66
11.	Sodium azide	5.65	-	1.33	2.67	2.67	4.33
12.	TCMTB	8.44	5.0	0.0	1.67	0.0	2.33
13.	Thiram	0.43	0.93	1.0	2.67	3.0	4.67
14.	Zineb	3.09	1.15	1.0	2.33	3.0	4.00
	Systemic fungicides						
15.	Benomyl	3.55	0.97	0.0	0.0	0.0	0.33
16.	Carbendazim	1.35	0.0	0.0	0.0	0.0	0.0
17.	Chloroneb	6.9	6.79	2.0	3.0	2.67	3.67
18.	Tridemorph	- [**]	-	- [***]	-	-	-
19.	Triforine	10.59	6.27	1.0	2.0	2.03	3.00
	Fumigants						
20.	Methyl bromide	22.32	47.54	3.33	4.67	3.33	4.33
21.	Methyl isothiocyanate (Di-trapex)	19.23	46.70	3.33	3.67	3.67	4.67
	Combination of fungicides/ fumigants and fungicides						
22.	Bordeaux mixture+benomyl	0.35 [*]	0.31	0.0	0.67	1.0	2.33
23.	Bordeaux mixture+tridemorph	-	-	0.0	0.0	0.0	1.33
24.	Chlorothalonil+Carbendazim	0.0	0.0	0.0	0.67	0.0	1.67
25.	Chlorothalonil+etridiazole	0.0	0.0	0.0	0.33	1.33	1.67
26.	Mancozeb+benomyl	0.0	0.06	0.0	1.00	0.67	3.67
27.	Mancozeb+Chlorothalonil	0.0	0.0	0.0	0.33	1.67	2.07
28.	Copper oxychloride+benomyl	0.0	0.0	0.0	0.0	0.67	1.0
29.	Copper oxychloride+mancozeb	0.0	0.48	1.0	1.33	2.0	2.67
30.	Methyl bromide+Bordeaux mixture+benomyl	0.0	0.0	0.0	1.33	3.0	4.67
	Non-chemical means						
31.	Solar heating	10.80	17.89	3.33	4.0	3.67	4.67
32.	Control (means of 7 replicate beds)	30.95	20.64	1.83	3.83	3.17	5.0

* Disease rating index; 0, No infection; 1, 1-10% seedlings affected; 2, 11-25%; 3, 26-50%; 4, 51-75%; 5, 76-110%.

** Seedlings killed due to severe phytotoxicity; beds resown.

*** Seedlings killed due to severe phytotoxity; beds resown but seeds failed to germinate.

Table 10.6. Effect of various treatments on height growth and number of leaf pairs of Eucalyptus seedlings in 1981-Nursery trials at Chandanathode (Mean of 3 replicates of 50 seedlings each)

Sl.No	Treatment	*E. grandis*				*E. tereticornis*			
		50-day-old seedlings		100-day-old seedlings		50-day-old seedlings		100-day-oldseedlings	
		Height(cm)	No.of leaf pairs	Height(cm)	No.of leaf pairs	(Height(cm)	No.of leaf pairs	Height(cm)	No.of leaf pairs
	Non-systemic fungicides								
1.	Bordeaux mixture	3.40	2.91	17.91	7.31	6.14	3.58	23.25	7.05
2.	Captafol	4.18	3.04	23.06	8.20	4.64	3.20	24,21	7.42
3.	Chlorothalonil	3.59	2.94	32.04	7.04	6.75	3.72	35.61	7.94
4.	Copper oxychloride	3.40	3.13	24.58	6.24	6.54	3.64	37.64	7.88
5.	Dodine	3.09	2.96	17.88	6.46	4.75	3.40	33.51	7.44
6.	Etridiazole	2.96	2.97	22.99	7.57	4.51	3.46	27.25	7.97
7.	Etridiazole+quintozene	3.37	2.98	23.68	7.38	4.66	3.31	26.35	7.51
8.	Mancozeb	5.13	3.51	32.98	7.96	7.41	3.71	32.31	8.53
9.	Metiram	5.84	3.68	40.00	8.18	7.53	3.90	39.086	8.50
10.	Quintozene	4.63	3.40	30.48	7.96	6.13	3.51	31.11	8.30
11.	Sodium azide	- *	-	-	-	-	-	-	-
12.	TCMTB	- *	-	-	-	-	-	-	-
13.	Thiram	3.79	3.04	25.61	6.67	5.42	3.42	26.02	6.35
14.	Zineb	4.21	3.20	24.57	7.66	5.30	3.14	28.27	7.32
	Systemic fungicides								
15.	Benomyl	2.76	2.73	15.74	6.46	2.70	2.72	18.36	6.79
16.	Carbendazim	4.76	3.03	30.48	8.34	6.19	3.46	34.78	8.87
17.	Chloroneb	4.42	3.11	20.52	6.98	4.57	3.26	24.59	6.62
18.	Tridemorph	- *	-	-	-	-	-	-	-
19.	*Triforine*	3.34	3.37	20.81	7.37	6.25	3.75	29.03	8.09
	Fumigants								
20.	Methyl bromide	3.92	2.96	27.25	8.61	5.77	3.30	26.51	8.00
21.	Methyl isothiocyanate **	5.90	3.03	27.44	7.67	7.85	3.14	32.87	7.83
	Combination of fungicides/ fumigants and fungicides								
22.	Bordeaux mixture+benomyl	4.54	3.02	27.15	8.14	6.85	3.60	32.86	8.39
23.	Bordeaux mixture+tridemorph	3.01	2.86	13.23	6.52	4.00	3.30	23.79	6.50
24.	Chlorothalonil+Carbendazim	4.30	3.16	32.39	8.48	4.81	3.16	37.88	8.14
25.	Chlorothalonil+etridiazole	4.15	3.13	34.02	7.33	7.08	3.80	36.12	7.61
26.	Mancozeb+benomyl	4.22	3.00	26.98	7.36	5.45	3.37	35.99	8.03
27.	Mancozeb+Chlorothalonil	5.49	3.42	29.23	6.69	6.07	3.46	31.59	8.01
28.	Copper oxychloride+benomyl	2.74	2.53	16.90	6.83	3.87	3.01	22.02	6.34
29.	Copper oxychloride+mancozeb	4.71	3.66	30.81	7.78	6.27	3.72	34.67	7.77
30.	Methyl bromide+Bordeaux mixture+benomyl	3.76	2.86	30.89	7.35	5.60	3.26	37.30	8.57
	Non-chemical means								
31.	Solar heating	5.04	3.53	27.27	7.64	7.33	3.68	31.87	8.04
32.	**Control**	4.38	3.27	24.65	7.47	6.65	3.75	33.72	8.00
	F-value (One-way ANOVA)	1.55	1.486	1.418	1.507	2.358***	1.146	1.339	0.804

* Seedlings killed due to severe phytotoxicity

** Higher values due to application of ash in one of the replicate bed when seedlings were 38-day-old

*** Significant at P = 0.05; other values are non-significant.

differences due to various treatments (Table 10.6). Certain fungicides viz. carbendatim, mancozeb, metiram, chlorothalonil and their combinations had beneficial effect on growth in both the eucalypt species; in *E. grandis* methyl bromide, Di-Trapex and solar heat treatments also showed better growth as compared to control.

1982 **Nursery trials**

Seeds of *E. grandis* and *E. tereticornis* germinated respectively on 5 and 7 days after sowing. A total of six seedling diseases recorded in succession as seedlings matured, were damping-off, web blight, seedling blight, seedling wilt, Cylindrocladium leaf blight and Rhizoctonia root rot. Details of efficacy of various treatments in controlling these diseases are given below in Tables 10.7, 10.8, 10.9 and 10.10.

Damping-off

The severity of damping-off was more in *E. grandis* than in *E. tereticornis*. The best treatment having minimum severity was T_3 with copper oxychloride, carbendazim and quintozene. None of the treatments controlled damping-off completely but persistance of the disease was reduced considerably in treated seedbeds as compared to control.

Web blight

Web blight, appeared simultaneously in both the eucalypt species, was severe in E. *grandis* than in *E. tereticornis*. The best treatment was T_6 (copper oxychloride, quintozene and carbendazim) where quintozene was applied to seedbeds one week before sowing; this was followed by T_3.

Seedling blight

Seedling blight, appearing earlier in *E. tereticornis* (20-day of emergence) than in *E. grandis* (26-day) was controlled completely in all the treatments of both the eucalypts, except $_2$ where benomyl alone was applied; the seedlings remained free of this disease throughout the nursery period.

Table 10.7. Effect of Fungicidal treatments on theincidence and severity of damping-off, web blight and and seedling blight of E.grandis is 1982 nursery trials at Chandanathode

Treat-ment No.	Damping-off				Web blight				Seedling blight			
	Age of seedlings (days) disease recorded	Total No. of days disease recorded	Mean No. of days seedlings remained healthy	Meal, DSR	Age of seedlings (days) disease recorded	Total No. of days disease recorded	Wean No. of days seedlings remained healthy	Hean DSR	Age of seedlings (days) disease recorded	Total No. of days disease recorded	Hean No. of days seedlings remained healthy	Wean DSR
11	2	11	0	1.43 ^{abcd*}	13	41	10de	1.18^{bcde}	-	0	107^{a}	0^{a}
12	2	11	0	1.86^{bcd}	13	41	10^{ef}	1.75^{ef}	26	-	56.6^{a}	0.6^{b}
73	2	-	0	1.4^{a}	13		85.8^{a}	0.2^{a}	-	0	111.6^{a}	0.2^{a}
14	2	11	0	1.86 ^{abcd}	13	41	12.6 ^{bcde}	1.45^{def}		0	107^{a}	0^{a}
T5	2	11	0	1 10^{abc}	13	41	12.6^{bcde}	1.31^{cdef}		0	107^{a}	0^{a}
16	2		0	2.2^{abc}	-	0	I07^{a}	0^{a}	-	0	107^{a}	0^{a}
77	2	11	0	1 9^{abcd}		41	10^{cde}	1.20^{bcde}	-	0	107^{a}	0^{a}
T8	2	11	0	2.10^{bcd}	-	41	10^{f}	1.96^{f}	-	0	107^{a}	0^{a}
T9	2	11	0	2.62^{d}	13	41	10^{g}	3.08^{g}	26	28	23^{b}	1.95^{c}

* For details of treatment schedule and dosage see Table 10.5.

** For details see text.

*** Value in a column with the same superscript(s) do not differ significantly at P(0.05.

Seedling wilt

Seedling wilt did not appear in control seedbeds of both the eucalupts. In *E. grandis* the treatments which remained free of the disease were T4 (Bordaux mixture + tridemorph) and T5 (Captafol), whereas in *E.* tereticornis T3, T4, T5 and T6 did not develop any disease.

Cylindrocladium leaf blight

E. tereticornis was found to be more susceptible to this disease than *E.* grandis as it appeared earlier in the former (49-day-old seedlings) and persisted for longer time with higher severity. For *E.* grandis the best treatments, which brought about complete control of

Table 10.8. Effect of fungicidal treatments on the incidence and severity of seedling wil and cylindrocladium leaf blight of *E. grandis* in 1982 Nursery trials at Chandanathode

Treat-ment No.	Seedling Wilt				Cylindrocladium leaf blight				Rhizoctonia root rot			
	Age of seedlings (days) disease recorded	No.of days disease recorded	Hean No. of days seedlings remained healthy	Mean DSR**	Age of seedlings (days) disease recorded	No.of days disease recorded	Hean No. of days seedlings rerained healthy	Hean DSR	Age of seedlings (days) disease recorded	No.of days disease recorded	Hean No. of days seedlings rerained healthy	Hean DSR
T1	47	63	82.8	1.06^{c}***	-	0	107^{a}	0^{a}	73	37	81^{abc}	0.69^{bcd}
T2	54	63	80.8	1.00^{c}	-	0	107^{a}	0^{a}	73	-	95.8^{b}	0.2^{a}
T3	110	0	106.6^{b}	0.4^{b}	-	0.	107^{a}	0^{a}	73	37	81^{c}	0.6^{bc}
T4	-	0	107.0^{a}	0^{a}	73	37	92.2^{ab}	0.4^{b}	73	37	95.8^{b}	0.5^{b}
T5	-	0	107.0^{a}	0^{a}	110	0	106.6^{ab}	0.4^{a}	73	37	73.4^{c}	0.89^{d}
T6	110	0	106.0^{b}	0.2^{b}	-	0	107^{a}	0^{a}	-	0	107^{a}	0^{a}
T7	59	56	62.0^{d}	1^{a}	110	0	106.6^{b}	0.2^{b}	-	0	107^{a}	0^{a}
T8	54	56	69.8^{d}	1.06^{c}	-	0	107^{a}	0^{a}	73	37	84.6^{c}	0.5^{b}
T9		0	107.0^{a}	0^{a}	73	37	88.5^{b}	0.5^{b}	73	37	79^{c}	1^{e}

* For full details of treatment schedule and dosage see Table 10.5.
** For details see text.
*** Values in a column with the same superscript(s) do not differ significantly at $P<0.05$.

the disease were T1 (carbendazim) T_2, T_3, T_6, T_8 (Captan+carbendazim); in *E. tereticornis* only T_1, T_3 and T_6 were effective.

Rhizoctonia root rot

This disease, appearing in 73-day-old seedlings of both the eucalypts, though more severe in *E.* tereticornis than in *E. grandis*, persisted for longer time in the latter than the former species. Treatments T_3, T_6 and T_8 were highly effective in controlling the disease completely.

Table 10.9. Effect of fungicidal treatments on the incidence and severity of damping-off, web blight and seedling bligbt of the *E.* tereticornisin 1982 Nursery trials at Chandanathode

Treat-ment No.	Damping-off				Web blight				Seedling blight			
	Age of seedlings (days) disease recorded	Total No. of days disease recorded	Mean No. of days seedlings remained healthy	Mean DSR**	Age of seedlings (days) disease recorded	Total No. of days disease recorded	Mean No. of days seedlings remained healthy	Bean DSR	Age of seedlings (days) disease recorded	Total No. of days disease recorded	Bean No. of days seedlings remained healthy •	Bean DSR
11	4	11	0	1.16^{cde***}	15	41	29.4^{bc}	0.8^{bxdef}	-	0	107^{a}	0^{a}
12	4	11	0	1.33^{ef}	15	41	10^{c}	1.2^{ef}	20	0	90.2^{a}	0.2^{a}
13	4	0	0	1.2^{a}	56	0	95.8^{ab}	0.2^{a}	-	0	107^{a}	0^{a}
14	4	11	0	1.23^{ef}	15	41	29.4^{bc}	0.86^{def}		0	107^{a}	0^{a}
15	4	4	0	1.2^{cd}	15	41	38.8	1.00^{def}	-	0	90.2^{a}	0.2^{a}
16	4	0	0	1.6^{bc}		41	107a	0^{a}	-	0	107^{a}	0^{a}
17	4	11	0	1.16^{cde}	15	41	34.6 b	0.8^{bcdef}	-	0	107^{a}	0^{a}
18	4	11	0	1.53^{f}	15	41	10^{c}	1.16^{f}		0	107^{a}	o^{a}
19	4	24	0	1.75^{g}	15	41	10^{d}	2.06^{g}	20	29	23^{b}	2^{b}

* ** tor details of treatment schedule and dosage see Table 10.5.

*** for details see text.

Value in a column with the same superscript do not differ significantly at P<0.05.

1983 Nursery Trials

Seeds of *E. grandis* germinated 5 days after sowing. Due to first application of fungicidal treatment just after sowing, damping-off and web blight diseases were completely controlled. Only seedling blight, cylindrocladium leaf blight and seedling wilt appeared. Details of treatments effective in controlling these diseases are given separately below.

Seedling blight

Disease appeared in 18-day-old seedlings of control and treatments T_4, T_5, T_6, T_9 and T_{10} while in other treatments when seedlings were

Table 10.10. Effect of fungicidal treatments on the incidence and severity of seedling wilt and cylindocladium leaf blight of *E. teroticornis* in 1982 Nursery trials at Chandanathode

Treatment No.,	Damping-off				Web blight				Seedling blight			
	Age of seedlings (days) disease recorded	Total No. of days disease recorded	nean No. of days seedlings remained healthy	Mean DSR**	Age of seedlings (days) disease recorded	Total No. of days disease recorded	nean No. of days seedlings remained healthy	Mean DSR	Age of seedlings (days) disease recorded	Total No. of days disease recorded	nean No. of days seedlings remained healthy	nean DSR
T1	75	37	92.0^{c}	0.6 ^{bc***}	-	0	107^{a}	0^{a}	75	37	77.2^{abc}	0.8^{bc}
T2	49	63	94.2^{c}	0.4^{ab}	112	0	106.8^{a}	0.4^{ab}	75	37	82.4^{bc}	1.4
T3	-	0	107.0^{a}	-	0	107^{a}	0^{a}					
T4	-	0	107.0^{a}	0^{a}	49	83	75.8^{bc}	0.64^{bc}	73	37	95.8^{abc}	0.5^{b}
T5	-	0	107.0^{a}	0^{a}	75	37	91.8^{b}	1.0^{cd}	73	37	84.6^{abc}	0.6^{bc}
T6	-	0	107.0^{a}	0^{a}	-	0	107^{a}	0^{a}	-	0	107^{a}	0^{a}
T7	56	56	77.0^{b}	0.8^{bc}	112	0	106.7^{a}	0.4^{ab}	-	0	107^{a}	0^{a}
T8	56	56	79.2^{b}	0.6^{b}	112	0	106.8^{a}	0.2^{ab}	73	-	95.8^{a}	0.2^{a}
T9	-	0	107.0^{a}	0^{a}	49	63	47.5^{c}	1.33^{d}	73	37	51.^{c}	1.25^{d}

* For details of treatment schedule and dosage see table 10.5.
** For details see text.
***Values in a column uith the sane superscrip(s) do not differ significantly at P<0.01.

25-to 54-day-old. T1 (carboxin, mancozeb, carbendazim) was the most effective treatment as the appearance of seedling blight was delayed considerably as compared *to* other treatments. It was followed by T3 (MEMC, mancoteb and carbendazim) where the DSR was similar to T1 but the seedlings remained healthy for a shorter, duration (69.6 days) than T1 (Table 10.11).

Cylindrocladium leaf blight

All the treatments were effective in controlling the disease to varying degrees. The best treatements were T3, T4 and T7 where no leaf blight was recorded (Table 10.11). It was closely followed by T1 and

T_6 where seedlings remained healthy for a longer duration with minimum disease severity. Treatment T_{10} (BSF) was not at all effective since the disease severity was higher than in control beds.

Seedling wilt

Treatments T_3 and T_9 were best in controlling seedling wilt as no disease appeared; T_2 and T_4 were the other treatments where disease severity was minimum and the seed1ings remained healthy for longer period of time (Table 10.11).

DISCUSSION

The results of 1981 nursery trials indicate that even five treatments of most of the effective fungicides could not provide a total protection against Cylindrocladium infection. Though fungicides in various treatments reduced the disease incidence, they varied greatly in their effectiveness in controlling the disease. The ones which survived against a heavy pathogen pressure and provided a total control were systemic fungicides, carbendazim and benomyl and a non-systemic, captafol. Though ED_{100} of benomyl in laboratory evaluation was at a dosage of 0.5%, application of even 0.2% or 0.1% was found very effective. These results are in conformity to earlier findings of Engelhard (1971) who reported the effectiveness of benomyl against cylindrocladium rot of Azalia cuttings caused by C. scoparium. However, contrary to our findings carbendazim did not control the disease. The effectiveness of benomyl and carbendazin even in high rainfall area of Wynad District could be attributed to favourable properties of the active principle. In this regard Fuchs and Bollen (1975) have reported that a significant fraction of benomyl applied remains in soil as such or methyl benzimidazole - 2-yl-carbonate (MBC), which is the fungitoxic principle of benomyl and carbendazim. Furthermore, they also found that benomyl and MBC are practically immobile in soil and they do not leach significantly from the site of application, which facilitates continuous uptake of the effective principle. Thus, a number of tretments would result in cumulative effect against the pathogen and provide an effective protection to plants. The effectivenss of captafol as against other non-systemic

Table 10.11. Effect of fungicidal treatments on the incidence and severity of seedling wilt, seedling blight and *Cylindrocladium* leaf blight of *Eucalyptus Grandis* - 1983 Nursery trials at Chandanathode

Treatment No.	Seedling wilt				Seedling blight				Cylindrocladium leaf blight			
	Age of seedlings (days) disease recorded	No.of days disease recorded	Mean No. of days seedlings remained healthy	Mean DSR**	Age of seedlings (days) disease recorded	No.of days disease recorded	Wean No. of days seedlings remained hea thy	Mean DSR	Age of seedlings (days) disease recorded	No.of days disease recorded	Mean No. of days seedlings remained hea lthy	Mean DSR
11	54	43	69.8^{a***}	0.66^{a}	54		81.20^{a}	0.4^{a}	41	13	79.6^{ab}	0.2
12	54		91.66^{a}	0.33^{a}	25	49	56.3^{ab}	0.66^{ab}	41	13	72.0^{ab}	0.33^{abc}
13			104.0^{a}	0^{a}	25	-	69.6^{ab}	0.4^{a}			91^{a}	0^{a}
14	54		92.6^{a}	0.2^{a}	18	36	43.4^{ab}	0.85^{ab}	-		91a	0^{a}
15	34		81.2^{a}	0.4^{a}	18	36	44.0^{ab}	1.05^{ab}	41	46	74.6^{ab}	0.65^{bc}
16	34	43	78.4^{a}	0.8^{a}	18	36	62.6^{ab}	0.6^{ab}	14	13	86.2^{ab}	0.2^{abc}
17	54		81.2^{a}	0.66^{a}	41	13	67.2^{ab}	0.6^{ab}	-		91.0^{a}	0^{a}
18	54	43	85.0^{aab}	0.66^{ab}	25	29	28.6^{b}	1.22^{b}	41	13	53.0^{ab}	0.66^{bc}
19			104.0^{a}	0^{a}	18	36	11^{c}	2.25	25^{c}	29	54.1^{ab}	0.5^{bc}
110	34	20	15.5^{ab}	1.25^{ab}	18	36	11^{c}	3.0^{c}	25	62	18d	2.4e
111	54	56	16.2^{b}	1.5^{gbc}	18	56	11^{c}	4.14^{d}	25	62	57.8'	1.43^{d}

** For details of treatment schedule and dosage see Table 10.9.

*** For details see text.

Values in a column with the same superscript(s) are not significant at P<0.01.

fungicides may be due to the fact that it is less affected by climatic variations than many other fungicides (Thomson, 1979).

Fumigation with chemicals has been used commercially for many years to control certain plant pathogens present in the upper few centimeters of soil. For example, *Rhizoctonia*, *Pythium* and *Phytophthora* diseases can be suscessfully controlled by such treatments. In our studies both the fumigants were effective in control l ing damping-off and web blight diseases. However, Cylindrocladium leaf blight could not be controlled. It means

microsclerotia of *Cylindrocladium* are not affected, either by MB or Di-Trapex. On the contrary Bell *et al.* (1973) reported a satisfactory reduction in intensity of black rot of peanuts caused by C. *crotaloriae,* by preplant fumigants such as MB. However, our results are in agreement with those of Van Asche *et al.* (1968) on R. *solani,* which was completely controlled by MB.

Solar heat treatment, where mulching with polythene sheet increased the soil temperature from 37.0°C to 43.5°C, resulted in reduced damping-off and seedling blight as compared to untreated control. The results are in conformity with those of Katan *et al.* (1976) who also found reduction in soil-borne diseases of tomato caused by *Verticillium dahaliae* and *Fusariom oxysporum* f.sp. *lycoperscii.* The solar heating treatment would have been more effective had the temperature increased atleast upto 50°C, which will be possible in the plains. Solar heat treatment gave some indications of its effectiveness and considering its superiority over fumigation with additional beneficial side effects (Katan *et al.,* 1976), detailed investigations will be fruitful.

In the first nursery trial conducted during 1981 only damping-off, seedling blight and Cylindrocladium leaf blight were recorded whereas during 1982 trials three new diseases i.e., web blight, seedling wilt and Rhizoctonia root rot also appeared. This increase in number of diseases could be either due to close observation of seedlings during 1982 or build up of inoculum in due course which resulted in the appearance of these diseases. However, during 1983 trials since damping-off and web blight were controlled completely only seedling blight, web blight and Cylindrocladium leaf blight appeared in some of the treatments; these treatments were partially effective against these diseases. Furthermore, the two eucalypts showed significant differences in their susceptibility to some diseases. Among the two eucalypts *E. grandis* appears to be more susceptible to seedling blight, web blight, and seedling wilt than *E. tereticornis* while the latter shows higher severity of Cylindrocladium leaf blight and Rhizoctonia root not than the former; high severity of damping-off was recorded in *E. tereticornis* during 1981 trials and *E. grandis* during 1982 trials. It is clear from these observations that owing to difference in the level of susceptibility to various diseases of the

two eucalypt species the fungicide dosage required to control a specific disease may not be the same.

Seedling wilt caused by *S. rolfsii* was the only disease, which appeared in 1982 trials in certain treatments (carbendazim and benomyl) and not in control. During 1983, however, seedling wilt was recorded in most of the treatments (except T3) and control. In a similar example Backman *et al.* (1975) found that unsprayed plots of peanuts had consistently lower levels of white mold (caused by *S. rolfsii),* those sprayed with benomyl consistently had the highest, and other fungicides (chlorothalonil, copper hydrpxide, thiophenate methyl), intermediate. They attributed this to indirect effect of benomyl on *Trichoderma viride,* a natural. antagonist of *S. rolfsii.* Appearance of disease even in controls of 1983 trials could be due to high inoculum build up during the two nursery trials is supported by observations of Punja (1985) that continous rotation with the same crop highly susceptible to *S. rolfsii* may increase disease incidence in subsequent years. The best treatments where seedling wilt did not appear were T4, T5 of *E. grandis* and T5, T4, T3 and T6 of *E. tereticornis* during 1982 trials and T3 and T9 during 1983 trials. This suggests that fungicides should be evaluated for their effect on non-target pathogens before disease control recommendations are made.

On comparison of efficacy of different fungicidal treatments against various diseases in three nursery trials during 1981, 1982 and 1983, carbendazim stands out as the best as it controlled besides CLB other diseases too. Its efficacy increases when used in combination with other fungicides such as MEMC, mancozeb and quintozene. During the 1983 trials when prophylactic treatments were given just after sowing of seeds, the best treatment where no damping-off, web blight and seedling blight appeared and other diseases were subsequently controlled effectively is T3 - a combination of MEMC, mancozeb and carbendazim in the first application followed by second and third applications of carbendazim alone. By applying the fungicides initially at pre-emergence stage the damping-off, web blight and seedling blight caused by *Cylindrocladium, Rhizoctonia,* and *Pthium* are controlled. Subsequently, carbendazim treatment controls effectively all the *Cylindrocladium* diseases.

11. Effect of Some Nursery Practices on Incidence and Severity of Diseases, and Growth of *Eucalyptus grandis* Seedlings

Under the conventional method practised in Kerala, the eucalypt seedlings are raised in seedbed nurseries during December/January, and pricked out in polythene containers during February/March. These container seedlings are maintained in the nursery till the time they are outplanted during June after the onset of monsoon. In this way the seedlings are exposed to disease hazards, if any, for nearly six months. During this period any lapse in the management of nursery may accentuate the disease situation, resulting in large scale mortality of seedlings as has been observed in a forest disease survey by Sharma et al. (1985). Some of the important nursery practices which appear to have direct bearing on the incidence and severity of seedling diseases are shading, watering frequency and quantity of water, and seed rate. Shading, usually of coconut leaf thatch, provided over the seedbed for initial three months to protect seedlings from sun scorch becomes so dark that seedlings start to etiolate. Similarly, indiscriminate watering of seed beds with excess quantity of water in an enthusiasm to germinate seeds at the earliest has been observed. The seed rate per standard bed (12m x 1.2m) has been found to be varying greatly from 25 to 240 g (i.e., 1.75 to 16.7 g m^{-2} of seed bed). The tendency being, if once the nursery had failed, either because of poor quality of seeds or improper nursery practices, to use a higher quantity of seeds to assure availability of desired number of seedlings.

The purpose of this study was to investigate the effect of different types of shading, moisture regimes and seed rates on the incidence and severity of nursery diseases and growth of seedlings of Eucalyptus grandis with a view to standardise nursery practices for raising healthy and disease-free seedlings.

MATERIALS AND METHODS

Experimental site and preparation of nursery

The experiments were conducted during 1983 at Chandanathode. Details of the experimental site and nursery preparation are provided in the previous chapter.

The beds were broadcast sown with seeds of E. grandis in January 1983. To study the effect of various nursery practices on the spectrum of diseases developing at different growth phases, the seedlings were retained in the seedbeds for 112 days after emergence, and no pricking of seedlings was carried out. There were three replicate seed beds for each combination of treatments - type of shading, soil moisture regime (MR) and seed rate (SR). A randomised block design was followed for assigning the seed bed with various treatments.

Type of shading

For shade treatment, besides conventional coconut leaf thatch (CLT), coir mat (CM) of 7 mm mesh was also used. Light intensity over the beds under CLT, CM and outdoors was measured using an Integrating Photometer (LICOR, USA) at hourly intervals from 0800 to 1600 hr during the study period.

Seed rate

There were two seed ratesi.e., 2.8 g m^{-2} (SR1)and 7.0 g m^{-2} (SR2) equivalent to 40 g and 100 g per standard seedbed (12 x 1.2 m).

Soil moisture regime

Two soil moisture regimes viz. 11 l m^{-2} (MR1) and 14 l m^{-2} (MR2) per day were regulated by appropriate frequency of watering, which was 4-5 times a day during the first ten days of sowing and 2-3 times after 25 days of emergence of seedlings. Soil water potential of beds was measured using a soil water tensiometer for 25 days after emergence of seedlings both under CLT and CM.

Soil temperature, ambient temperature and relative humidity

Soil temperature was recorded daily under CLT and CM shading using a soil thermometer, at a depth of 10 cm upto 45 days after emergence of seedlings. Wet and dry bulb temperatures were recorded daily in CLT and CM nurseries; r.h. was deduced from these tempertures.

Disease incidence and severity

Incidence and severity of seedling diseases viz. damping-off, web blight, seedling blight and seedling wilt were recorded in all the seedbeds under CLT and CM from the day of their first appearance. Observations were recorded daily till the twentieth day of emergence of seedlings, and later at weekly or fortnightly intervals.

Since the degree of patchiness of infection is considered to be a direct function of the degree of inoculum patchiness (Griffin and Tomimatsu, 1983), the progress of damping-off and web blight, was recorded by counting the number of disease foci (patches). At each observation, foci were marked by placing small coloured reed bamboo splints, soaked in 0.05% a.i. solution of Bavistin (carbendazim) (BASF, Bombay, India) and air dried, at the periphery to ascertain whether the focus was still expanding. In the case of expanding foci, the splints were removed from the old periphery and placed at the new periphery at the next observation.

From the cumulative number of foci m^{-2}, area under the disease progress curve (AUDPC) (Smith *et al.* 1988) and growth rate of diseases were calculated in each treatment combination under CLT and CM. AUDPC was calculated for each replicate seed bed with the mid point rule (Shaner and Finney, 1977) and the mean and S.E. calculated for each treatment.

$$\text{AUDPC} = \sum_{i-1}^{n-1} (Y_{i+1} - Y_i)/2 \times (t_{i+1} - t_i),$$

where t_i = time in days, i = 0 to n, and Y_i = number of foci on day i. Growth rate was calculated using an exponential model, $Y_t = Y_o e^{rt}$ (van der Plank, 1963; Kranz, 1974),
where Y_t = number of foci present after a given time t, Y_o = initial number of foci, e = exponential function, and r = rate of growth.

In cases where disease severity was zero, 0.001 was added to all the observations before transforming them to log scale.

For seedling blight and seedling wilt diseases, only severity ratings were recorded. The disease severity was assessed using a rating scale (0-5)given below. The data were subjected to analysis

of varience (ANOVA) and the treatments were grouped by methods reported by Calinski and Corsten (1985).

Severity of disease	Disease rating
Nil	0
1-25 foci of infected seedlings in 3 x 1 m of seed bed	1 (0.1 - 1)
26 - 50 "	2 (1.1 - 2)
51 - 75 "	3 (2.1 - 3)
76 - 100 "	4 (3.1 - 4)
>100 "	5 (4.1 - 5)

Growth of seedlings

Data on emergence of seedlings and development of first, second and third leaf pairs were recorded for seedlings under CLT and CM. Growth of seedlings in terms of root and shoot lengths of 25 seedlings, selected at random, was measured at about weekly intervals from the twentieth day of emergence upto 105 days in MR1-SR1 and MR2-SR1 treatments; shoot:root ratio of seedlings was calculated for each treatment. The data were subjected to ANOVA followed by comparisons made through cluster analysis (Calinski and Corsten, 1985).

RESULTS

Microclimatic conditions under two shade treatments

Microclimatic conditions under coir mat (CM)and coconut leaf thatch (CLT) varied significantly. The average light intensity under CLT was about 15 times less as compared to CM; in comparison with outdoors (without shade) the light intensity was reduced 45 times under CLT whiie only three times under CM (Fig. 11.1). Average ambiant temperature and soil temperature were higher under CM (26°C and 24.3°C, respectively) than under CLT (25°C and 22.5°C, respectively) (Figs. 11.2 and 11.3). Also, the soil water potential (SWP) was generally higher under CM than in CLT except on a few days (Fig. 11.2); SWP was higher in seed beds with low moisture regime

(MR1) than in high moisture regime(MR2). There was no significant difference in r.h.under the two shade treatments.

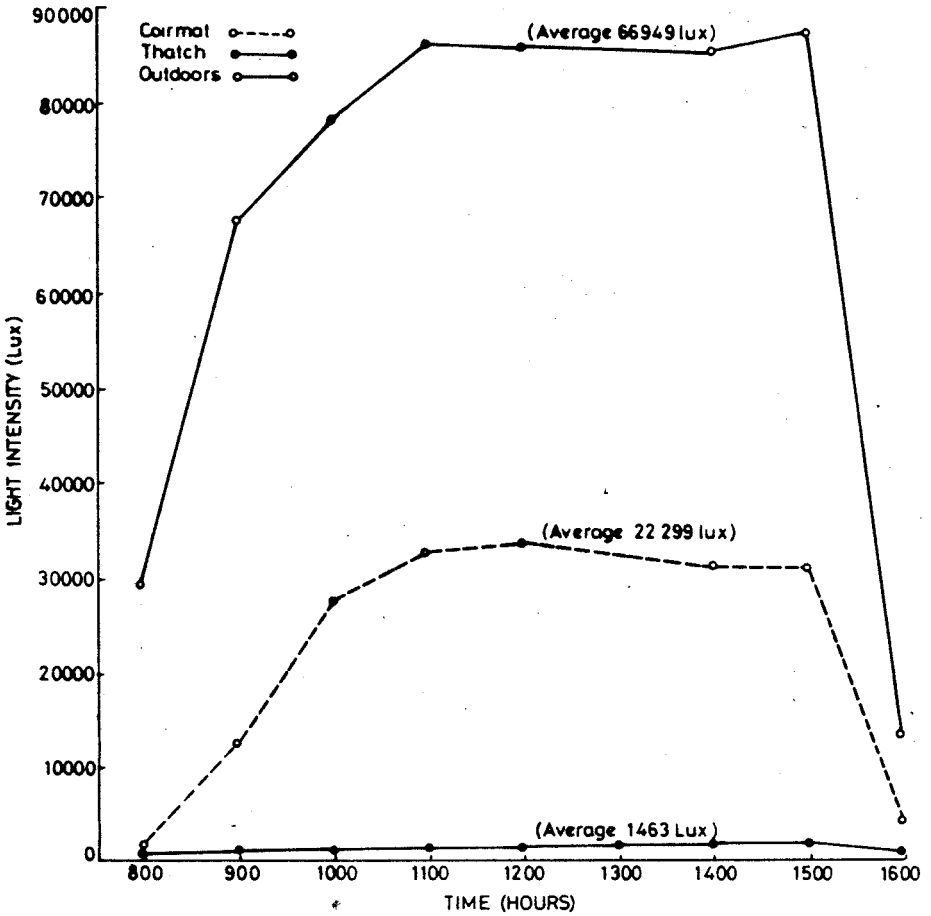


Fig. 11.1. Average light intentisity outdoor and over the nursery beds in two shade treatments - coiraat and thatch.

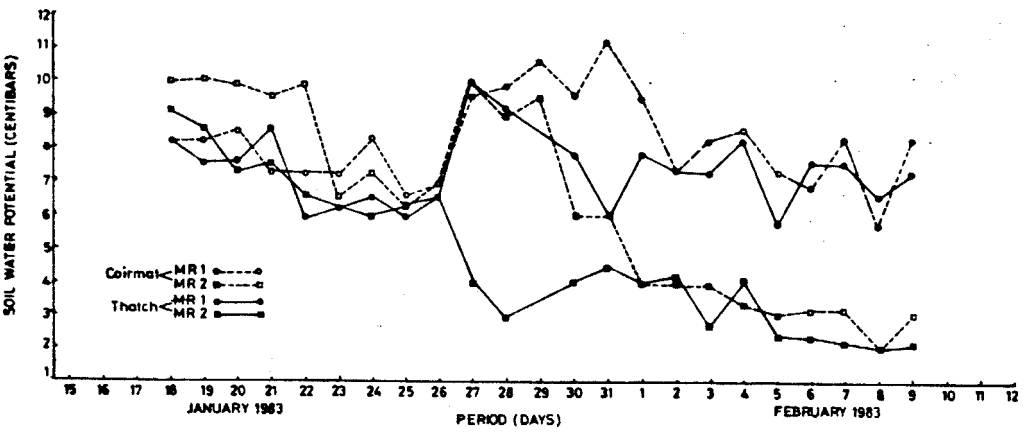


Fig. 11.2. Soil water potential of nursery beds under coirrat and thatch shading.

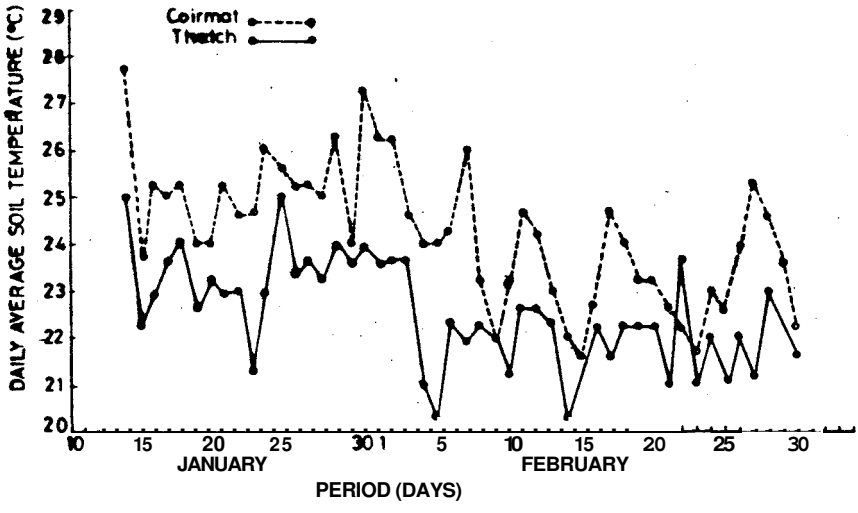


Fig. 11.3. Average soil temperature of nursery beds under coirmat and thatch shading.

Incidence and severity of seedling diseases

Four seedling diseases viz. web blight, damping-off, seedling blight and shoot wilt were recorded during the experiment. .Details in respect of these diseases are provided below.

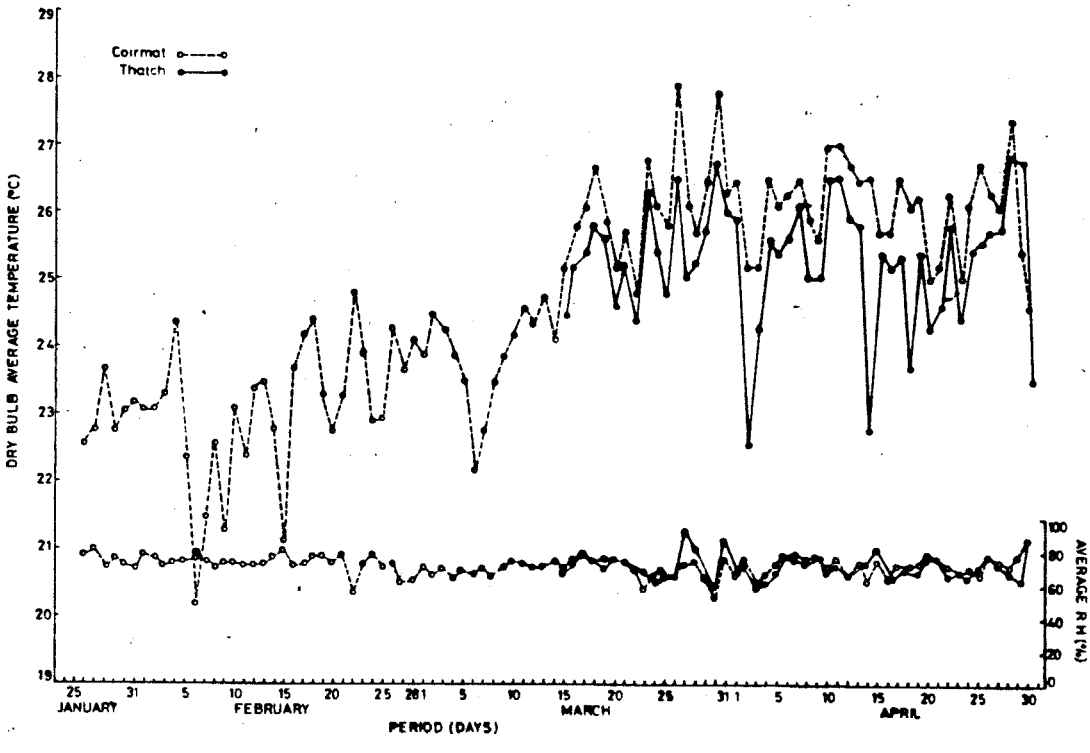


Fig. 11.4. Average ambient temperature and relative humidity in the nursery.

Web blight

lncidence and severity of web blight was significantly affected by various nursery practices. Except under MRl –SRl treatment web blight appeared early and persisted for a longer duration under CLT than in CM; in MR1–SR1 of both the shade treatments no disease was recorded (Table 11.1). Average number of foci (m^{-2}) and AUDPC were significantly higher for CLT shading than those of CM. High moisture regime (MR2) and high seed rate (SR2) had significantly higher disease severity than low moisture regime (MR1) and low seed rate (SR1) as evidenced by average number of foci (m^{-2}), AUDPC and the disease progress rate.

Table 11.1, Effect of various nursery practices on the incidence and severity of web blight of *E. grandis*

tloisture regime (MR) I of water a^{-2} per day	Seed rate gr^{-2} (SR)	Type of shading: Coir mat (CM) — Days after emergence disease appeared	Days fresh infection recorded	Av. no. foci a^{-2}	AUDPC[a] (S.E.)	Disease progress rate* (S.E.)	Type of shading: Coconut leaf thatch (CLT) — Days after emergence disease appeared	Days fresh infection recorded	Av. no. foci m^{-2}	AUDPC[a] (S.E.)	Disease progress rate* (S.E.)
	2.8 (SRl)					-			-		
11(MR1)											
	7.0 (SR2)	5	3	1.35	1.0 ±1.0	0.104 ±6.97 10^{-4}	9	7	3,04	2.42 ±0.75	0.152 ±0.164
	2.8 (SR1)	7	2	0.69	0.44 ±0.44	0,266 ±3.15 10^{-4}	8	4	1.72	1.39 ±0.6	0.216[b] ±7.68 10^{-4}
14 (HR2)											
	7.0 (SR2)	10	6	3.87	3.0 ±0.5	0.450[b] ±0.158	6	10	5.27	4.44 ±0.41	0.858 ±0.770 10^{-5}

a, Area under disease progress curve

f, foci m^{-2} per day

Damping-off

Incidence of damping-off was also affected significantly by the type of shading as it appeared first and persisted for a longer duration under CLT than CM; a similar trend was also observed for MR2 and SR2 treatments (Table 11.2). However, disease severity as expressed by AUDPC and the disease progress rate did not differ in both the shade treatments. Within a shading type, MR2 and SR2 treatments had significantly higher AUDPC and disease progress rate as compared to MR1 and SR1.

Table 11.2. Influence of various nursery practices on the incidence and severity of damping-off of *E. grandis*

Moisture regime (MR) 1 of Water m^{-2} per day	Seed rate gm^{-2} (SR)	Type of shading: Coir mat (CM)					Type of shading: Coconut leaf thatch (CLT)				
		Days after emergence disease appeared	Days fresh infection recorded	Av. no. foci m^{-2}	AUDPC[a] (S.E.)	Disease progress rate* (S.E.)	Days after emergence disease appeared	Days fresh infection recorded	Av. no. foci m^{-2}	AUDPC[a] (S.E.)	Disease progress rate* (S.E.)
	2.8 (SR1)	14	5	5.52	5.998 ±1.782	0.773 ±0.158	10	13	5.54	6.055 ±0.974	0.919
11 (MR1)											
	7.0 (SR2)	8	13	9.94	14.832 ±0.162	2.04 ±0.279	6	37	15.13	16.75 52.25	2.496
	2.8 (SR1)	12	8	11.68	8.391 ~0.618	1.164 0.208	10	28	7.82	9.165 ±0.193	1.327 ±0.127
14 (MR2)											
	7.0 (SR2)	7	14	1'4.12	22.498 ~5.678	3.087 ±0.544	6	43	16.15	22.25 ±4.751	2.520 ±0.232

a, Area under disease progress curve

*, foci m^{-2} per day

Seedling blight

Unlike the above two diseases, seedling blight was recorded first under CM and a week later under CLT but it persisted for longer duration in the latter than in former (Table 11.3). The disease severity was significantly higher in MR2 of CM than of CLT; MR1 treatments of both CM and CLT did not differ in disease severity. Though high disease severity was correlated well with high seed rate (SR2) of all the treatments of CM and CLT, significantly higher disease severity in MR2 than in MR1 was observed only in CM.

table 11.3. Effect of various nursery practises incidence and severity of seedlings blight of E. grandis

Moisture regime (MR) 1 of Water m^{-2} per day	Seed rate gm^{-2} (SR)	type of shading						
		Coir mat (CM)				Coconut leaf thatch (CLT)		
		Weeks after emergence seedling blight appeared	Days fresh infection recorded	Av. disease severity	Disease severity rating	Ueeks after emergence seedling blight appeared	Daysfresh infection recorded	Av. disease severity
11(MR1)	2.8(SR1)	3	57	1.66[a]*	2	4	50	1.48[a]
	7.0(SR2)	3	57	2.1[b]	3	4	72	2.4[b]
14(MR2)	2.8(SR1)	3	57	2.52[c]	3	4	64	1.72[a]
	7.0(SR2)	3	57	3.6[d]	4	4	61	2.4[b]

* Values with the same script do not differ significantly at p = 0.05.

Shoot wilt

This disease was recorded simultaneously in seed beds under CM and CLT on 44-day of emergence, but it persisted for a longer duration in the former than in the latter (Table 11.4). In both the types of shading, MR2 and SR2 treatments had significantly higher disease

severity than MR1 and SR1. Though average disease severity was slightly higher in CM as compared to CLT, severity ratings did not differ, except in the case of MR1 – SR1 treatments.

Table 11.4. Effect of various nursery practices on the incidence and severity of shoot wilt of *E. grandis*

Moisture regime (MR) l of Water m^{-2} per day	Seed rate gr^{-2} (SR)	Type of shading: Coir rat (CM) — Weeks after emergence seedling blight appeared	Days fresh infection recorded	Av. disease, severity	Disease severity rating	Type of shading: Coconut leaf thatch (CLT) — Weeks after emergence seedling blight appeared	Days fresh infection recorded	Av. disease severity
11 (MR1)	2.6 (SR1)	6	56	1.35[a]	2	6	26	0.99[a]
	7.0 (SR2)	6	61	1.9[b]	2	6	33	1.33[b]
14 (HR2)	2.8 (SR1)	6	44	1.53a	2	6	33	1.22[b]
	7.0 (SR2)	6	68	2.1[b]	3	6	33	2.33[c]

* Values within a column with be the same script do not differ significantly at p = 0.05.

Growth of seedlings

Since the microclimatic conditions differed considerably under CM and CLT, tne growth of seedlings of E. grandis also showed variation under the two shade treatments. Development of leaves was much faster under CM than under CLT; in the former, the first leaf pair appeared on the 15-day of emergence, while in the latter, on the 20-day, when leaf in the former had already grown 5 mm in length. Similarly, on 27-day of emergence, while seedlings under CM had second leaf pair having 3-4 mm long leaves, under CLT it was either absent or found to be just appearing. Third leaf pair was also recorded earlier in most of the seedlings of CM on 45-day of emergence as compared to CLT

seedlings. Furthermore, leaves and stems of seedlings under CM developed respectively light and dark purple pigmentation. On the other hand, CLT seedlings remained green, except for the basal part of the stem which developed some pigmentation.

The shoot growth was exponential in both the shade treatments. At 105-day of emergence, the length of root and shoot was significantly higher in seedlings of both the moisture regimes under CM than CLT (Figs. 11.5-11.8). Uithin one shade treatment, moisture regime affected the shoot and root lengths significantly and consequently the S:R ratio; at 105-day of emergence, the S:R was higher in seedlings ofMR1 treatment with high soil water ,potential than MR2 with low soil water potential. In the beginning, from 20- to 45-day of emergence of seedlings the S:R was higher in both MR1 and MR2 of CLT as compared to CM while at 75-day it was lower than CM seedlings. But later, S:R appeared to be higher in seedlings under CM (Fig. 11.9).

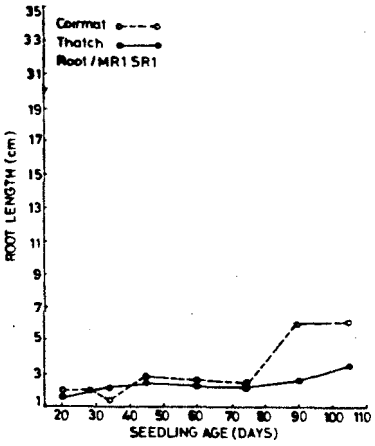


Fig. 11.5.

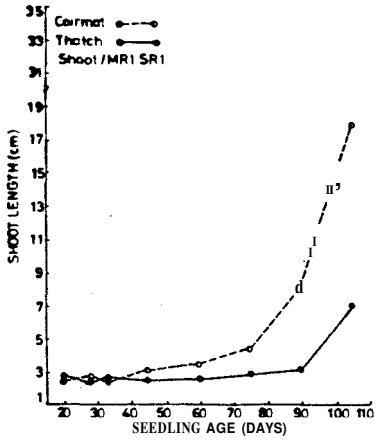


Fig. 11.6.

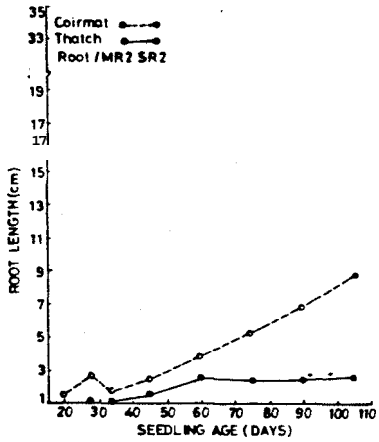


Fig. 11.7.

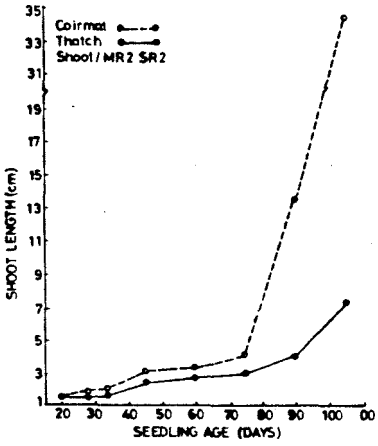


Fig. 11.8. Seedling shoot length in relation to moisute regime (MR2) and seed rate (SR2) under coiraat and thatch shading.

Fig. 11.5. Seedling root len th in relation to moisture regime (MR1-11 l of water m^{-2} per day) and seed rate (SR1-2.8g m^{-2}) under coirrat and thatch shading.

Fig. 11.6, Seedling shoot length in relation to roisture regime (MR1) and seed rate (SRl) under coirrat and thatch shading.

Fig. 11.7. Seedling root len th in relation to moisture regime (MR2-14 1 of water m^{-2} per day) and seed rate (SR2-7.0 g m^{-2}) under coirmat and thatch shading.

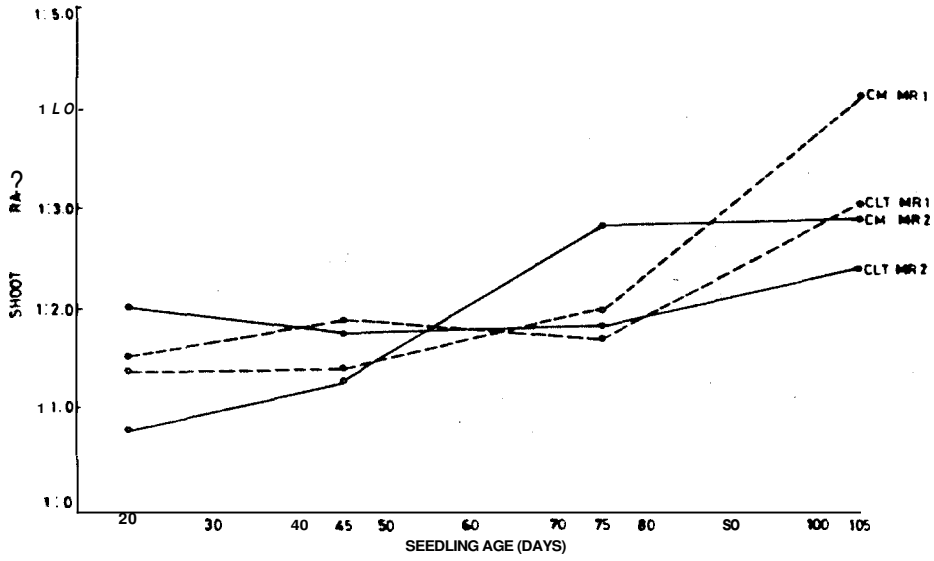


Fig. 11.9. Seedling shoot: Root ratio under various treatments. CH MR1:Coirmat shading and moisture regime 1 (11 l of water m^{-2} per day). CM MR2: Coirmat shading and noisture regine 2 (14 l of water m^{-2} per day) : CLTMR1: coconut leaf thatch and moisture regime 1: CLT HR2: coconut leaf thatch and moisture regine 2.

DISCUSSION

The epidemiology of forest nursery diseases has been studied much less than their etiology or ecology, especially in relation to nursery practices (Bloomberg, 1985). Besides exhibiting different frequencies, forest nursery diseases vary with respect to growth stage of the host attacked, infection rate, symptom development and spatial distribution (Bloomberg, 1985). Each of these epidemiological parameters, chiefly governed by the nursery practices has implications in disease management. All the four diseases, fairly specific to the host growth stage (Sharma *et al.*, 1984), appeared in distinct patches (foci). The occurrence of diseased plants in identifiable patches is probably the usual case for soil-borne pathogens (Campbell and Noe, 1985; Punja, 1985). Moreover, microsclerotia of ***Cylindrocladium*** are aggregated spatially in naturally infested soil and infected plants initially appear in foci (Campbell and Noe, 1985). Thus, disease foci provide a good quantitative measure of severity of soil-borne diseases.

Appearance and severity of diseases in nursery are known to be influenced by many factors, including type of shading over seed beds, seedling density, soil moisture and temperature, and other ambient conditions (Hartley, 1920; Vaartaja, 1952; Gibson, 1956; Vaartaja et *al.*, 1961; FAO, 1581; Burdon and Chilvers, 1982; Bloomberg 1985). Results presented here confirm the earlier findings and provide clear indication that nursery practices affect significantly the microclimatic conditions in the nursery and consequently the incidence and severity of seedling diseases of Eucalyptus grandis. To evaluate the influence of various nursery practices such as shading moisture regime and seed rate on the incidence and severity of diseases we shall discuss these factors separately.

Shading

Among the various nursery practices, the type of shading provided over the seed beds to prevent sun scorching of eucalypt seedlings (Doran, 1977). appears to be the most important factor affecting the microciimatic conditions in the nursery and consequently the incidence and severity of diseases as also has been reported by Vaartaja (1952). Low light intensity due to CLT shade lowers the ambient temperature, soil temperature and soil water potential, which favour the development of web blight and damping-off diseases. Conversely, seed beds under CM received fifteen times more light which results in higher ambient temperature, soil temperature and soil water potential than CLT, favouring the development of seedling blight and shoot blight. This confirms Vaartaja's (1952) observations that different shade treatments result in differences in temperature and moisture, and also high light intensity increases the dryness of soil which decreases the incidence of damping-off. Roth and Ricker (1943) have also reporteo severe damping-off under shade than on exposed sites having low soii moisture.

Soil moisture regime.

The purpose of watering the seed beds is to keep the soil moist but not sodden. Observations like 'watering twice a day is desirable in many countries, especially in hot dry areas' (FAO, 1981) are

misleading since it is not the frequency alone but also the quantity of water per day that is important. Qadri (1971) has reported 2.94 l m^{-2} per day (6 gal. 100 ft^{-2} per day) for seed beds of *E.* tereticornis Sm. whereas we find that atleast 111 m^{-2} per day is required to keep the soil moist under coirmat. From this it is obvious that quantity of water will depend upon the local climatic conditions, texture of nursery soil, age of seedlings and type of shade used in the nursery (FAO, 1981).

Soil moisture regime of seedbeds, which is influenced by the type of shading used and quantity of water applied is another important factor affecting directly the incidence and severity of diseases. This is evident in all the four diseases where severity is higher in MR2 than in MR1 treatments of both CM and CLT shades. The tendency of increased disease with increased moisture has also been reported by Vaartaja et al. (1961) for damping-off of pines caused by Pythium. This is possibly beacuse Rhizoctonia solani, the web blight and main damping-off pathogen of eucalypt seedlings in Kerala (Sharma et al., 1984), is favoured by high soil moisture. On the contrary, recently Sharma and Sankaran (1987) reported adverse effect of high soil moisture on the mycelial growth of *R.* solani and development of web blight of Albizia falcataria. This type of behaviour of *R.* solani could be due to the involvement of various pathogenic strains differing in H_2O and O_2 requirements (Parmeter, 1970). Low severity of shoot wilt under CLT as compared to CM may find an explanation in observations of Mustafa and Chattopadhyay (1971) who reported that growth of *S.* rolfsii is progressively less with increasing moisture content.

Seed rate

In the literature different sowing rates have been recommended for eucalypt seeds. While for *E, grandis* a sowing rate of 12 g m^{-2} has been recommended by Barrett (1978), on the other hand for *E.* tereticornis it is as high as 41 g m^{-2} (Qadri, 1971). It is not known whether in the latter species pure seeds or seeds with chaff were used.

Seed rate directly influencing the host density also plays a significant role in the host-pathogen-environment disease triangle.

Table 11.5. Second degree polynomial regression equation (SDCI) fitted to root and shoot growth of *E grandis* seedlings in different treatments.

Shade	Moisture regime (MR) (l of water m^{2-1}	Root and Shoot	SDCX ($y = a + bx + cx^2$)	R^2	F-value
Coir mat (CM)	11 (MR-1)	Root	Y= 1,119 + 0.007464 x + 0.0006156 x^2	0.9818	134.9**[b]
		shoot	Y = 16.10 - 0.7536 x + 0,008553 x^2	0.9303	33.39**
	14 (MR-2)	Root	Y = 2.817 - 0.05390 x + 0.0008605 x^2	0.8198	11.37*[a]
		shoot	Y = 7.066 - 0.2909 x + 0,003613 x^2	0.9394	38.78*[a]
Coconut leaf thatch (CLT)	11 (MR-1)	Root	Y = 0.02360 x + 0,0446 x - 0.0001953 x^2	0.8052	10.331
		Shoot	Y = 2.852 - 0.06352 x + 0,0009524x^2	0.94	37.84*
	14 (MR-2)	Root	Y = 1.162 - 0.010 59 x + 0.0003067 x^2	0.9178	27.91**
		Shoot	Y = 2.899 - 0.06217 x + 0.0008978 x^2	0.8545	14.65**

a, significant at 5%; b, significant at 1%.

Results provide positive relationship between increased disease incidence and severity, and high seedling density due to high seed rate (SR2). These results are in agreement with the earlier recommendations that sowing densities may need to be modified in accordance with degree of risk of damping-off and web blight (FAO, 1981; Sharma and Sankaran, 1984).

In our experiments high seed rate (SR2) is two and half times of low seed rate (SR1). Corresponding two and half to three times increase in disease incidence has been recorded only for web blight and damping-off, both under CM and CLT shade treatments. Since, for seedling blight and shoot wilt quantitative data are lacking this trend is not apparent. These observations conform to those of Burdon and Chilvers (1975a) who deduced from studies on damping-off of Lapidium sativum caused by Pythium irregulare that a four-fold

increase in host density produced an identical four-fold increase in the number of plants receiving primary infection. The proportion of host plants becoming diseased, therefore, remained constant. They concluded that there is no reason to doubt that host density effects acted directly on the incidence of damping-off through simple changes in the number of host targets. Since, Burdon and Chilvers' results are based on glass house trials with artificial inoculation and ours are field trials with natural infection, the latter are unlikely to show the exactness in the increase of disease with the corresponding increase of host density.

Disease progress rate

All the four diseases recorded can be categorised as rapid-limited duration rate diseases for this type of disease progress rate is often associated with diseases specific to a host growth stage (Bloomberg, 1985). There are only a few records of disease progress rates in nurseries. From the evidence available, it is obvious that disease progress rate in forest nurseries varies greatly among pathogen species, tree species or provenance within species, and especially from year to year (Bloomberg, 1985). Our studies provide first evidence that AUDPC and disease progress rates of damping-off and web blight are also greatly influenced by the forest nursery practices such as moisture regime, shading and seed rate (host density); majority of positive correlations between these factors and disease incidence and severit reported earlier are based on comparisons made at a single point of time. Increase in seed rate and moisture regime also increase both the parameters of disease severity. However, the effect of light intensity (depending upon type of shading) on AUDPC and disease progress rate appears to be disease specific. With regard to host density, Burdon and Chilvers (1975a,b, 1982) have reported that both the infection rate and rate of spread of advancing disease fronts of damping-off caused by P. irregulare have similar curvilinear relationship to host density.

The results obtained by using AUDPC as a measure of severity of damping-off and web blight over a period of time are comparable well with the disease infection rates. Thus, AUDPC provides excellent and simple measure of disease severities influenced by various nursery

practices. Since calculation of AUDPC uses all data available it does not obscure the variation in rate of disease' development because of transformations (Shaner and Finney, 1977). As stipulated, AUDPC was calculated from a common time base in order to compare treatments, because it is a product of time and severity.

Seedling growth

Seedling quality, which reflects the integration of a multitude of physiological and morphological characteristics, is a prerequisite to intensive forestry practice because upon it depends the success of a plantation programme (Duryea, 1984; Ritchie, 19841; a stock of good quality seedlings may even compensate for inadequate site preparations (Iverson, 1984). Thus, the nursery practices employed in raising seedlings for any afforestation programme need important attention.

In most of the studies on growth of seedlings, usually root dry weight and shoot dry weight are taken into account for determining the shoot:root (S:R) ratio (Lavender 1984; Jones 1984). However, in the present study, shoot length and root length are used for calculating the S:R ratio. Though it may not be an appropriate method for S:R ratio, certainly it provides some indications of the effects of nursery practices on growth of seedlings. Nevertheless, both root dry weight and root length are known to exhibit more or less similar pattern of growth (Turner and Burch, 1983).

It is evident from the results that the seedling growth is greatly influenced by various nursery practices, especially the type of shading and soil moisture regime. Seedlings under CM record higher shoot and root lengths than those CLT. Solberg (1978) has also found that seedlings of Pinus caribaea respond strongly to different shade treatments. Shoot:root ratio of seedlings, also affected significantly by various treatments shows, however, a differnt trend as-compared to shoot and root lengths. Initially S:R ratio is higher in CLT seedlings than in CM but later, towards the end of observation at 105-day of emergence, seedlings of MR2 treatment under CM and CLT record higher S:R ratio than MR1. This possibly indicates that effect of moisture regime is more pronounced on S:R ratio than that of the shade. High S:R ratio of seedlings growing in high moisture regime has also been recorded by Zimmerman and Brown (1971). Within one

moisture regime, S:R ratio of seedlings is higher under CM than under CLT. This could be because of higher soil temperature and light intesity under CM as compared to CLT. Similarly, Davidson (1969) has also reported that at high temperature S:R ratio also shows increase. Considering all the microclimatic factors over the experimental period of 105-day of emergence when the S:R ratio shows a gradual increase in all the treatments, the soil water potential and soil temperature decrease whereas ambient temperature shows an increase. These results are in conformity with those of Brouwer (1966) who found that S:R ratio is sensitive to environment and it changes with the age of plant; older plants, generally, have high ratio than younger ones (Jones, 1984).

12. Effect of Seed Rate and Seed Viability on the Number of Prickable Seedlings of *Eucalyptus grandis*

In Kerala, eucalypt seedlings are raised usually in seedbed nurseries and when seedlings attain a height of over 10 cm they are pricked into the polythene containers. The seed rate, has been found to be varying greatly from 25 g to 210 g per seedbed (i.e., 7.75 to 16.7 g m^{-2}) and the main reason for this is believed to be using seeds of poor or unknown viability. High seed rate leads to high density of seedlings in seedbed, producing conducive microclimate for the incidence and spread of diseases. Lately, there is a 3 to 4 fold increase in the cost of eucalypt seeds. In order to minimise disease hazards in the eucalypt nurseries, bring down the wastage of seedlings and to ensure availability of required number of healthy seedlings it is essential to standardise the seed rate for eucalypt nurseries. With this in view, the effect of seed viability and seed rate on the density as well as number of prickable seedlings of Eucalyptus grandis was studied.

MATERIALS AND METHODS

Seeds of local E. grandis utilised in this experiment had a viability of ca. 95%. Three seed rates viz. 2.8, 5.6 and 7.0 g m^{-2} equivalent to 40g, 80g and 100g respectively for a standard seedbed were selected for the study. Each seed rate was replicated in three small experimental beds of 1 m x 1 m, provided with shade of coir mat (1.3 cm mesh). The beds received 10 l of water m^{-2} per day. When the seedlings were 3-week-old, seedling density was estimated in each replicate bed by counting number of seedlings in a quadrate of 15 cm x 15 cm placed at five places of bed at random. From each observation seedling density in a standard bed was estimated. The data were analysed by one-way ANOVA.

Seedlings, 10 cm and above in height were pricked from the above seedbeds at weekly intervals from 65 day of emergence and it was continued up to 122 day of emergence. Number of prickable seedlings in a standard bed (12 m x 1.2 m) was estimated from number of

seedlings pricked in three replicate seedbeds, of 1 m x 1 m. Mean and standard error (SE) were calculated for each seed rate and the data were analysed statistically by one-way ANOVA followed by multiple comparison of means.

In another experiment seeds of old stock having poor viability viz. ca. 35%, 55% and 75% were utilised for studying the effect of seed viability (SV) and seed rate (SR) (2.8, 5.6, and 7.0 g m^{-2}) on the number of prickable seedlings. Three replicate seedbeds, 1 x 1 m, for each SV and SR combination were raised under coir mat and water was given at the rate of 10 l m^{-2} per day. Seedling density for each SR and SV combinations was estimated as in the previous experiment. Mean and SE were calculated and the data were analysed statistically by one-way ANOVA.

Seedlings, 10 cm and above were pricked from 65 day of emergence and it was continued till 122 day. Observations were recorded at weekly interval. From each observation, total number of prickable seedling in a standard bed was calculated and the date were analysed statistically.

RESULTS

Prickable seedlings in relation to seed rate

Seed rate affected significantly ($P < 0.01$) the seedlings density. As expected the density increased with the seed rate (Table 12.1). Similarly, the cumulative number of prickable seedlings also significantly affected by the seed rate i.e., higher the seed rate higher the number of prickable seedlings. Seed rates SR1 and SR2 and SR1 and SR3 had significantly different ($P < 0.05$) number of prickable seedlings; the difference between SR2 and SR3 was non-significant. Interestingly, the percentage of prickable seedlings in respect of seedling density decreased as the seed rate increased.

Prickabie seedlings in relation to seed rates of different seed viabilities

Both seed rate as well as seed viability affected significantly ($P < 0.01$) the seedling density in a standard bed; seedling density increased with increasing seed viability and seed rate (Table 12.2).

Table 12.1 Estimated prickable seedlings (10 cm and above) of E. grandis is a standard bed (12 x1.2 m) in relation to seed rate

Seed rate (SR) (gm^{-2})	Estimated curulative number of prickabie seedlings at different periods after erergence (mean and S.E. of 3 replications)							Mean estimated density of seedlings in a 122 standard bed and S.E.	% of prick-able seedi-ings
	65th day	73rd day	82nd day	90th day	98th day	106th day	114th day		
2.8(SRl)	71	521	3891	5959	7802	9890	13854	14556	65,578	22.1
	±29.78	±158.95	± 671.18	720.0	±678.14	±547.25	±437.85	±299.21	±3238	
5.6 (SR2)	350	1492	5428	8141	10203	12401	17330	18127	1,05,856	17.12
	±97.28	±184.31	±404.87	637.9	±803.99	±967.72	2930.96	±888.67	55714	
7.0 (SR3)	590	1881	6123	8096	9848	12771	18065	18856	1,49,888	12.58
	±302.5	±565.03	±374.80	±300.17	2115.67	±203.78	±220.56	2199.70	±9119	

LSD = 215.8912

Generally, in the initial stages more prickable seedlings were obtained from the seedlings having lower seed rate (SR1, SR2) as compared to those with high seed rate (SR3). Later, however, more seedlings were available from SR3 than SR1 or SR2, except in SV2 SR2 where higher number of prickable seedlings were available than that of SR3. For a given seed viability, as the seed rate increased the cumulative number of prickable seedlings also increased gradually. However, the percentage of prickable seedlings decreased as the seed rate increased. In SVI, the three seed rates did not differ significantly in their output of prickable seedlings. Significantly higher percentage of seedlings was available in SV2–SR2, and SV3–SR1 treatments; highest seed rate (SR3) had lowest percentage of prickable seedlings. In ANOVA, the effect of seed rate and seed viability on the number of prickable seedlings was highly significant at $P<0.01$, but their interaction was not significant. This indicates that the pattern of number of prickable seedlings available at different time period was not different for the three seed viabilities.

Table 12.2 Estimated prickable seedlings (10 cm and above of *E. grandis* in a stantard bed (12 x 1.2m) in relation to seed viability and seed rate

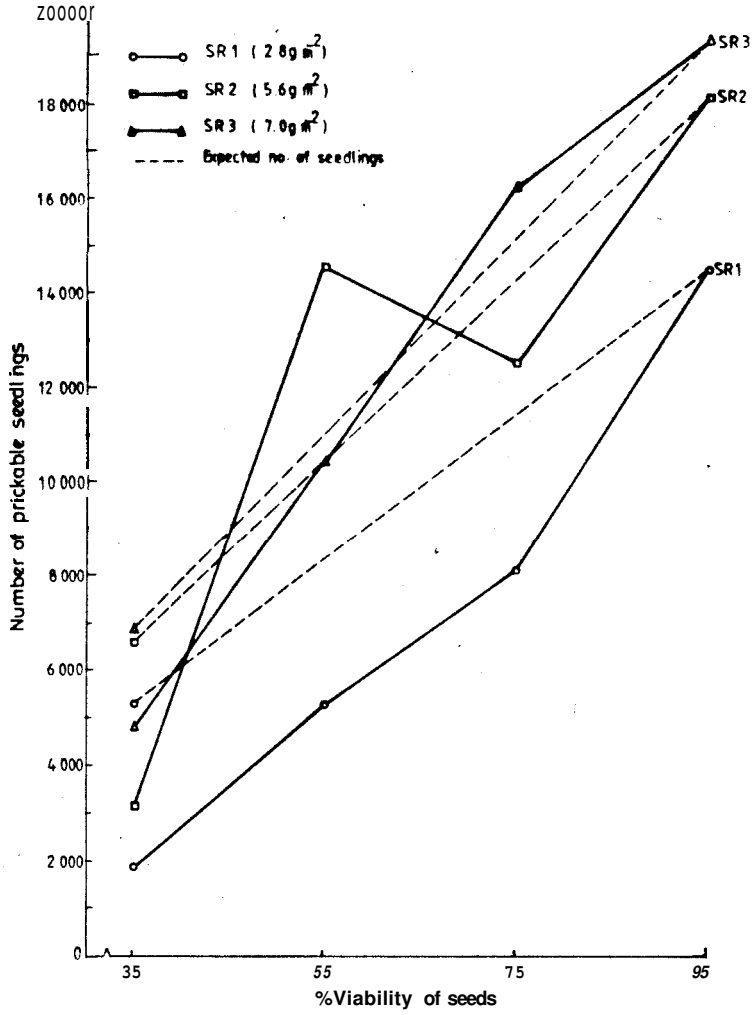
Seed viability (SV) (%)	Seed rate (SR) (gm^{-2})	Estimated cumulative number of prickable seedlings at different periods after emergence lmean of 3 replications)								Mean estimated density of seedlings in a standard bed S.E.	% of prickable seedlings
		65th day	73rd day	82nd day	90th day	98th day	106th day	114th day	122nd day		
35 (SV1)	2.8 (SR1)	0	14	215	416	790	1193	1654	1899	24,320 ± 3275	7.8
	5.6 (SR2)	0	14	230	446	1007	1919	2835	3281	49,021 + 5885	6.74
	7.0 (SR3)	0	0	57	158	791	2101	4131	4851	11552 + 3993	6.78
55 (SV2)	2.8 (SR1)	14	100	834	1338	2015	3095	4578	5370	54656 + 5156	9.90
	5.6 (SR2)	43	244	3081	4838	6436	9129	13118	14601	14496 + 4880	19.60
	7.0 (SR3)	0	43	120	1713	3038	5515	9417	10482	152704 + 10687	7.54
75 (SV3)	2.8 (SR1)	86	345	2030	2692	3527	5111	7357	8163	67600 + 2811	14.17
	5.6 (SR2)	101	302	2174	3585	5823	7804	11749	12541	90752 + 5821	13.9
	7.0 (SR3)	0	43	1627	3441	6500	9503	15248	16311	113440 + 24101	9.45

DISCUSSION

Careful selection of planting stock is critical in any afforestation programme. A good choice of planting stock may even compensate for inadequate site preparations (Iverson, 1984). For raising disease-free healthy seedlings, appropriate seed rate is one of the mportant component of nursery management. Since high seedling density promotes development and spread of disease it is always advisab e to use right quantity of seeds per bed to avoid disease problem as well as to obtain healthy stock. Furthermore, this view gets support from the outplanting results of Iverson (1981) and Duryea (1984) who compared the performance of seedlings grown at various densities. They found that survival did not differ significantly but seedlings initially grown at lower density grew taller than grown at higher densities. It is clearly evident from the results that seed

rate affects significantly the number of prickable seedlings as with the increase in seed rate percentage of prickable seedlings show decline. Our results are in conformity with earlier observations on seedlings of a number of temperate trees species that increased seedling density is negatively correlated with height growth (Baron and Schubert, 1963), and stem diameter and dry weight (Edgren, 1976; (verson, 1981).

Seed viability, which governs the seed rate, influences significantly the availability of prickable seedlings. Generally, the percentage of prickable seedlings declines with increasing seed viability. This could be due to high density of seedlings which has negative effect on height growth due to cpmpetition and overcrowding (Thompson, 1984). Seed rate and viability of seeds show significant interaction in the estimation of seedlings in seedbeds. The same appears to be true for prickable seedlings in respect of seed rate and seed viability a5 evident from Fig. 12.1. Deviation of actual number of prickable seedlings from the expected values, calculated from the prickable seedlings available in 95% viable seeds is also shown in Fig 12.1. As the seed rate decreases the difference between estimated number and actual values increases, converse is true for the high seed rates. It is not clearly understood whether this phenomenon is either due to the loss in seedling vigour because of poor seed viability or the seedling density or a negative relationship between seed germinability and prickable seedlings. The results indicate that due to the unexpected decline in the availability of prickable seedlings it may not be possible to estimate the number of prickable seedlings for a given seed rate from the values obtained for seeds having high viability. The important point that emerges from these results is that even when the seeds are of poor viability, the required number of healthy nursery stock can be ensured by choosing the appropriate seed rate as seen from SV2 treatment. However, a high seed rate with seeds of poor viability will have negative effect on the availability of seedlings. It is, therefore, suggested to use always seeds of known viability so that an appropriate quantity of seeds can be determined and sown for the required number of seedling as a part of sound nursery management.



Fig, 12.1. Prickable seedlings in relation to seed rates SR1, SR2, SR3) of different seed viabilities.

This study clearly shows that a low percentage (maximum **22.1%** in **SR1)** of prickable seedlings is available from seedbed nursery (Table **12.1).** Obviously, the unpricked seedlings will be left in the mother bed to be pricked later for casualty replacements, for which such a large number of seedlings will not be required. It means a good percentage of seedlings are likely to be wasted. Though seedbed nursery method of raising seedlings is in vogue for a long time, considering the wastage of seedlings and cost of good quality seeds of forest tree species ever increasing, it will be advisable to adopt polyurethane foam technique (Chacko, **1983)** or direct container sowing technique (see chapter **13),** wherever possible for raising healthy eucalypt seedlings economically.

13. Comparison of Direct-Sown and Transplanted Eucalypt Seedlings in Nursery and the Field

Since the occurrence of serious diseases in eucalypt nurseries in Kerala are mainly due to lapses in management practices followed, effective disease control may be brought about by adopting appropriate nursery practices (Sharma et al., 1984). In order to minimise the disease hazards, the cost and also the length of nursery period a direct container sowing method for raising the seedlings was attempted. Seedlings of Eucalyptus grandis Hills ex Maid.. raised in containers and seedbeds were outplanted and their performance was compared in respect of incidence of diseases and height growth in the nursery and field.

MATERIALS AND METHODS

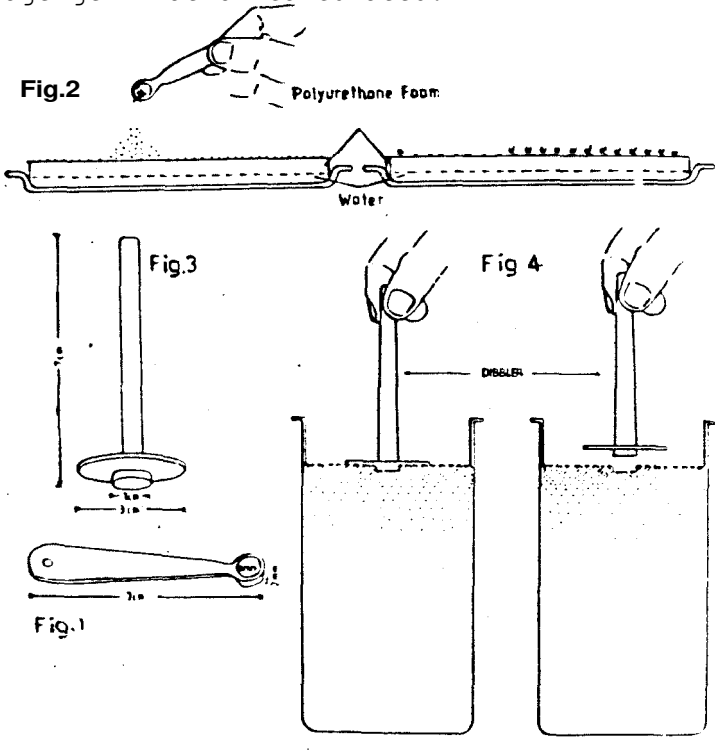
Nursery site and seedbed nursery

The nursery was raised at Chandanathode during 1982 as described earlier in Chapter 10. Brief details in respect of various nursery operations such as sowing of seeds, emergence of seedlings, application of fertilizer and fungicides, etc'. are provided in Table 13.1. Healthy seedlings from treated and control seedbeds were pricked out into containers (18 cm x 12 cm) in March when the seedling height was in excess of 10 cm. Initially, the container seedlings were kept under coir mat shade for two weeks, later the coir mats were removed gradually.

Direct-sown container seedlings

Polythene bags (25 cm x 18 cm for large containers, and 15 x 8 cm for small containers) with at least 3 holes at the base were filled with sieved forest soil, leaving about 0.5 cm at the top to hold the water. Large and small containers were placed on raised platforms 12 m x 1.2 m and 10 m x 1.2 m respectively, which were lined all around with reed bamboos for support. The containers were arranged leaving a small space between them. From the seed lot, excess of chaff was

removed using an ordinary kitchen sieve and pure seeds of *E.* grandis having high germinability were used. Viability of seeds was determined by spreading about 100 seeds on a piece of moist polyurethane foam (10 x 10 cm x 2 cm) (Chacko, 1983); approximately 100 seeds were taken from the seedlot using a scoop (Fig. 13.1). The foam was kept adequately moist, not flooded with water, by placing it in a plate/dish containing sufficient water (Fig. 13.2). The dish was covered with another dish so as to maintain high humidity around the seeds. After five days, number of germinated seeds was counted and the percentage germination calculated.



Figs. 13.1-4. 1. A scoop standardized to carry approximately 100 seeds of *E.* grandis and *E. tereticornis.* 2. A diagrammatic representation of a method for testing viability of eucalypt seeds. 3. A dibbler for making a depression in the soil. 4. Use of dibbler for making a depression in the container

Before sowing the seeds, the containers were watered at least two times a day. For sowing, a shallow depression, not more than 3 mm deep and 1 cm across, was made in the soil by placing a specially designed dibbler (Fig. 13.3) gently at the centre of the container soil (Fig. 13.4). A small pinch of seeds, 3 to 8 in number of known viability were put and the depression covered with fine sieved soil.

After the emergence, when seedlings were in the second leaf pair stage they were thinned by hand to two per container. A second culling to reduce the number to one seedling per container was done during the sixth week. The container beds were watered two to three times a day depending upon the climatic conditions with 30 litres of water using a fine spray rosecan. The container seedlings were provided with shade of coir mat (7 mm mesh) for initial two months.

Fungicide treatment

Fungicidal treatments for seedbeds/container seedlings were the same as given earlier for 1982 nursery trials (see Chapter 10). Details of fungicides applied to small and large container seedlings are provided in Tables 13.2 and 13.3 respectively. In treatments T1 to T4 of small containers, the bags were first filled half with the sieved soil and the top half with soil mixed with PCNB @ 150 mg per container. Each treatment had six replicates of 50 seedlings each, except for T3 and T4 which had only 25 seedlings in each replicate. Equal number of control seedlings were maintained for each treatment. Foliar application of carbendazim and copper oxychloride was made in April when Rhizoctonia solani infection was recorded. Second application of carbendazim was given a week before planting out the seedlings.

Disease incidence and severity

Incidence and severity of diseases in seedbeds have been described earlier in Chapter 10. In container seedlings, the severity of diseases such as Rhizoctonia stem infection, and Phaeoseptoria leaf spot was recorded; except for the former where percentage of affected or dead seedlings was calculated, severity of the latter disease was rated on a 0-3 scale (0, no disease; 1, low;2 medium; 3, severe).

Fertilizer application

Diammonium phosphate (DAP) was applied to container seelings to enhance their growth. Details of fertilizer application are given in Table 13.1.

fable 13.1. Details of various operations carried out in seed bed and direct-sown container nurseries at Chandanathode during 1982

Particulars	Age of seedlings (days)	Seedbed/transplanted seedlings	Direct-sown large container seedling	Age of seedlings (days)	Direct-sown small container seedlings
1. Sowing of seeds		30 January	30 January		17 Harch
2. Seedling emergence	0	5 February	5 February	0	22 Harch
3. Fungicidal application	5	10 February	10 February		
4. Fertilizer (1st) application	15	20 February	20 February	10	1 April
5. Pricking of seedlings into containers 918x12cm)	40	17 March		-	
6. Fungicide application	74	20 April	20 April	30	20 April
7. Fertiliter (2nd) application	79	25 April	25 April	40	1 Hay
8. Height (Cm) of seedlings on the day of field planting	125	33.9±3.06	42.85±3.80	81	19.15±2.11
9. Field planting	125	10 June	10 June	81	10 June

Field performance

The field performance of direct container-sown and transplanted seedlings of *E. **grandis*** was assessed in an area of 0.75 ha at Vattappoil (Wynad). The area, part of 1979 failed *E. **tereticornis*** plantation, was surrounded by natural forest on three sides and eucalypt plantation on the other side. The site preparations were initiated during the last week of April 1982; the area was clear-

weeded and planting alignment done and pits of 30 cm x 30 cm x 30 cm were taken at an espacement of 2 m x 2 m.

Within one treatment all treated seedlings were pooled for planting out as no significant difference in height and disease incidence was observed; untreated control seedlings were kept separately. Healthy seedlings of ***E. grandis,*** similar in height and vigour were selected from the three treatments: direct container-sown (small) (TI), direct container-sown (large) (T II) and transplanted seedlings (T III). Each treatment had fungicide treated and control seedlings. The details of fungicidal application are provided in Table 13.1. These seedlings were outplanted after the onset of monsoon during the first week of June 1982. In all, a total of 1335 seedlings were planted, 300 in TI, 450 in T II, and 585 seedlings in T III, the control. Prior to planting all the container seedlings were drenched with aldrin 30 EC (0.02% a.i.) (Nair and Varma, 1981). Because of high pressure of termites in the area the planting pits were also drenched with aldrin 30EC (0.01% a.i.). Height and survival percentage of seedlings were recorded after 7 and 21 months of planting. Incidence of pink disease and Cryophonectria stem canker was recorded during the second observation.

RESULTS

Occurrence of diseases

Transplanted seedlings

Details of incidence of diseases in transplanted seedlings are given in Chapter 10. After pricking the seedlings in containers during March 1982 the only disease which killed 1.71% of seedlings in the month of April was Rhizoctonia root rot; during Nay only one seedling died. Slight foliar infection of ***Phaeoseptoria*** appeared in lower leaves of ca < 5% seedlings; ***E. tereticornis*** seedlings were more susceptible than those of *E.* grandis. No Cylindrocladium leaf blight was recorded in any of the treatments.

Small direct-sown container seedlings

The best treatments were T1 and T4 where except mild infection of Phaeoseptoria leaf spot no other disease was observed. Rhizoctonia

Table 13.2. Effect of fungicidal treatments on the occurance of disease of direct-sown small container seedlings at Chandanathode during 1982

Treatment	Fungicide (% a.i.)		DAP foliar application on 1 April and 1 May 1982 (%)	Disease incidence in small container seedlings							
				E. grandis				*E. tereticornis*			
	Pre-sowing soil application	Post-emergence foliar application		% Seedlings dead due to Rhizoctonia stem infection		Phaeosep-toria (P) leaf spot	Coniella (C) leaf spot	% Seedlings dead due to Rhizoctonia stem infection		Leaf spot (P)	Leaf spot (C)
				30 April	27 Hay			30 April	27 Hay		
T1*	PCNB	Carbendazim (0.05)	0.5	0	0	+		0	0	+	-
T2*	PCNB	Carbendazim (0.05) Copper oxy-chloride 10.0251		0.66	0	+		0	0	+	-
T3*	PCNB	Carbendazim (0.05) Copper oxy-chloride (0.025)	0.5	0.66	0	+		0	0	+	
T4*	PCNB	Carbendazim (0.05) Copper oxy-chloride (0.0251		0	0	+		0	0	+	
T5	Control		0.5	8.66	1.0		+	0	0.33	+	+
T6	Control	-	-	13.0	1.66	-	+	0	0.33		+

[1] Treatment had 30 seedlings in 6 replications of 5G seedlings each; T5 and T6 had only 150 seedlings each.

stem infection was the major disease recorded in small container seedlings. Initially, the disease incidence was high (<15%) but later

it decreased as the seedlings matured; ***E. grandis*** was found to be more susceptible than ***E. tereticornis***, which recorded the disease only during May. Both the species appeared to be equally susceptible to Pheaoseptoria leaf spot but interestingly no infection was recorded in the control seedlings of ***E. grandis***. Low infection of ***Coniella*** was recorded only in the control seedlings of ***E. tereticornis*** and ***E. grandis*** (Table 13.2).

Large direct-sown container seedlings

In large container seedlings no Rhizoctonia stem infection was recorded. Phaeoseptoria leaf spot appeared during May in all the treatments; ***E. tereticornis*** appeared to be more susceptible than ***E. grandis*** in which moderate infectionn was recorded in five treatments. Low infection of ***Coniella*** was recorded only in a few treatments of both the species (Table 13.3).

Height growth of seedlings

The growth of transplanted seedlings was good in all the treatments, except those of T3 and T6 where PCNB was applied as pre-sowing treatment. The average height of seedlings before outplanting was 33.91 cm.

Average height of 81-day-old direct-sown small container seedlings before planting was 19.15 cm, which was significantly less than the other two treatments viz. transplanted seedlings and large container seedlings. Initially, the growth was slow, however, due to application of DAP on I April the seedlings showed improvement.

The height of large container seedlings was significantly higher than those of two other treatments; the seedlings were tall but slender,possibly because of two DAP treatments which accelarated the height growth.

Field performance

After seven months of planting the trend of height growth was similar to initial height just before planting with overall survival of 83.53%; maximum height was recorded for large container seedlings (Table 13.4–13.6). ANOVA showed significant difference among the various treatments at $P<0.05$ (Table 13.5). The control seedlings in

Table13.3. Effect of fungicidal treatment on the occurance of diseases in direct-son large container seedlings at Chandanathode during 1982

treatrent	Fungicides @ Pre-sowing soil application	Fungicides @ Post-emergence foliar application	Disease incidence in large container seedlings *E. teretricornis* Rhizoctonia stem infection	*E. teretricornis* Phaeoseptoria leaf spot	*E. teretricornis* Coniella leaf spot	*E. grandis* Rhizoctonia stem infection	*E. grandis* Phaeoseptoria leaf spot	*E. grandis* Coniella leaf spot
11	-	Carbendazis (1,2,3)*		+		-	+	
12		benomyl (1,2,3)		+		+	+	
13	PCNB	Copper oxychloride Carbendazim(1) Carbendazir (2,3)		+			+	-
T4		Bordeaux mixture (1,2,3)	-	++	-	-	++	+
T5		Captafol(1,2,3)	-	+	+	_	++	
T6	PCNB	Copper oxychloride Carbendazim 1) Carbendazin (2,3)	-	++			++	
T7		Copper oxychloride Carbendarid 1), Carbendazim(2,3)	-	+	+		+	
T8	-	Captafol Carbendazimi1l, Carbendazim (2,3)	-	+		+	++	
T9	Control	-		+			++	

@ For details of dosage, see Nursery trials of 1982 in Chapter 10.

4 Respectively first, second and third application

all the treatments had higher height growth than the treated seedlings. However, at 21 months no significant difference was observed in the height of treated and untreated plants. But the percent survival of seedlings showed some significant differences. In direct-sown container seedlings, percentage survival of treated seedlings was higher than that of compared to untreated ones; in transplanted seedlings it was just the reverse as the percentage survival of untreated seedlings was high. The lowest percentage survival (22.58) was recorded for small container-sown seedlings.

Table 19.4. Height growth of 7-month-old outplanted seedlings of *E. grandis* at Vattapoyil during 1982

Planting	Average height (Cm) of plants in replicate rows					
	Direct-sown small containers		Direct-sown large containers		Transplanted [seedbed seedlings	
	Treated (1)	Untreated (2)	Treated (3)	Untreated (4)	Treated (5)	Untreated
1	152.09	139.68	154.00	166.34	135.64	161.02
2	128.68	-	163.65	167.07	145.75	142.19
3	122.50	-	158.20	136.07	137.20	139.51
4	133.46	-	151.08		146.42	141.79
5	125.50	-	141.47	-	145.47	
6		-	149.25	-	152.00	-
7	-	-	-	-	132.28	-
8	-	-	-	-	127.10	-
9	-	-	-	-	114.14	-
10	-	-	-	-	144.76	
	132.44	139.68	152.94	156.49	138.07	146.1215

As regards the mortality of seedlings a total of 3.90% of seedlings died on account of stem canker caused by pink disease (2.34%) and ***Cryophonectria*** (1.56%). Both the stem diseases appeared

one year after planting and generally, all the affected seedlings died within one year. High mortality of container-sown seedlings was partly due to weeds and cattle damage.

Table 13.5. One-way Anova of Data presented in Table 19.4

Source	SS	DF	MS	F
Treatment	1987.06	5	397.41	3.1285*
Total	4908.75	28		
Error	2921.68	23	127.02	

* P=0.05

Table 13.6, Height growth performance and percent survival of direct-sown container and transplanted container seedlings of *E. grandis* at Vattapoyil 1982

Treatments (No. of replicate rows)		Age of plants (months)	Mean height (m)	Percent survival
Direct-sown smallcontainer	Treated (5)	7	1.32	
		21	6.26	69.57
	Untreated (1)	7	1.39	
		21	7.04	22.58
Direct-sown large container	Treated (6)	7	1.53	
		21	6.91	58.34
	Untreated (3)	7	1.56	
		21	6.65	54.40
Transplanted container seedlings	Treated (10)	7	1.30	
		21	7.27	64.86
	Untreated	7	1.46	
		21	6.56	73.44

DISCUSSION

Sound nursery management practices, which can avert the occurrence of many serious diseases coupled with some prophylactic treatments can ensure a disease-free nursery. Comparison of direct-sown and transplanted seedlings has yielded some interesting results. All the fungicidal treatments given to seedlings controlled effectively the Cylindrocladium leaf blight (CLB). However, some diseases of minor importance such as Rhizoctonia stem infection (RSI), Phaeoseptoria leaf spot and Coniella leaf spot could not be controlled effectively in the nursery; possibly either the fungicides used were not effective or the dosage of these fungicides was not sufficient to control these diseases. Except for **<15**% mortality of seedlings in small containers due to RSI there was no mortality in other treatments. After the seedlings were outplanted no CLB was recorded. However, pink disease and Cryphonectria stem canker caused some mortality of seedlings after one year of planting. This early infection could be due to high disease pressure in nearby eucalypt plantations.

Though, initially, the three treatments showed significant difference in height growth, later there was no difference; This clearly indicates that the small container seedlings are equally good for field planting. But the low percentage survival of direct-sown container seedlings as compared to those of transplanted seedlings raises some doubt on the feasibility of the method. Higher percentage survival of transplanted seedlings as compared to direct-sown container seedlings has also been observed by Solberg (1978) in *Pinus caribaea*. Besides the weed problem and cattle damage to direct-sown seedlings, another reason for the relatively higher rate of survival of transplanted seedlings may be the unintentional selection of the most healthy seedlings from the seedbed while pricking them out.Furthermore, pricking up surplus seedlings from the direct-sown containers may also have caused slight interuption to the remaining seedlings.

view of economic considerations and various other advantages and disadvantages involved in carrying out various operations, a comparison between transplanted and direct-sown container nurseries is

made in Table 13.7. The direct-sown container method appears to be economical as it avoids preparation of seedbeds and it has lesser period of maintenance of seedlings in the nursery as compared to seedbed nursery, thus bringing down the overall cost of raising the nursery. Evidently, direct-sown container nursery appears to be a viable alternative.

Table 13.7. Comparison seedbed and direct-sown container nursery

Particulars	Seedbed nursery	Direct-sown container nursery
1. Formation and maintenance cost of raising seedling for 10 ha	Rs. 9,500/-	Rs. 6,500/-
2. Period of maintenance of nursery	Five-six months	Three months
3. Disease problems	numerous	negligible
4. Cost of prophylactic fungicidal treatment	Rs.290/-(High rainfall area)	Rs. 166/-(High rainfall area)
5, Availability of seedlings	A large number of seedlings are available for planting and casualty replacement.	A large number of extra seedlings (atleast 7000–8000 per 1000 direct-sown containerlare available for casualty replacement After thinning seedlings can be transfered to new containers.
6. Advantage1 disadvantage at planting tine	Containers large (18x 12 cm) and heavy; a person can carry a maximum of 10 containers at a time.	Containers small (15x9 cm) and light; a person can carry a maximum of 15 containers at a tire

Prices at 1986 rate

From the above, it may be concluded that direct-sown technique (small containers) may be feasible for large-scale planting programmes provided adequate protection is given during the first year against weeds, and cattle damage to circumvent low survival of seedlings. The growth of direct-sown seedlings may be further enhanced by preponing sowing of seeds to 1st week of March as well as by judicious application of fertilizer in the field at the time of planting. Nevertheless, multilocation pilot-scale field trials will be necessary before direct-sowing technique can be adopted for large-scale plantation programmes.

14.General Discussion and Conclusions

Since the genus ***Cylindrocladium*** was originally established by Morgan(1892) for a Mucedinaceae fungus, ***C. scoparium*** Morgan on dead pods of honey locust ***(Gleditschia triacanthus*** L.) in Indonesia, several ***Cylindrocladium*** species have frequently been reported as pathogenic. ***C. quinqueseptatum*** Boedijn & Reitsma, isolated in Indonesia in 1941 by W.C. Sloof from clove leaves and published by Reitsma and Sloof(1950) after establishing its pathogenicity, has emerged as one of the serious pathogens of ***Eucalyptus*** in Australia, Brazil, India, Indonesia, Malaysia and Mauritius (Peerally, 1974; Sharma, 1984; Bolland ***et al.***, 1985; Ferriera, 1989). Association of 10 species of ***Cylindrocladium*** viz. ***C. camelliae*** Venkataramani & Venkata Ram, C. ***clavatum*** Hodges & May, ***C. curvatum*** Boedijn & Reitsma, C. ***floridanum*** Sobers, C. ***ilicicola*** Boedijn & Reitsma, C. ***parvum*** Anderson, ***C. quinqueseptatum, C. scoparium, C. theae*** Loos reported in this study and ***C. colhounii*** Peerally recorded by Nair and Jayasree (1986) with various diseases in Kerala indicates their potential threat to susceptible ***Eucalyptus*** in exotic environment. Among these species, C. ***quinqueseptatum, C. theae*** and C. ***ilicicola*** are the major pathogens affecting eucalypts at different growth stages in nurseries and plantations. In Brazil, which has the largest area under ***Eucalyptus*** plantations, 13 species of ***Cylindrocladium*** have been recorded, the prominent species being ***C. crotalariae*** (Loos) Bell & Sobers, ***C. scoparium, C. quinqueseptatum*** and ***C. ilicicola***. However, in Australia, the home of eucalypts, only ***C. quinqueseptatum*** and ***C. scoparium*** have been reported and only the former species is known to cause severe shoot blight of *E.* ***microcorys*** F. Muell. in Queensland (Fitkethley, 1976; Bolland ***et al.***, 1985). This variation in dominant species in different geographical areas appears to be closely related to eucalypt species grown and climatic conditions, and to a lesser extent, the presence of hosts other than eucalypts on which different ***Cylindrocladium*** species occur. The specialized nature of ***Cylindrocladium*** species is clearly evident from their distribution pattern within Kerala and their causing diseases of specific plant parts. For example, ***C. quinqueseptatum*** is widespread throughout Kerala whereas, ***C. ilicicola*** and ***C. theae*** are localised in high

elevation areas. Similarly, on one hand C. ***quinqueseptatum*** causes diseases of all plant parts, except roots at all growth stages, on the other hand ***C. floridanum*** affects the root of saplings; and ***C. camelliae*** and ***C. clavatum*** cause only seedling diseases. There appears to be an ecological balance between various ***Cylindrocladium*** species which governs their temporal and spatial distribution within a geographica1 area.

The present study reveals that ***C. quinqueseptatum*** has specialised into physiologic strains varying greatly in virulence to adopt eucalypts. *E.* ***tereticornis***, commonly called Mysore hybrid, possibly has considerable genetic variability. This variability in the host may have exerted the selection pressure on C. ***quinqueseptatum*** to evolve into different physiologic strains. Origin of strains is further substantiated by the fusion of germ tubes, originating from the same conidium or different conidia observed on the leaf surface. Of the five strains of ***C. quinqueseptatum*** identified, four (Nos. 755, 897, 947 and 1080) have specific virulence or wide variability in their reactions which possibly means that eucalypt differential provenances may have some common genes for resistance. The fifth strain, No.968, possesses general or uniform virulence within the sampled population as it gave identical reactions to all eucalypt genotypes. This clearly shows that the dynamics of virulence in the population of ***C. quinqueseptatum*** is much more complex than expected. Since the production of conidia in slime will limit the CLB spread among the field sites the population of ***C. quinqueseptatum*** strains will be stabilised during ***Eucalyptus*** rotations. However, by the introduction of resistant provenances, fresh selection pressure will be applied on the pathogen to mutate to suitable strains. Before Contemplating any introduction of new eucalypt provenances in Kerala a detailed survey will be advisable to find out the spectrum of physiologic strains in ***Cylindrocladium*** species ***so*** that appropriate provenances are chosen after testing their field resistance to all the strains.

Different eucalypt provenances show differential susceptibility to three CLB pathogens viz. ***C. ilicicola*** (least virulent), ***C. clavatum*** (the most virulent) and ***C. qvinqueseptatum*** (intermediate). Most significantly, a number of provenances possess resistance to

Cylindrocladium which can be exploited for the management of the disease in eucalypt plantations.

Only certain provenances differentiated one isolate from the other. Except four differential provenances viz. *E.* ***brassiana*** 13412, *E.* ***urophylla*** 12895,E. *grandis* 13020, *E.* ***grandis*** TN local, the rest gave identical reactiions to all the five CQ isolates. This possibly means that they are closely related and, therefore, one provenance each may be selected of the three groups i.e., (i) ***E. tesseilaris*** 12967 and ***E. urophylla*** 12896 with ***S***, R, R, R, R, reactions of the isolates 755, 897, 947, 968 and 1080, (ii)E.***tereticornis*** 13398, ***E. saligna*** 13027 and ***E.*** *brassiana* 13415 with R, R, S,R, R reactions and (iii) ***E. propinqua*** 12800 and ***E.*** *brassiana* 13397 with *S*, R, HS, R, R, reactions to the respective isolates. From the first group ***E. tessellaris*** 12567 may be selected as ***E. urophylla*** 12896 failed to differentiate any of the isolates in multiple comparison of means. *E.* ***saligna*** 13027 may be selected from the second group, though all behaved similarly and differentiated only one isolate 947 from the others; as it belongs to Section Transvaria of Subgenus Symphyomyrtus of ***Eucalyptus*** it may behave differently to other CQ isolates. On the other hand, from the third group ***E. brassiana*** 13397 may be chosen as it differentiated two isolates. Hence, a total of only seven ***Eucalyptus*** provenances may from a set of differential in identifying physiologic strains of ***Cylindrocladium quinqueseptatum.***

Highly pathogenic nature of C. ***quinqueseptatum*** is evident from infection studies which show production of multiple germ tubes by a conidium, and their potential in causing multiple infections of CLB within a short duration through direct penetration. As expected, due to mucilage-borne conidia which are dispersed by water drops, development and spread of CLB is rain-dependent . There is a positive correlation of CLR severity with high rainfall. Since taungya crop of tapioca in young eucalypt plantations provided a conducive environment for rapid buildup of CLB, it may be desirable to replace tapioca with some other crop or with a dwarf variety of tapioca which does not cover the eucalypt seedlings completely. Since, currently the taungya practice has been stopped in eucalypt plantations due to possible soil erosion problems, it may prove to be counter productive as far as protection to young saplings is concerned during the first three years

of establishment. Therefore, it is essential that the plantations are intensively managed, adequate weeding operations are undertaken and protection against grazing is provided.

It is essential to ensure healthy nursery stock for a large-scale plantation programme of eucalypts. Raising healthy seedlings depends largely upon the nursery cultural practices, besides the quality of seeds. Considering the immense pressure of ***Cyliindrocladium*** spp. in Kerala, growing provenances with durable field resistance is the only viable alternative in combating CLB in nursery and plantations. Results of this study provide ample evidence that how nursery practices can influence the seedling growth, and occurrence of seedling diseases, especially those of economically important damping-off and seedling blight. The best treatment combination where growth of seedlings in terms of S:R ratio is optimal and disease incidence and severity are within reasonable limits to be controlled by preventative measures, such as prophylactic fungicidal application, is MR1-SR1 having low moisture regime and low seed rate under coir mat (CM) shading. This means that low sowing density with seeds of known germinability and avoidance of overcrowding of seedlings, overshading and over watering of seedbeds can do much towards reducing the serious problems and providing required number of healthy seedlings. Hence, to overcome the problem of disease hazards nursery practices which are very critical in the production of healthy seedlings, need to be standardised for a particular climatic zone. Results presented here are an important step in the direction of standardisation of nursery practices for eucalypts.

A Chemical control, though justifiable in nursery, is not feasible in plantations due to prohibitive operation costs . The study shows that effective control of CLB and other seedling diseases in the nursery is possible through prophylactic chemical treatment and adopting standard nursery practices. The latter should be given due importance as they can influence significantly the availability of desired quality of plantable seedlings. Though there were a number of fungicides effective, only carbendazim controlled the CLB effectively in nursery trials conducted at Chandanathode. The effectiveness of carbendazim in high rainfall areas like Chandanathode is possibly due to the favourable properties of its active principle, MBC. Since a

significant fraction of carbendazim applied remains in soil and MBC is immobile there is no significant leaching from the site of application which facilitates continuous uptake of the effective principle.

Nursery trials indicate that three prophylactic fungicidal treatments ensure the disease-free seedlings not only in nursery but also in field, atleast for a couple of months after outplanting. First treatment of carbendazim, MEMC and mancozeb to be given prior to seed germination, and two treatments of carbendazim just after pricking out the seedlings in containers and prior to field planting have been found to control all the nursery diseases of eucalypt in Kerala. Since no separate schedule is required . for these three treatments they easily form part of the nursery management practices. It may be concluded that even though CLB could be a derastating disease in eucalypt nurseries, especially those situated in high rainfall areas of Kerala, studies have shown that it can be managed effectively provided adequate timely measures are taken. However, in low rainfall areas where the CLB incidence is generally low, the disease can be controlled even after its appearance and spread using suitable fungicides.

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