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Cryptosporiopsis leaf spot and shoot blight of eucalypts

K. M. Old[A,F], *M. J. Dudzinski*[A], *K. Pongpanich*[B], *Z. Q. Yuan*[C], *Pham Quang Thu*[D] *and Nguyen Tran Nguyen*[E]

[A]CSIRO Forestry and Forest Products, PO Box E4008, Kingston, ACT 2604, Australia.
[B]Royal Forest Department, Bangkok 10900, Thailand.
[C]School of Agricultural Science, University of Tasmania, Hobart 7001, Australia.
[D]Forest Science Institute of Vietnam, Hanoi, Vietnam.
[E]Forest Science Sub-Institute of Vietnam, Ho Chi Minh City, Vietnam.
[F]Corresponding author; email: ken.old@csiro.au

Abstract. *Cryptosporiopsis eucalypti* has been associated with foliar disease of eucalypts in many parts of the world, especially in South-East Asia where *Eucalyptus camaldulensis* can be severely affected. In a field trial in southern Vietnam of 150 open-pollinated families of seven tropical provenances of *E. camaldulensis, C. eucalypti* was the main pathogen associated with leaf blight and crown dieback. Variation in susceptibility to foliar disease occurred at the family, provenance and sub-species levels offering excellent opportunities for selection of resistant trees. Pathogenicity tests using seedlings have shown that the fungus can infect stems as well as leaves. Stem inoculation may offer opportunities for rapid screening for resistant germplasm, but a connection between the results of such tests and field performance has not been made.

Additional keywords: Cylindrocladium quinqueseptatum, endophyte.

Introduction

Eucalyptus spp. are grown throughout many tropical and sub-tropical regions of the world. They have formed the basis for large-scale plantations and their associated forest product industries, farm and communal plantations, and social plantings. In addition, eucalypts grow well on low fertility, stony or eroded sites, and on sloping ground not suited for cultivation of staple food crops.

Eucalyptus camaldulensis has been grown in parts of South-East Asia for most of the twentieth century, particularly during the past 40 years, in regions that experience seasonally dry conditions combined with extended wet seasons. The smooth bark and self-pruning stems of this species make it particularly useful for pulp logs, piles, scaffolding and simple building structures (Midgley and Pinyopusarerk 1996).

After several decades of success in growing eucalypts in Vietnam, the late 1980s saw severe epidemics of leaf and shoot blight, especially in central regions and in the south-east of the country. Similarly, in Thailand, expansion of industrial plantations, largely based on *E. camaldulensis,* has required action to counter chronic leaf and shoot blight problems in both seedling-based and clonal stands. One of the most common pathogens, to be found associated with foliar spots and shoot blight in Vietnam and Thailand, is *Cryptosporiopsis eucalypti*.

Species of the fungal genus *Cryptosporiopsis* are well known as stem pathogens of woody hosts in temperate regions, including maple, hazel and fruit trees. Verkley (1999) accepted at least 23 species of *Cryptosporiopsis* and, where found, teleomorphs are in the genus *Pezicula* or in *Neofabraea*. *C. eucalypti* was first formally described in 1995, but the fungus had attracted attention from eucalypt pathologists considerably earlier. Sankaran *et al.* (1995) noted that specimens were lodged with the International Mycological Institute (IMI) as early as 1972 from collections made in north east Australia, India and the Hawaiian Islands.

Pathogenicity tests were carried out on *E. grandis* and *E. tereticornis* seedlings in humid conditions (Sankaran *et al.* 1995) and characteristic leaf spots resulted. In 1986, Old (unpublished) found the fungus in association with leaf spots on several *Eucalyptus* spp. growing in central Honshu, Japan and, during the early 1990s, Pongpanich found this fungus to be the most common cause of leaf and shoot blight of *E. camaldulensis* in Thailand (reported in Ciesla *et al.* 1996). Ferreira *et al.* (1998) reported a leaf spot caused by *C. eucalypti* in Brazil affecting *E. grandis* and *E. saligna*. Leaf spot symptoms associated with the fungus have also been reported to be widespread in New Zealand on planted *Eucalyptus* and *Corymbia* spp. (Gadgil and Dick 1999). Old and Ivory (1999) and Lee (1999) included *C. eucalypti* in summaries of pathogen threats to short rotation forest

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plantations in South-East Asia but there has been little published information on the pathogen, its impacts on plantations, and options for control.

The objectives of the research reported here were to: describe the range of foliar and stem symptoms associated with infection by *C. eucalypti* in eucalypt plantations in South-East Asia and to isolate the fungus from diseased tissues; assess the level of foliar crown damage, associated with *C. eucalypti* infection, in seven tropical provenances consisting of 150 families of *E. camaldulensis* in a field trial in southern Vietnam as the basis for future selection of disease-resistant germplasm; and assess the status of *C. eucalypti* as a shoot pathogen and develop methods for screening for disease-resistant germplasm by artificial inoculation of *E. camaldulensis* seedlings.

Methods

Isolation of Cryptosporiopsis eucalypti

Eucalypt plantations were regularly inspected for disease in many locations across Vietnam, Thailand and north Queensland during the period 1995–1999. Pure cultures of *C. eucalypti* were obtained from lesions on leaves or small-diameter twigs. Small pieces of excised tissue were sterilised in 70% ethanol for 30 s, 3% sodium hypochlorite for 3 min, rinsed in sterile distilled water (SDW), plated onto potato-dextrose agar (PDA) or malt-extract agar (MEA) (Dhingra and Sinclair 1985) and incubated in the dark at 25°C. Alternatively, spore masses, which exuded from conidiomata after moist incubation of infected plant parts for 72 h, were streaked onto agar. Cultures were maintained on slants in sealed McCartney bottles at room temperature (approximately 20°C). For long-term storage, Australian isolates and several cultures from Vietnam were immersed in sterilised paraffin oil and are held in quarantined storage at CSIRO Forestry and Forest Products in Canberra.

Disease impact rating in field trials

In Vietnam, a 3.5 ha provenance and progeny trial was established at Chon Thanh in Binh Phuoc province in southern Vietnam (11°24′N, 106°36′E) in 1996, using 150 open-pollinated families of *E. camaldulensis* (ssp. *simulata* and var. *obtusa*) from seven provenances across the Northern Territory (Katherine and Fergusson River) and north Queensland (Petford, Kennedy River, Kennedy Creek, Laura River and Morehead River). The trial consisted of eight replicates, laid out in a two-dimensional incomplete block row–column design with each plot consisting of a line of four trees of one family. Trees were at 2 m spacing with 3 m between rows to allow for the trial to be later converted into a seed orchard. All trees were scored for crown health condition and presence of foliar pathogens and stem heights and diameters were measured during the first and second year after planting. Assessments were carried out during late October 1997 and 1998, towards the end of the rainy season, to allow maximum disease expression.

A scale of 1–6 was developed based on the severity of foliar symptoms, defoliation and shoot blight as follows: categories 1 and 2 included trees with good crown retention and no evidence of shoot blight; category 3 trees had suffered lower crown defoliation with some minor shoot tip dieback; category 4 (Fig. 1*C*) included trees with leaf spots and terminal shoot dieback associated with infection by *C. eucalypti*; categories 5 and 6 had suffered major to complete defoliation with shoot death, associated with infection by *C. eucalypti* and stunting of form.

Analysis of Variance using Genstat 5 (Lawes Agricultural Trust, Rothamsted Experimental Station) was conducted for comparisons of crown condition. Significances between means were determined using an LSD test. A simple linear correlation analysis was used to relate mean family foliar crown condition (1–6) with tree height and diameter.

Pathogenicity experiments

Cultures of *C. eucalypti* (I00062, I00111, I00108) used in inoculation experiments were isolated in northern Queensland and were selected for their capacity for good growth on PDA and profuse sporulation. Pathogenicity tests were undertaken by spraying intact seedlings with spore suspensions or by means of stem inoculations. Following symptom development, samples of tissue from the margins of lesions were plated onto PDA. All seedlings were raised in a partially sterilised standard glasshouse potting mix from seed supplied by the Australian Tree Seed Centre, CSIRO, Canberra.

Spray-inoculation of seedlings. Conidial suspensions were produced by growing mycelial mats of *C. eucalypti* (isolate I00062) on PDA in 9-cm Petri dishes for 2 weeks at 25°C in darkness and flooding with SDW containing a drop of Tween 80. Concentrations of spore suspensions were assessed by using counts from 1 µL sample droplets on microscope slides and were diluted to 2×10^5 conidia/mL in sterile SDW containing 0.05% Tween 80 or an organic-based spreader. Controls were minus the fungus.

Two Western Australian provenances (Wiluna and Leonora) of *E. camaldulensis* var. *obtusa*, two Victorian provenances (Edenhope and Lake Coorong) of *E. camaldulensis* var. *camaldulensis* and one seedlot each of two closely related red gum species *E. amplifolia* (Nerriga, NSW) and *E. blakelyi* (Mendo, NSW) were selected for inoculation. Two-month-old seedlings with six to eight fully expanded leaf pairs were spray-inoculated to run-off. A randomised block design with six blocks was used. The tubed seedlings were placed in compartmentalised punnet trays with each tray representing a replicate. After inoculation, each replicate was enclosed in a large polyethylene bag and then placed into a high humidity plant growth chamber with a 27/22°C 12 h day/night regime with illumination by fluorescent lights. Earlier observations had shown that growth and sporulation were reduced at 32°C compared with 24°C, that temperatures in the range of 23–30°C favoured symptom development and that high humidity was a requirement, at least for initial infection. An inoculum level of $2–5 \times 10^5$ spores/mL also consistently produced disease in spray inoculation tests. Plants were inspected regularly for symptoms and the number of leaves with spots and frequency of spots per leaf were scored after 2 weeks.

Stem inoculation. Two provenances of *E. camaldulensis* ssp. *simulata* (Kennedy Creek and Morehead River), two of *E. camaldulensis* var. *obtusa* (Petford and Emu Creek, Queensland) and one provenance of *E. tereticornis* (Helenvale, Queensland) were grown for 3 months in the glasshouse. The four *E. camaldulensis* provenances were chosen as they were also represented in the field trial in Vietnam. Small scalpel cuts were made to stems of these seedlings, to the depth of the xylem, and 4-mm^2 agar discs bearing mycelium from 9-day-old PDA cultures of isolate I00111 were placed in the wounds. Control inoculations with sterile agar were made on each stem at least 10 cm above the fungal inoculation sites. The wounds were then wrapped with laboratory film and inoculated seedlings were held in high-humidity, plant-growth cabinets set at 28/22°C and 12 h day/night light regime supplied by fluorescent lights. For comparison with recognised foliar pathogens of eucalypts, seedlings were also inoculated with single, north Queensland isolates of *Cylindrocladium quinqueseptatum* (I00057) and *Coniella fragariae* (I00104). Inoculum consisted of agar discs bearing mycelium taken from the margin of 9-day-old cultures. A randomised block layout with four replicates of each treatment combination was employed and lesion length was measured after

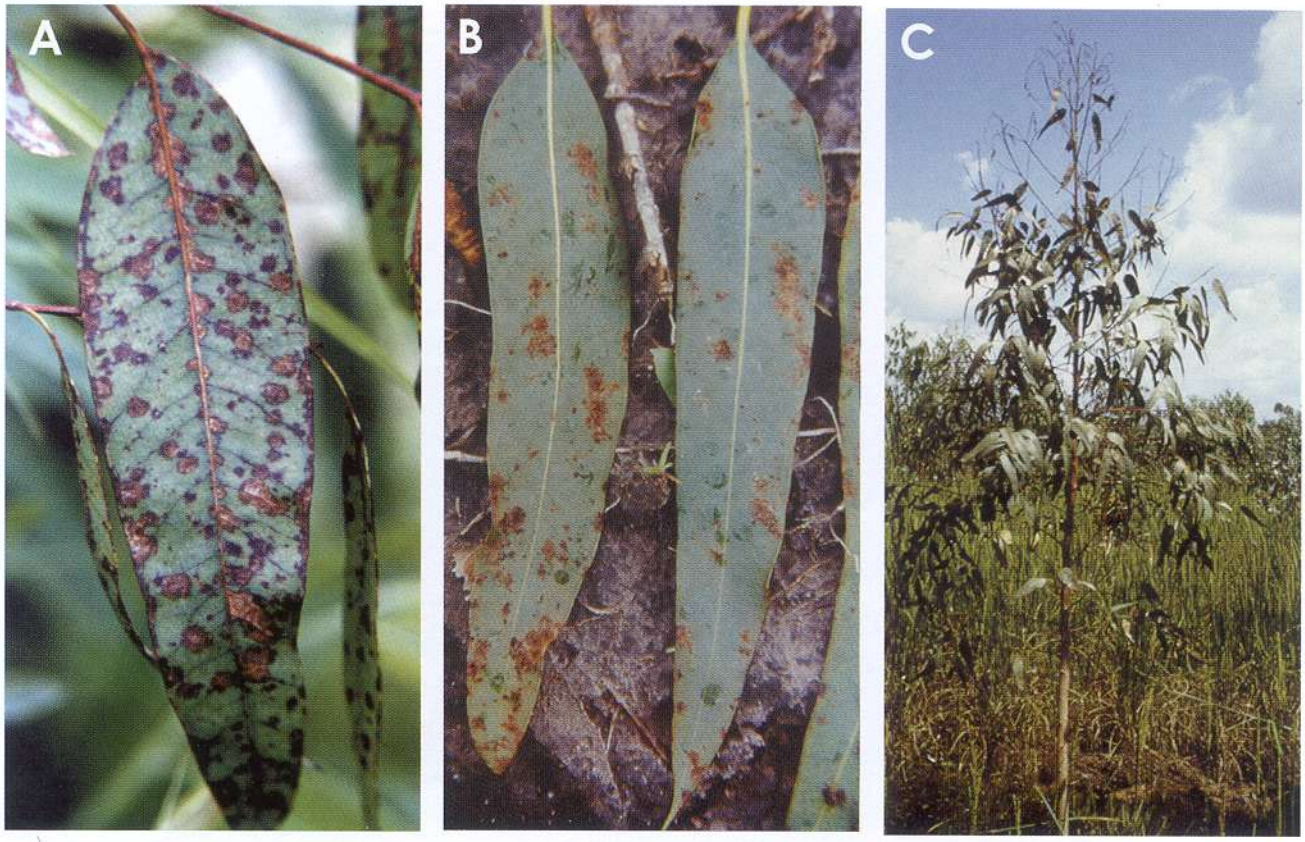

Fig. 1. Disease symptoms associated with *Cryptosporiopsis eucalypti* on *Eucalyptus camaldulensis* in the field. (*A*) Irregular brown leaf spots; (*B*) irregular roughened or corky lesions with localised eruption and necrosis of epidermal leaf tissue; (*C*) defoliation, leaf blighting and shoot dieback in a 1-year-old *Eucalyptus camaldulensis* plantation in southern Vietnam.

3 weeks. For the purpose of data evaluation, control lesion lengths were subtracted from lesion lengths associated with fungal inoculation.

A second stem inoculation technique, avoiding stem injury, of 3-month-old seedlings was also used. A broader range of tropical *E. camaldulensis* provenances was used, with representatives of *E. camaldulensis* var. *obtusa* from north Queensland, the Northern Territory and Western Australia, and one seed lot of *E. camaldulensis* ssp. *simulata* from Kennedy Creek, north Queensland.

Seedlings were approximately 1 m tall when inoculated at a point on the stem between 600–700 mm stem height. This portion of the stem was green, without visible secondary periderm development, and stem diameters ranged from 0.25–0.45 cm. Three days before inoculation, the selected infection court on each stem was wiped with 70% ethanol, wrapped in autoclaved wet cotton wool and sealed with laboratory film. Six replicate seedlings from each of six seedlots of *E. camaldulensis* were pretreated in this way before arrangement in a randomised block design. An additional two control plants were pretreated for each seedlot.

After 3 days, the films were removed and PDA discs, 5 × 3 mm in size and bearing mycelium from 9-day-old *C. eucalypti* cultures (I00108) grown at 25°C, were placed on the stem surfaces before film replacement. Sterile agar discs were used as controls. The seedlings were placed on a laboratory bench adjacent to a window receiving indirect sunlight, with temperatures during the experimental period ranging from 15–23°C. All protecting films were removed after 2 weeks. After a further 5 weeks, lesions were measured, infected stems cut into 1 cm pieces, split, surface sterilised and plated onto MEA.

Data analysis

Analysis of Variance with Genstat 5 was used to evaluate all foliar and stem inoculation experiments. Where percentage data were used, an arcsin transformation was applied.

Results

Disease symptoms in plantations

Symptoms of *C. eucalypti* infection develop on both leaves and shoots of eucalypts. Leaf spots occur on both sides of the leaves and vary in size, shape and colour, within and between eucalypt species. There are at least four lesion types on *E. camaldulensis*, namely, large brown spreading necrotic lesions leading to a leaf blight symptom; circular or sub-circular spots 1–2 cm in diameter; irregular chocolate brown or greyish spots covering much of the leaf area (Fig. 1*A*); and irregular roughened or corky lesions with eruption and necrosis of epidermal tissue, sometimes localised along veins, on which the fungus fruits (Fig. 1*B*). Terminal shoots of young trees can be totally defoliated and are commonly blighted (Fig. 1*C*).

Conidiomata develop on foliar lesions, on blighted shoots and have also been found associated with cankers on small diameter woody branches. Fruiting bodies are cupulate when

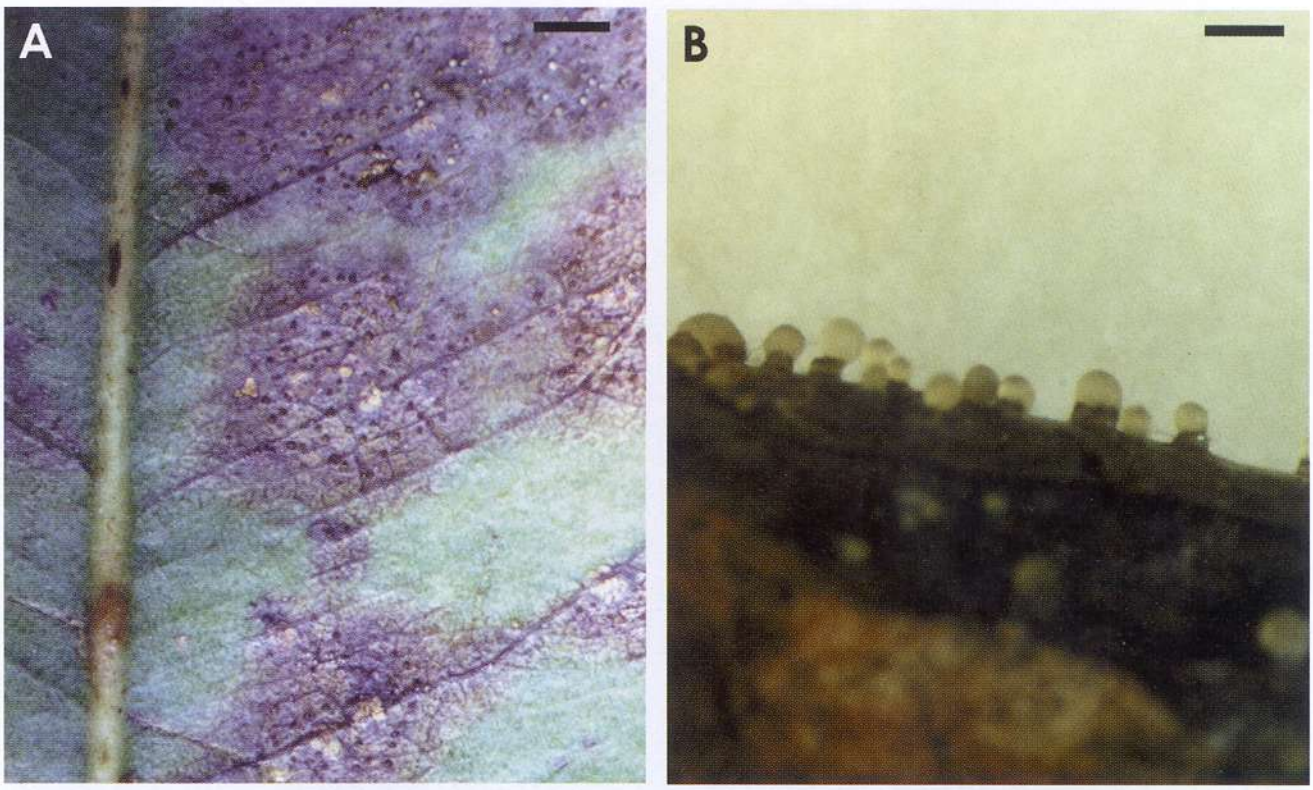

Fig. 2. Creamy masses of conidia of *Cryptosporiopsis eucalypti* oozing from cupulate conidiomata on moist incubated *Eucalyptus camaldulensis* components. (*A*) Leaf; bar = 2 mm; (*B*) branchlet; bar = 375 µm.

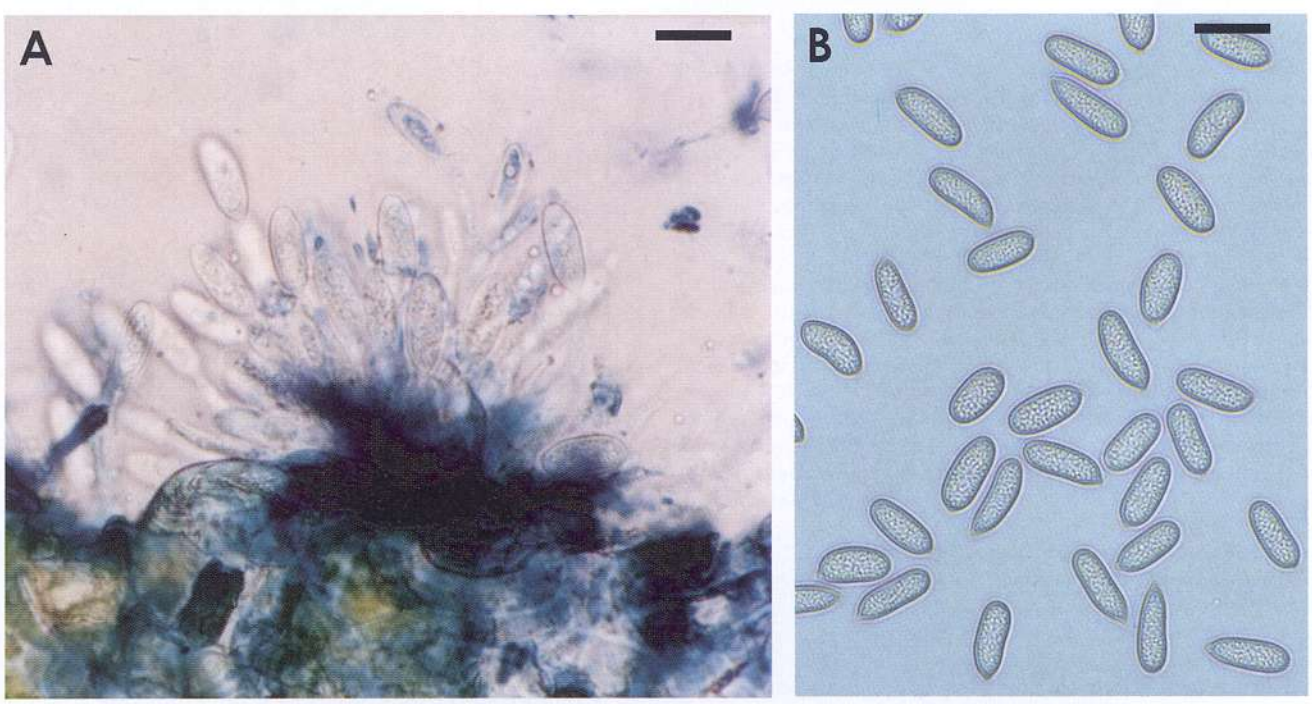

Fig. 3. *Cryptosporiopsis eucalypti.* (*A*) Vertical section through acervulus on leaf showing conidia; bar = 18 µm; (*B*) conidia from pure culture; bar = 20 µm.

moist with pigmented margins, bearing creamy masses of macroconidia (Figs. 2*A*, *B*). The conidiomata are scattered irregularly on lesions and erupt through the epidermis or stem periderm (Fig. 3*A*), but can be quite inconspicuous when leaves are dry. Macroconidia are thick-walled and ellipsoid to elongate-ellipsoid in shape with distinctive protuberant scars (Fig. 3*B*).

C. eucalypti has been collected by the authors from the following eucalypts with leaf spot or shoot blight symptoms in Australia, Japan, Laos, Sri Lanka, Thailand and Vietnam: *E. camaldulensis* (VPRI 20397, 20396); *E. camphora* (DAR 58686); *E. cinerea* (DAR 58682); *E. cypellocarpa* (DAR 58684); *E. nova-anglica* (DAR 58685); *E. urnigera* (FPH 6610); *E. nitens* (DAR 58681); *E. pellita* (DFR 0300); *E. tereticornis* (DFR 0976); *E. viminalis* (DAR 58683); and *E. urophylla* (DFR 0466). It has also been isolated from soil below cryptosporiopsis-affected eucalypt plantations in Thailand. Specimens prefixed FPH are now held in Herb. TFM, Tsukuba, Ibaraki, Japan. Those designated DFR are held in the forest pathology herbarium of CSIRO Forestry and Forest Products in Canberra, Australia.

Impacts of C. eucalypti *in plantations*

Leaf spots caused by *C. eucalypti* occurred on both juvenile and adult leaves with major defoliation being associated with shoot blight in the crown. Successive defoliation and shoot blight of susceptible trees caused loss of apical dominance, flattening of the normally conical crown of otherwise vigorously growing eucalypts, and loss of height and diameter growth. Stems became forked, form was lost, and secondary canker-inducing fungi such as *Cytospora eucalypticola* and *Cryphonectria gyrosa* invaded stems and caused dieback of main branches.

During 1997 and 1998, all trees in the Chon Thanh trial in Vietnam were scored for crown condition, growth and the presence of foliar and stem pathogens. Crown condition and growth in 1998 are shown in Fig. 4. Crown health condition, on a scale of 1–6, was strongly correlated ($P < 0.001$) with tree growth (height $R^2 = 0.475$, diameter $R^2 = 0.536$). The major influence on crown health after 2 years was infection by *C. eucalypti,* especially the shoot blight phase of the disease. *Cy. quinqueseptatum* was recorded within the trial on a few occasions but occurred so infrequently that it had no impact on tree health.

In the 1998 assessment in the Chon Thanh trial, there were highly significant family differences in the incidence of leaf spot and shoot blight associated with *C. eucalypti* infection. Approximately 35% of trees showed symptoms of *C. eucalypti* infection and the frequency of diseased trees per family ranged from 8.34 to 96.8%. Highly significant differences ($P < 0.001$) in crown health class were found between sub-species of *E. camaldulensis* (ssp. *simulata* performed better than var. *obtusa*) and between families within all seven provenances ($P < 0.001$). Provenance differences ($P < 0.05$) also occurred, with Laura River and Kennedy Creek showing least disease.

Pathogenicity tests

Spray inoculation of seedlings. Leaf spots were produced on eucalypt seedlings of four provenances of *E. camaldulensis* and one each of *E. amplifolia* and *E. blakelyi* sprayed with spore suspensions of *C. eucalypti.* Symptoms closely resembled those observed in the field on susceptible trees. A mean of approximately 54% of sprayed leaves developed leaf spots on two provenances of *E. camaldulensis*, and on *E. blakelyi*, compared with

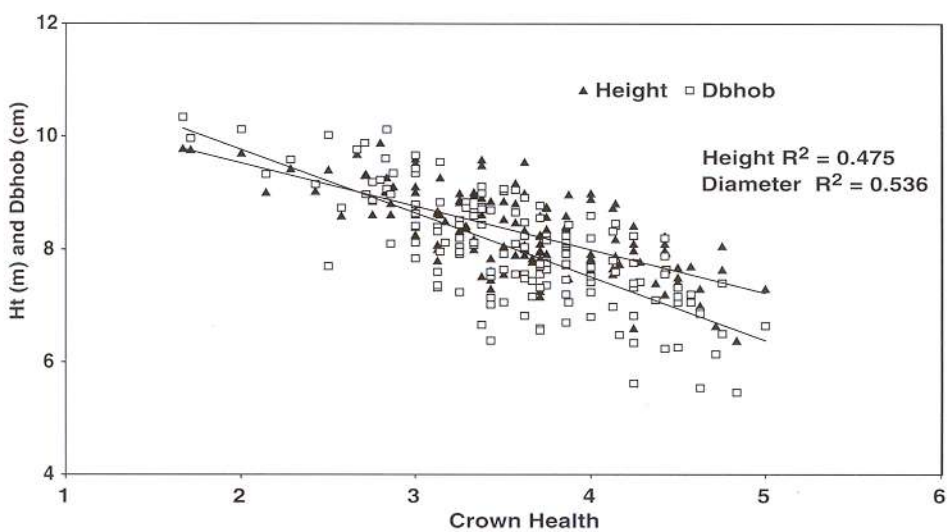

Fig. 4. Relationship of mean family tree height, and overbark diameter at 1.3 m (Dbhob), with foliar crown health score (1 is most, and 6 least, healthy) for a 2-year-old provenance/progeny trial affected by *Cryptosporiopsis eucalypti* at Chon Thanh, Binh Phuoc, southern Vietnam, for 150 families of *Eucalyptus camaldulensis.*

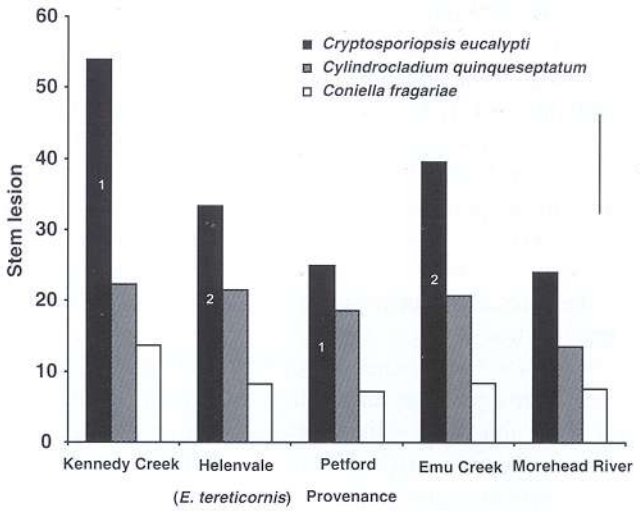

Fig. 5. Comparisons of mean lesion lengths (minus control on same plants) at 3 weeks after wound inoculation of seedling stems from four provenances of *Eucalyptus camaldulensis* and one of *E. tereticornis* with mycelial plugs of *Cryptosporiopsis eucalypti* (I00111), *Cylindrocladium quinqueseptatum* (I00057) and *Coniella fragariae* (I00104). Numbers on columns indicate number of stems completely girdled resulting in top death. Bar represents $LSD_{0.05}$.

E. amplifolia with approximately 17% infected leaves, and this difference was significant at $P < 0.05$. Two additional provenances of *E. camaldulensis* developed fewer leaf spots (34% and 42% infected leaves, respectively). *C. eucalypti* was re-isolated from all inoculated trees but not from the small number of spots which occurred on three control trees.

Stem inoculations. Four young seedlings from each of four provenances of *E. camaldulensis* and one of *E. tereticornis* inoculated with *C. eucalypti* developed more extensive lesions than resulted from inoculation with *Cy. quinqueseptatum* or *Co. fragariae* (Fig. 5). Six of these 20 seedlings inoculated with *C. eucalypti* were completely girdled, resulting in death of the terminal portions of main stems.

Lesions caused by *C. eucalypti* ranged from 6–73 mm in length (mean length less control was 35 mm) compared with 6–33 mm (19 mm) for *Cy. quinqueseptatum* and 5–12 mm (9 mm) for *Co. fragariae*, neither of which girdled the stems. Lesions associated with controls were small, with a mean length of less than 5 mm. Re-isolations of all three inoculated fungi were made from the respective lesions on inoculated plants. All five provenances were equally susceptible to *Cy. quinqueseptatum*. Significant differences in lesion size were recorded between provenances infected with *C. eucalypti* but the small number of provenances included in the trial did not allow relationships between the degree of damage and species, sub-species or region of provenance to be tested.

Inoculations of stems with *C. eucalypti* without wounding induced discrete or diffuse lesions, or sparse spotting of the epidermis and phloem tissues. Lesion extension after 7 weeks measured from 0–63 mm, with mean lesion length between seedlots varying from 22.7 to 42.7 mm (Table 1). Seventeen of the 36 seedlings inoculated with *C. eucalypti* developed necrotic lesions that girdled stems, and three plants suffered death of their terminal shoots.

As with the stem-wounding inoculations, significant differences between seedlots were observed. For example, seedlot 16563 (Mitchell River) showed lesions only 22.7 mm in mean length compared to seedlot 18242 (Kennedy River) with lesions 42.7 mm in length. *C. eucalypti* was isolated consistently from up to 15 mm beyond visible surface lesions with recovery lengths across the trial ranging from 20–70 mm. Even where lesions were sparse, in some cases consisting of a small number of minor spots, the fungus was readily isolated, indicating that the fungus is capable of some degree of growth within asymptomatic tissue.

Discussion

Although *C. eucalypti* is widely distributed in many parts of the tropics and sub-tropics, and has also been collected in several more temperate regions, the pathogen has assumed importance only in parts of India (Sankaran *et al.* 1995), Sri Lanka (Old, unpublished information) and in South-East Asia (Lee 1999; Old and Ivory 1999). The association of *C. eucalypti* with significant disease of eucalypts in South-East Asia contrasts with lesser symptoms in northern Australia, Brazil, Japan and New Zealand. In Vietnam and Thailand, where *E. camaldulensis* is the most commonly

Table 1. Mean lesion circumference, length and *Cryptosporiopsis eucalypti* recovery length 7 weeks after stem inoculation of *Eucalyptus camaldulensis* seedlings without wounding ($n = 6$)

Seedlot	Provenance	Lesion circumference (°)	Lesion length (mm)	Cryptosporiopsis recovery (mm)
12347	Manning Creek (WA)	297	24.5	39.8
13929	Cockatoo Creek (NT)	226	25.2	36.5
13941	Victoria River (NT)	268	32.0	38.2
15827	Kennedy Creek (Qld)	280	27.0	37.8
16563	Mitchell River (Qld)	222	22.7	28.3
18242	Kennedy River (Qld)	301	42.7	50.5
		N.S.	$LSD_{0.05}$ 14.6	$LSD_{0.05}$ 9.05

grown eucalypt species, leaf and shoot blight associated with *C. eucalypti* infection impacts severely on plantation productivity.

Although previously reported as being associated with leaf spots, *C. eucalypti* has not previously been considered to affect twigs and small diameter branches. Crown damage associated with *C. eucalypti* can be confused with cylindrocladium leaf blight (CqLB, caused by *Cy. quinqueseptatum*) and in some plantations both pathogens can be found. The latter fungus is well known as the cause of cylindrocladium leaf blight in South-East Asia and India (Sharma and Mohanan 1982). Cylindrocladium leaf blight is favoured by mean annual rainfall maxima of more than 1600 mm (Booth *et al.* 2000) and epidemics are highly episodic. In Vietnam and Thailand, however, defoliation and shoot blight of *E. camaldulensis* associated with *C. eucalypti* infection is more widespread than CqLB and occurs across a broader range of rainfall and temperature regimes.

The provenance and progeny trial at Chon Thanh in southern Vietnam was designed to enable the selection of individual trees that would be disease resistant and of good form and growth. *E. camaldulensis* ssp. *simulata* provenances, such as Kennedy Creek and Laura River from north Queensland, consistently showed a higher proportion of trees resistant to *C. eucalypti* infection than *E. camaldulensis* var. *obtusa* e.g. Petford (Queensland) and Katherine (Northern Territory). Between-provenance, within-family variation in susceptibility to leaf and shoot blight associated with *C. eucalypti* infection was highly significant ($P < 0.001$). *E. camaldulensis* is readily propagated from cuttings and tissue cultured plants. Resistant trees can therefore be used to establish clonal seed orchards as sources of seed with enhanced resistance to disease. Alternatively, clonal selections could be readily multiplied for widespread planting in disease-prone areas.

Although field observations and trials are essential aspects of tree improvement, an aim of this research was to develop reliable and sensitive rapid screening methods for testing seedlings or clonal plants for disease resistance. This objective was only partially realised. Ideally, rapid screening methods should have been developed in Vietnam using cultures of fungi isolated in the field trials. The unavailability of facilities at the outset of the research, however, precluded this. Therefore, screening methods were developed in Australia with the intention for their subsequent use in Vietnam and Thailand. Australian quarantine regulations required that only isolates originating within Australia could be used as inoculum and consequently only north Queensland isolates of foliar pathogens were used.

Screening trials, both spraying seedlings with conidial suspensions and stem inoculations, were characterised by a high level of variability in seedling susceptibility to disease, even within seedlots (half-sib seedling families). Leaf and juvenile shoot infection by conidia seems to be the most likely avenue of infection in plantations. However, conditions favouring infection are poorly understood. Spray inoculation with *C. eucalypti* adequately reproduced symptoms characteristic of field infections, but the relatively long incubation period at high relative humidity created problems and several trials failed due to inadequate control of environmental conditions.

Stem inoculation was initially carried out in order to establish the capacity of *C. eucalypti* to invade wounded and intact stems. Comparison of *C. eucalypti* with *Cy. quinqueseptatum*, a major cause of eucalypt leaf blight in the tropics and sub-tropics, and with *Co. fragariae*, which is similarly associated with leaf spots, confirmed field observations that *C. eucalypti* is an aggressive shoot pathogen. *Co. fragariae* was the least pathogenic of the three fungi with very limited capacity to invade stems. Both the stem wounding and the less invasive stem inoculation methods developed here showed significant differences in lesion length between seedlots.

The rapid screening methods described here did not show any trends in the susceptibility of *E. camaldulensis* sub-species and provenances to *C. eucalypti* infection. This is probably a reflection of the small number of seedlots from a limited number of provenances included in these trials and a high level of variation in this trait between seedling families within provenances. As discussed above, such provenance differences were highly significant in the Chon Thanh field trial where 150 families from seven provenances were replicated eight times, a level of replication not possible in our laboratory and cabinet screening trials. There is a strong trend for clonal forestry to replace seedling plantations, especially in Thailand. The methods developed here, both spray and stem inoculation, could be more suitably applied to screening of individual genotypes (clonal selections) for disease resistance and are being further tested for this purpose in Vietnam.

Disease surveys, observations from the Chon Thanh field trial and contrasting inoculation methods developed here support the report of Sankaran *et al.* (1995) regarding the status of *C. eucalypti* as a significant eucalypt pathogen. In this respect, *C. eucalypti* is unusual. The only other *Cryptosporiopsis* sp. reported to be a significant foliar pathogen is *C. citri,* which causes leaf spotting on citrus in Cook Islands and Niue in the South Pacific region (Johnston and Fullerton 1988). In contrast, of the 23 *Cryptosporiopsis* species described in Verkley (1999), 21 are associated with stems and roots and are mostly endophytic in habit.

Cryptosporiopsis abietina, although initially identified as causing a serious resinous canker disease of *Chamaecyparis obtusa* (Kobayashi *et al.* 1990), was later regarded as an endophyte and unlikely to be the causal agent (Kaneko *et al.* 1996). This fungus has been widely reported as endophytic within the tissues of both gymnosperms and angiosperms

including *Sequoia sempervirens* (Espinosa-Garcia and Langenheim 1990) and *Acer macrophyllum* (Sieber and Dorworth 1994). *Cryptosporiopsis radicicola* is endophytic in fine roots of hardwoods and softwoods (Ahlich and Sieber 1996). An undescribed *Cryptosporiopsis* sp. has also been reported associated with asymptomatic green leaves of the myrtaceous species *Metrosideros fulgens* in New Zealand (McKenzie *et al*. 1999).

There is much still to learn regarding the biology of *C. eucalypti,* including its relationship to other *Cryptosporiopsis* species on different woody hosts and the possibility that, in common with some of these species, *C. eucalypti* may be able to exist as an endophyte. In one of our stem inoculation trials, *C. eucalypti* also appeared to have some capacity for growth in stem tissue in advance of visible lesion development. Rommert (1998) has indicated that there could be both endophytic and pathogenic strains within *Cryptosporiopsis* species. There are several published examples of significant pathogens of trees, such as *Sphaeropsis sapinea* and *Botryosphaeria dothidea* existing as endophytes in asymptomatic tissues (Smith *et al*. 1996). There is a need for further study of the etiology of cryptosporiopsis leaf diseases of eucalypts. An endophytic phase in asymptomatic trees could assist in explaining its wide distribution, variable symptoms and association with trees showing chronic shoot blight.

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