New foliar pathogens of *Eucalyptus* from Australia and Indonesia

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*Mycosphaerella tasmaniensis* is newly described from Mycosphaerella leaf blotch symptoms occurring on *Eucalyptus nitens* in Tasmania, Australia. Single ascospore cultures produced a *Mycovellosiella* anamorph, described here as *M. tasmaniensis*. Both states occurred together, as well as separately on leaf spots. *Phaeophleospora epicoccoides* (= *Kirrmyces epicoccoides*) is commonly associated with leaf spots of *Eucalyptus* spp. in Australia. The teleomorph, *Mycosphaerella suttoniae*, previously known only from Indonesia, was also collected on *E. grandis* leaves from Australia. A Cylindrocladium leaf blight disease of young *E. grandis* trees in Indonesia was found to be associated with a new species of *Calonectria*. *Calonectria multiseptata* and its anamorph *Cylindrocladium multiseptatum* is newly described and distinguished from other species based on their larger, multi-septate ascospores and conidia.

Species of *Eucalyptus* L’Hér. provide an important source of fibre to the international paper and pulp industry (Turnbull, 1991). Concurrent with their growing importance, there has been an intensification of management practices including production through vegetative propagation and hybridization. Diseases of eucalypts have also gained considerable importance, particularly where management practices have led to an increase in the genetic uniformity of plantations.

An extraordinary number of fungal species have already been reported from *Eucalyptus* (Sankaran, Sutton & Minter, 1995). Many of these are pathogens, which are specific to certain species or subgenera of *Eucalyptus* (Wall & Keane, 1984; Walker, Sutton & Pascoe, 1992). Although losses incurred by leaf spotting fungi in either plantations or nurseries have been poorly documented, these pathogens have formed the basis of several studies. In recent years Mycosphaerella leaf blotch disease (MLB), associated with several species of *Mycosphaerella* Johanson (Crous & Wingfield, 1996), has received considerable attention. Likewise, Cylindrocladium leaf spot and shoot blight diseases (CLB) are considered to be amongst the most important threats to *Eucalyptus* spp. (Ferreira, 1989; Crous, Phillips & Wingfield, 1991; Sharma & Mohanan, 1991). While several *Mycosphaerella* species are highly host specific on *Eucalyptus* (Crous & Wingfield, 1996), *Cylindrocladium* spp. are much less so, also infecting many other diverse hosts (Crous & Wingfield, 1994).

The present study reports on two newly discovered MLB and CLB diseases, and one new MLB disease record. The new MLB disease was discovered on *E. nitens* (Deane & Maid.) Maid. in Tasmania, while the new disease record was on *E. grandis* Hill ex Maid. in Brisbane, Australia, and the new CLB disease occurred on young *E. grandis* trees in northern Sumatra, Indonesia.

**MATERIALS AND METHODS**

Single-conidial isolates of the *Calonectria* sp. were cultured on 2% malt extract agar (MEA) (Biolab), plated onto carnation-leaf agar (CLA) (Fisher et al., 1982; Crous, Phillips & Wingfield, 1992), incubated at 25 °C under nuv light, and examined after 7 d. Only material occurring on carnation leaves was examined. Leaves with Mycosphaerella leaf blotch symptoms were collected from several plantations in Tasmania. Lesions were excised from leaves, and single ascospore cultures were established on MEA using the technique described by Crous, Wingfield & Park (1991). Germinating ascospores were examined after 24 h, germination patterns determined, and transferred to MEA. Colonies were subcultured onto divided plates with one half containing CLA and the other MEA, incubated at 25 °C under continuous nuv light. Linear growth on agar for each culture was determined after 6 d for *Calonectria*, and 1 mo for *Mycosphaerella* (Crous & Wingfield, 1996). Colony colours were scored using the colour charts of Rayner (1970). Wherever possible, 30 measurements were made of structures mounted in lactophenol, and the extremes are given in parentheses.

**TAXONOMY**

*Mycosphaerella tasmaniensis* Crous & M. J. Wingf., sp. nov. (Figs 1–4, 9–11)

*Anamorph:* *Mycovellosiella tasmaniensis* Crous & M. J. Wingf., sp. nov.

*Etym.*: Named after its country of origin
Figs 1–8. Mycosphaerella tasmaniensis and Calonectria multiseptata. Figs 1–4. Mycosphaerella tasmaniensis and its anamorph Mycoceilloidea tasmaniensis. Fig. 1. Obovoid ascus. Fig. 2. Ascospores germinating parallel to their long axis on MEA. Fig. 3. Conidiophore with thickened, refractive, darkened, terminal, conidial loci. Fig. 4. Ellipsoidal, aseptate conidia with thickened, darkened hila. Figs 5–8. Calonectria multiseptata and its anamorph Cylindrocladium multiseptatum. Fig. 5. Fusoid ascospores. Figs 6, 7. Curved to straight, multiseptate macroconidia, and 1–3-septate microconidia. Fig. 8. Vertical section through the side wall of a peritheciun showing the various cell layers (bars, 10 µm).
medium brown textura angularis, subhyphal layer at base consisting of 1–2 layers of hyaline cells. Asci fasciculate, bitunicate, subcylindrical to ellipsoid, rarely obvoid, straight or curved, 8-spored, 30–40 × 7–11 μm. Ascospores tri- to multiserial, overlapping, hyaline, guttulate, thin-walled, straight, fusoid-ellipsoid with obtuse ends, widest in middle of apical cell, marginally 1-septate, not constricted at septum, tapering toward both apices, but with more prominent taper towards lower end (10–)11–12–13 × (2.5–)3–(4) μm. Spermogonia not observed. Mycelium mostly external, consisting of septate, branched, smooth, pale to light brown hyphae, 2–4 μm wide. Conidiophores arising singly from superficial mycelium, medium brown, smooth to finely verruculose, 3–12-septate, subcylindrical, straight to slightly curved, mostly unbranched, or branched above, 50–150 × 4–6 μm. Conidigenous cells terminal, mono- to polyblastic, proliferating sympodially, pale brown, smooth, subcylindrical, tapering toward subobtuse apices with flat-tipped loci that are thickened, darkened and refractive, 6–15 × 3–4 μm. Conidia catenulate, chains branched, olivaceous, smooth, eguttulate, subcylindrical, narrowly ellipsoid or fusiform, tapering toward rounded ends with flattened, darkened, refractive, thickened loci, straight to slightly curved, 0(–1)-septate, (4–)8–12–(20) × 2–2.5 μm in vivo and in vitro.

Ascospore germination on MEA. Germinating from both ends, not darkening or distorting upon germination, becoming constricted at the septum, (3–)4 μm wide, with germ-tubes growing parallel to the long axis of the spore, and occasionally developing lateral branches after 24 h (frequently from original ascospore).

Cultural characteristics. Colonies up to 30 mm diam. on MEA after 1 mo at 25 °C in the dark, margins smooth, regular, surface not sectored, smooth, aerial mycelium moderate, grey, colonies iron grey, 25°/−1 (bottom).

Host. E. nitens.

Distribution. Tasmania, Australia.


Mycosphaerella tasmaniensis is morphologically similar to M. heimi Crous (anam. Pseudocercospora heimi Crous), M. heimioides Crous & M. J. Wingf. (anam. Pseudocercospora heimioides Crous & M. J. Wingf.), M. ellipsioida Crous & M. J. Wingf. (anam. Uvelvraaninia ellipsiodea Crous & M. J. Wingf.), M. irregulariramosa Crous & M. J. Wingf. (anam. Pseudocercospora irregulariramosa Crous & M. J. Wingf.) and M. molleriana (Thüm.) Lindau (anam. Colletogloeopsis molleriana Crous & M. J. Wingf.) (Crous & Wingfield, 1996, 1997 a, b). Other than slight differences in ascospore dimensions, the mode of ascospore germination of M. tasmaniensis is similar to that of M. ellipsiodea and M. irregulariramosa. None of the
Anamorph:

- Phaeophleospora epicoccoides
- Cercospora epicoccoides
- Mycosphaerella suttoniae (Cooke) Hansf.

constricted at septum, tapering prominently towards lower obtuse ends, widest near apex, medianly l-septate, not Ascospores thin-walled, straight to curved, obovoid with thecia being inconspicuous, intermingled between pycnidia. 

Fruiting hypophyllous, with spermogonia and pseudo-

from small specks to larger medium brown spots up to 6 mm dark brown to purple with diffuse purple margins, ranging Leaf spots amphigenous, irregular to angular or subcircular, 

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Additional synonyms listed in Walker et al. (1992). Leaf spots amphigenous, irregular to angular or subcircular, dark brown to purple with diffuse purple margins, ranging from small specks to larger medium brown spots up to 6 mm diam. Fruiting hypophyllous, with spermogonia and pseudo-thecia being inconspicuous, intermingled between pycnidia. Ascospores thin-walled, straight to curved, obovoid with obtuse ends, widest near apex, medianly l-septate, not constricted at septum, tapering prominently towards lower end, (10–)11–12(–13) × (2.5–)3–3.5 μm. Spermatia rod-shaped, hyaline, 4–7 × 1–1.5 μm, straight or slightly curved. Conidia exuding from ostiole in long cirri, subcylindrical to narrowly obclavate, apex subobtuse, tapering slightly from the basal septum to a narrowly truncate base, straight or slightly flexuous, thick-walled, medium brown, verruculose, guttulate, 3–7-euseptate, (30–)40–55(–60) × (3–)3.5–5 μm, thus very similar to the description given by Crous & Wingfield (1997b).

Ascospore germination on MEA. Germinating from both ends, becoming 3.5–5 μm wide, spore and germ-tubes becoming uniformly olivaceous upon germination, with germ-tubes parallel or perpendicular to the long axis of the spore.

Cultural characteristics and hosts. As previously described by Crous & Wingfield (1997b).


The anamorph, Phaeophleospora epicoccoides, occurs on several eucalypts in the subgenus Symphyomyrtus (Nichol, Wingfield & Swart, 1992), and has been recorded from most countries where eucalypts are grown (Sankaran et al., 1995). The teleomorph, M. suttoniae, has previously only been reported from Indonesia (Crous & Wingfield, 1997b). In the present study, lesions on E. grandis leaves colonized by P. epicoccoides were soaked in water, and placed over MEA dishes. After 24 h several ascospores were released from pseudothecia. The pseudothecia and spermagonia were inconspicuous, and intermingled with pycnidia of the anamorph. Ascospores germinated in a mode similar to that of the Indonesian type collection, and after 1 mo sporulated on MEA to produce long cirri of conidia. Because the teleomorph was inconspicuous among pycnidia on infected leaves, leaves infected with P. epicoccoides from Brazil and South Africa were treated in a similar fashion to determine if the teleomorph also occurs in these countries. No evidence of a teleomorph could be found, and its role in the genetic structure of populations of P. epicoccoides in plantations world-wide remains uncertain.

Calonectria multisepata Crous & W. J. Wingf. sp. nov. (Figs 5–8, 15–18)

Anamorph: Cylindrocladium multisepatum Crous & M. J. Wingf., sp. nov.

Etym.: Named after its multi-septate ascospores and conidia
Perithecia crocea deinde croceo-rubra, globosa vel ovooidea, 300–500 μm alta, 250–400 μm lata. Peritheci paries stratorum duorum; strato exteriori texturae globulosae parietibus crassis, ad 40 μm lato, cellulis 20–50 μm altis, 15–35 μm latis; strato interiori texturae angularis ad 20 μm lato, cellulis 15–20 μm altis, 7–11 μm latis. Asci unituniciati, clavati vel fusoidi, petiolo longo basali, 80–150 × 12–20 μm. Ascospores aggregated in upper third of the ascus, hyaline, fusoid with rounded ends, curved, rarely straight, 1–9-septate, not constricted at septa before ascus dehiscence, guttulate, (45–)65–75(–110) × (5–)6–7(–8) μm. Macroconidiophores rarely observed, comprising a stipe, a sterile elongation and a few phialides. Stipe elongation rarely formed, septate, thin-walled, terminating in a narrowly clavate vesicle; primary conidiophore branches subcylindrical, straight to slightly curved, 30–60 × 4–5 μm. Phialides arising directly from the stipe or from primary branches, occurring singly, or in groups of 2–4, cylindrical, apex truncate, 15–55 × 4–5 μm; periclinal thickening minute. Conidiophora cylindrical to subcylindrical, rounded at apex, flattened at base, straight to slightly curved, widest in middle of conidium, (120–)150–170(–200) × 8–9(–10) μm, 6–10-septate, occurring singly, rarely in small packets. Macroconidiophores comprising a stipe, a stipe elongation and a penicillate or subverticillate arrangement of fertile branches. Stipe elongation septate, thinned, terminating in a narrowly clavate vesicle. Primary branches 0(–1)-septate, subcylindrical, straight to curved, 30–45 × 2–3 μm; secondary and additional branches 0–1-septate, 10–30 × 2.5–3.5 μm, terminating in 1–4 phialides that are cylindrical, straight to slightly curved, 15–30 × 2–3 μm; apex with minute periclinal thickening. Microconidium cylindrical, mostly straight, rounded at apex, flattened at base, 20–65 × 2.5–3.5 μm, 1–3-septate, held in packets by colourless slime. Dark brown, thickened chlamydospores are formed throughout the medium in moderate numbers, and aggregate to form microsclerotia.

Cultural characteristics. Colonies 14–18 mm diam. on MEA after 6 d at 25° in the dark, aerial mycelium moderate, colonies umbre (13°); Rayner, 1970) underneath.

Host. E. grandis.


Calonectria multiseptata is easily distinguished from other species by its large (45–)65–75(–110) × (5–)6–7(–8) μm, 1–9-septate ascospores. Ascospores of Ca. quaesepsetata Figueirado & Namek. are (1–3)-septate, 54–100 × 4–8 μm, and are most similar, while those of Ca. indusiata Seaver [= Ca. theae (Petch) Subraman.] and Ca. retenubii (Bugnic.) Booth are only up to 70 μm long. Cyl. multiseptatum has large
conidia (120–200 × 8–10 µm, 6–10-septate) and clavate stipe extensions, and therefore must be compared with Cy. heptaseptatum Sobers, Aliieri & Knauss (conidia 1–8-septate, 96–144 × 6–9 µm) and Cy. quinquespatatum Boedijn & Reitsma (conidia 1–6-septate, 61–101 × 5–7 µm). Although the conidia of Cy. multiseptatum are much larger than those of the other two species, they are also peculiar in that they are frequently curved (Figs 6, 18), and that they have their widest points in the middle of conidia, rather than at their bases. Furthermore, this is the only species of Cylindrocladium known to have a multi-septate microconidial state. Where known, microconidial states of all other Cylindrocladium species have been observed to be 1-septate (Crous & Wingfield, 1994). Cy. multiseptatum is also peculiar in the sense that it is the teleomorph state that forms predominantly in culture. It appears that although the fungus sporulates occasionally, it forms conidia on phialides which occur separately on unbranched conidiophores, rather than in branched conidiophores with tight, penicillate arrangements of phialides, which is characteristic of the genus.

Although Ca. multiseptatum was collected from large, brown leaf blots and small leaf spots typically associated with Cylindrocladium leaf spot or blight disease, perithecia were common on dying shoots and branches of young trees throughout the plantation. Little is known about the pathogenicity of Ca. multiseptata. As no inoculations have yet been done, more attention will have to be given to these aspects of its biology in order to elucidate its role as a Eucalyptus pathogen.

The authors thank the South African Foundation for Research Development for financial support.

REFERENCES


(Accepted 24 June 1997)