Mycol. Res. **97** (5): 582–584 (1993) *Printed in Great Britain*

Mycosphaerella parkii and *Phyllosticta eucalyptorum*, two new species from *Eucalyptus* leaves in Brazil

P. W. CROUS

Department of Plant Pathology, University of Stellenbosch, 7600 Stellenbosch, South Africa

M. J. WINGFIELD

Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, 9300 Bloemfontein, South Africa

F. A. FERREIRA AND A. ALFENAS

Departamento de Fitopatologia da Universidade Federál de Viçosa-MG, Brazil

Mycosphaerella parkii sp. nov. and *Phyllosticta eucalyptorum* sp. nov. isolated from *Eucalyptus* leaves in Brazil are described. Both fungi are associated with leaf spots of *E. grandis*.

Although a number of leaf pathogens have been recorded on *Eucalyptus* in Brazil (Ferreira, 1989), several other foliicolous pathogens remain to be identified. The fungi described in this study were encountered in *Eucalyptus* plantations in Espirito Santo (ES).

Eucalyptus grandis Hill: Maid. leaves bearing large necrotic lesions that were light brown in colour, mostly round, and with raised margins were subjected to a closer examination. A *Mycosphaerella* sp. was consistently found to be associated with these symptoms. Eleven species of *Mycosphaerella* are now known to occur on *Eucalyptus* (Park & Keane, 1982*a*, 1984; Corlett, 1991; Crous, Wingfield & Park, 1991). Based on the morphology of the ascospores, mode of ascospore germination and cultural behaviour the *Mycosphaerella* sp. on *Eucalyptus* leaves from Brazil is best placed in a new species.

Mycosphaerella parkii Crous, Wingf., Ferreira & Alfenas, sp. nov.

Etym.: Named after Dr Robert Park in recognition of his significant contributions to the taxonomy of *Mycosphaerella* spp. on *Eucalyptus*.

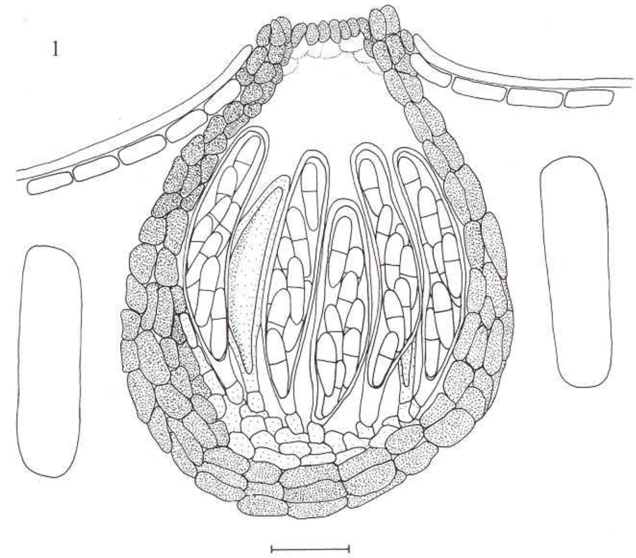
Laesiones amphigenae, maculae parvae vel magnae, rotundae vel subirregulares, pallide brunneae, cinctae linea exili rufa vel brunnea, saepe marginem elevatam formanti. *Ascocarpi* amphigena, solitaria vel aggregata, nigra, immersa, et erumpescentia, punctiformia, globosa, glabra, usque ad 90 µm lata, 100 µm alta; ostiolum apicale papillatum usque ad 18 µm diam.; paries consistens in cellis medio-brunneis, 3–4 stratis texturae angularis, basis consistens in 2–3 stratis cellarum hyalinarum (Fig. 1); *Asci* in fascia una, bitunicati, aparaphysati, subsessiles, octospori, ellipsoidei vel obclavati vel cylindrici, recti vel incurvi, numerosi, 22·0–40·0 × 6·25–11·0 µm in vivo, 25·0–40·0 × 6·25–8·0 µm in vitro. *Ascosporae* 2–3 seriatae vel irregulariter dispositae, obliquae, superpositae, recte ellipsoidales, obtusae ad extrenum utrumque, hyalinae, laevae, uniseptatae, eguttulatae, non colligatae ad septum, latissimae ad mediam cellam superiorem, prominentius attenuatae ad extremum hoc quam ad illud, 8·75–(10·0)–14·0 × 2·5–3·0 µm in vivo (Fig. 2), 6·25–(9·0)–9·5 × 2·0–2·5 µm in vitro.

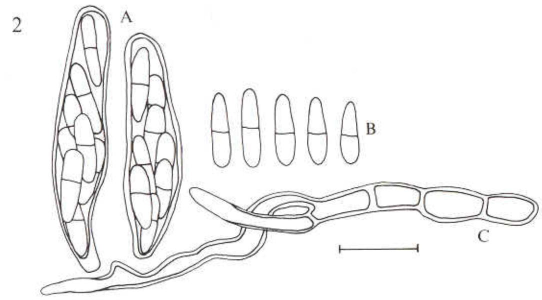
Holotypus PREM 50668, in foliis vivis *Eucalyptus grandis*, Aracruz Florestal nursery, Brazil, 24 Feb. 1990, M. J. Wingfield.

Lesions amphigenous, varying from small to large, round or slightly irregular spots, light brown in colour, surrounded by a thin red to brown line, forming a raised margin in most cases; *Pseudothecia* amphigenous, single or aggregated, black, immersed becoming erumpent, globose, glabrous, up to 90 µm wide, 100 µm high; apical papillate ostiole up to 18 µm diam.; wall consisting of medium brown cells, 3–4 layers of *textura angularis*, base consisting of 2–3 layers of hyaline cells (Fig. 1); *Asci* in a fascicle, bitunicate, aparaphysate, subsessile, 8-spored, ellipsoid to obclavate or cylindrical, straight or curved, numerous, 22·0–40·0 × 6·25–11·0 µm *in vivo*, 25·0–40·0 × 6·25–8·0 µm *in vitro*; *Ascospores* 2–3 seriate or irregularly arranged, oblique, overlapping, straight ellipsoidal, obtuse at each end, hyaline, smooth, 1-septate, eguttulate, not constricted at the septum, widest in the middle of the upper cell, tapering more prominently to the one end than the other, 8·75–(10·0)–14·0 × 2·5–3·0 µm *in vivo* (Fig. 2), 6·25–(9·0)–9·5 × 2–2·5 µm *in vitro*. No spermatogonial or anamorph state was found or produced in culture.

Specimens examined: on leaves of *E. grandis*, Aracruz Florestal nursery, Brasil, 24 Feb. 1990, M. J. Wingfield, PREM 50668 (holotype); *E. grandis*, Teixeira de Freitas, BA, Brazil, 12 Apr. 1991, A. M. Moreira, PREM 50670; *E. dunnii* Maid., Brazil, Aug. 1991, M. J. Wingfield, PREM 50900.

Several small-spored *Mycosphaerella* spp. occur on *Eucalyptus*. Of these, *M. heimii* Bouriquet (Bouriquet, 1946) lacks





Figs 1–2. Diagrammatic representation of a pseudothecium, asci and ascospores of *M. parkii*. Bar = 10 µm. **Fig. 1.** Transverse section through a pseudothecium. **Fig. 2(A–C).** Asci and ascospores. A, ellipsoid to obclavate asci; B, ellipsoidal ascospores; C, germinating ascospore after 24 h on malt extract agar.

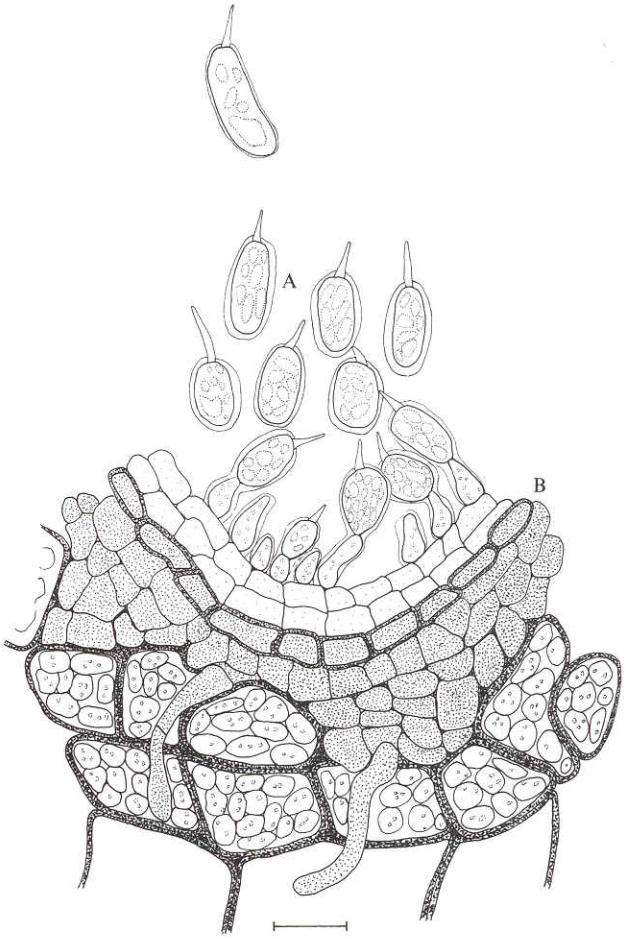


Fig. 3(A–B). Pycnidial wall with conidiogenous cells and conidia of *P. eucalyptorum*. Bar = 10 µm. A, conidia with apical appendages; B, pycnidial wall with conidiogenous cells and developing conidia.

a Latin description, and is therefore not validly published. Furthermore, based on the illustration of Bouriquet (1946), Park & Keane (1984) are of opinion that this species is similar to *M. cryptica* (Cke) Hansf. *M. martinae* Hansford is described to have ascospores of 11·0–13·0 × 2·5–3·0 µm (Hansford, 1956), being larger than those of *M. parkii*, which are 8·75–14·0 × 2·5–3·0 µm. Furthermore, *M. martinae* is associated with angular lesions (Park & Keane, 1982*a*), unlike the circular lesions of *M. parkii*. Ascospores of *M. parva* Park & Keane are 7–10 × 1–3 µm (Park & Keane, 1982*a*), being narrower than those of *M. didymelloides* Petrak, which are 7·5–10·0 × 3·0–3·5 µm (Corlett, 1991).

Mycosphaerella parkii can clearly be distinguished from all small-spored *Mycosphaerella* spp. occurring on *Eucalyptus* by the following characteristics: ascospores are generally median septate, other than in ascospores at the extreme of the range, where the bottom cells can be slightly longer than the upper wider cells; ascospores are not constricted at the septum; ascospores taper more prominently towards the one end than the other; germinating ascospores frequently have more than one germ-tube per cell, sometimes at right angles, but more commonly parallel to the long axis of the spore (Fig. 2C); and ascospores do not darken after germination, further distinguishing it from *M. parva* (Park & Keane, 1982*a*).

Cultures of *M. parkii* were produced from single ascospores on 2% malt-extract agar. Colonies that developed were olive green, with a loose texture and aerial mycelium of a similar colour, becoming darker green to almost black with age. Pseudothecia of *M. parkii* developed with asci and ascospores within 3 weeks after the fungus had been inoculated onto carnation-leaf agar (Fisher, Burgess, Toussoun & Nelson, 1982). Cultures grew faster than has been observed for isolates of *M. cryptica*.

Inoculations with a concentrated mycelium and spore suspension (Park & Keane, 1982*b*) of *M. parkii* on juvenile and mature *E. grandis* leaves in moist chambers, attached to young plants in the laboratory (Wall & Keane, 1984), indicated that this fungus was pathogenic. Hyphae penetrated leaf laminas after 1 week and prominent lesions developed 2 weeks after inoculation.

Young juvenile *E. grandis* leaves displaying leaf spot symptoms were collected in a nursery at Aracruz (ES). Necrotic areas were extensively colonized with pycnidia of a *Phyllosticta* sp. As far as we are aware, four *Phyllosticta* spp. are known from *Eucalyptus*, while another, as yet undescribed species is known from *Eucalyptus* in Florida, U.S.A. (Farr *et al.*, 1989). *Phyllosticta extensa* Ellis & Everh. was described from *Eucalyptus* leaves in California, and is regarded as the earlier name for *P. eucalypti* Ellis & Everh., having conidia 5·0–8·0 × 1·5–2·5 µm (Saccardo, 1899). *Phyllosticta eucalypti* Thüm. was described from *E. globulus* Labill. leaves in Portugal, having conidial dimensions of 4·0 × 1·5 µm