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

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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*.

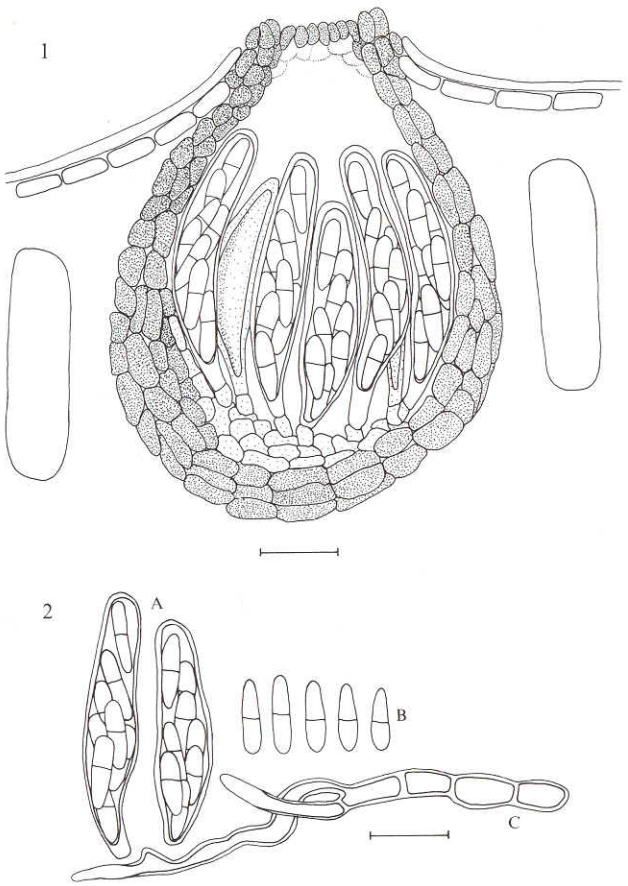
Laesiones amphigenae, maculae parvae vel magnae, rotundae vel subirregulares, pallide brunneae, cinctae linea exili rufa vel brunnea, saepe marginem elevatam formanti. *Ascocarpi* amphigena, solitaria vel aggregata, nigra, immersa, et erumpescentia, punctiformia, globosa, glabra, usque ad 90 µm lata, 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.

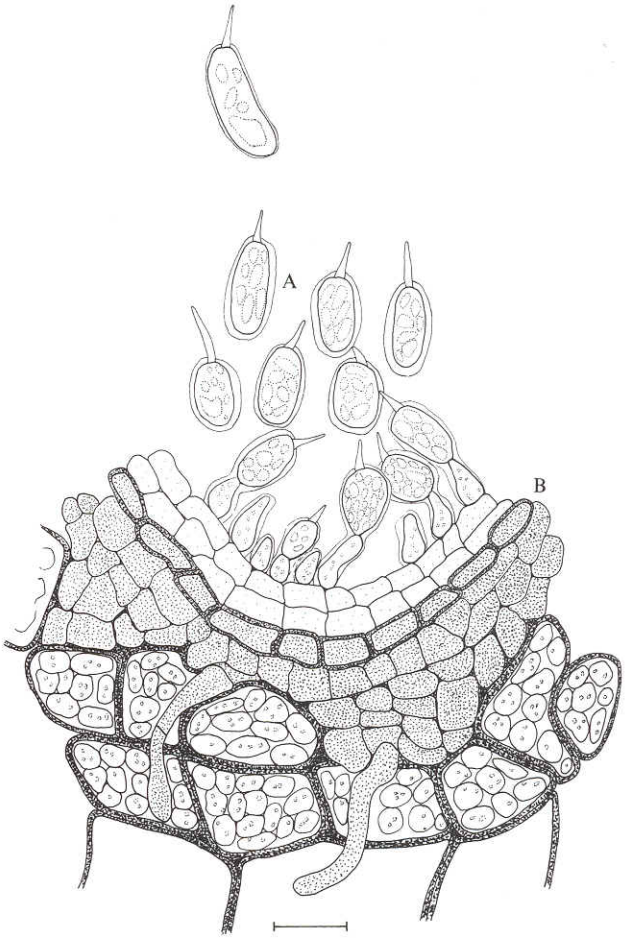


Fig. 3(A–B). Pycnidial wall with conidiogenous cells and conidia of *P. eucalyptorum*. Bar = 10 µm. A, conidia with apical appendages; B, pycnidial wall with conidiogenous cells and developing conidia.

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

(Saccardo, 1884). *P. eucalyptina* Pat. was described from the same host as *P. eucalypti* Thüm. in Tunisia, with conidia 18–20 × 5–6 µm (Saccardo, 1899). Both *P. extensa* and *P. eucalypti* Thüm. have conidia that are much smaller than those of our collection, while those of *P. eucalyptina* are much larger.

Species of *Phyllosticta* are separated mainly based on their hosts, and to a lesser extent on differences in symptoms, pycnidia and conidia (Van der Aa, 1973). Because our collection from Brazil is clearly different from the other *Phyllosticta* spp. known on *Eucalyptus*, it is placed in a new species.

Phyllosticta eucalyptorum Crous, Wingf., Ferreira & Alfenas, sp. nov.

Laesiones amphigenae rotundae vel irregulares, maculae parvae (3 mm diam.) vel maiores, pallide brunneae, marginibus rufis vel purpureis et subelevatis. *Mycelium* immersum, intracellulare, constitutum ex hyphis pallide vel medio-brunneis, septatis, 3·0–4·5 µm diam. *Pycnidia* plerumque aggregata, nigra, immersa erumpescentia, amphigena, subglobosa vel globosa, 70–250 µm diam.; parietes brunnei, 9–21 µm crassi; stratum conidiogenum 6–9 µm crassum; regio ostiolaris papillata poro circulari 6–20 µm diam. *Cellae conidiogenae* lageniformes vel ampulliformes, 5–15 × 2–3 µm, infixae in mucilagine. *Conidia* unicellularia, ellipsoidea, 7·5–(11·0)–20·0 × 5·0–(6·0)–6·5 µm, tunicis guttulatis et persistentibus, ca 1 µm crassis; appendices apicales, 3–10 µm longae, ca 1·0–1·5 µm diam. ad basim et decrescentes apicem obtusum versus (Fig. 3).

Holotypus PREM 50671 in foliis vivis *Eucalyptus grandis*, Aracruz nursery, ES, Brazil, 24 Feb. 1990, M. J. Wingfield.

Lesions amphigenous, round to irregular, small spots (3 mm diam.) to larger blotches, light brown, with a red to purple and slightly raised margin. *Mycelium* immersed, intracellular, composed of pale to medium brown, septate hyphae, 3·0–4·5 µm diam. *Pycnidia* mostly aggregated, black, immersed becoming erumpent, amphigenous, subglobose to globose, 70–250 µm diam.; walls brown, 9–21 µm thick; conidiogenous layer 6–9 µm thick; ostiolar region papillate with a circular pore measuring 6–20 µm diam. *Conidiogenous cells* lageniform to ampulliform, 5–15 × 2–3 µm, invested in mucilage. *Conidia* unicellular, ellipsoid, 7·5–(11·0)–20·0 × 5·0–(6·0)–6·5 µm, guttulate, with persistent mucous coats, *ca* 1 µm thick; apical appendages 3–10 µm long, *ca* 1·0–1·5 µm diam. at the base and tapering to a blunt tip (Fig. 3).

Specimen examined: on *Eucalyptus grandis* leaves, Aracruz nursery, ES, Brazil, 24 Feb. 1990, M. J. Wingfield, PREM 50671.

Mycosphaerella parkii is one of two *Mycosphaerella* spp. occurring on *Eucalyptus* leaves in Brazil. The other species closely resembles *M. molleriana* (Thüm.) Lindau, but needs to be studied in more detail once cultures have been obtained. *M. molleriana* (= *M. nubilosa* (Cke) Hansf.) is widely distributed throughout the *Eucalyptus* growing areas of the world (Crous *et al.*, 1991). The limited occurrence of *M. parkii* suggests that this fungus might have originated on one of many native myrtaceous plants in Brazil. This would not be unusual for *Eucalyptus* pathogens, of which *Puccinia psidii* Winter is believed to have originated on native plants such as *Psidium* L. and *Syzygium* Gaertn. spp. (Joffily, 1944; Ferreira, 1989). Such 'new' pathogens pose a significant threat to *Eucalyptus* spp. in their areas of origin and elsewhere in the world.

Both *M. parkii* and *P. eucalyptorum* belong to well-known genera of biotropic fungi, and we therefore assume that they are pathogens of *Eucalyptus* in Brazil. Clearly, their significance must still be evaluated. The discovery of two previously undescribed pathogens in a preliminary survey suggests that many additional pathogens of *Eucalyptus* have yet to be discovered. Urgent attention should therefore be given to *Eucalyptus* disease surveys in this part of the world.

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