Mycol. Res. **95** (9): 1108–1112 (1991) *(Printed in Great Britain)*

Mycosphaerella marasasii sp. nov. and its *Pseudocercospora* anamorph on leaves of *Syzygium cordatum*

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, PO Box 339, 9300 Bloemfontein, South Africa

A prominent leaf spot disease was found to occur on *Syzygium cordatum* in South Africa. A *Mycosphaerella* was associated with these symptoms and proved to be different from other species occurring on genera in the Myrtaceae. The names *Mycosphaerella marasasii* for the teleomorph and *Pseudocercospora marasasii* for the anamorph are formally introduced for this fungus. The connexion between the anamorph and teleomorph was proven through cultural studies.

Eucalyptus spp. are currently being planted extensively in South Africa as important sources of mining timber and pulp (Directorate National Forestry Planning, 1988). All diseases occurring on this genus are, therefore, regarded as potentially important to the forestry industry. The first report of *Mycosphaerella* leaf-spotting and defoliation of *Eucalyptus* spp. was made early this century (Doidge, 1950). Since then, *Mycosphaerella* Leaf Blotch (MLB) has become one of the most important diseases of *Eucalyptus* spp. in South Africa (Crous, Wingfield & Park, 1991). A measure of its importance is that *E. globulus* and certain progenies of *E. nitens* can no longer be planted commercially in this country (Lundquist & Purnell, 1987).

A detailed examination of MLB on *Eucalyptus* (Crous *et al.*, 1991) has shown that only one *Mycosphaerella* sp., namely *M. molleriana* (Thüm.) Lindau is associated with the disease on *Eucalyptus* in South Africa. During subsequent collections, however, a MLB not unlike that occurring on *Eucalyptus* was noticed on leaves of *Syzygium cordatum* Hochst. Unlike *Eucalyptus* spp., *S. cordatum* is a tree native to South Africa. Because it also belongs to the Myrtaceae, the question has been raised as to whether the MLB could be the same as the one that affects *Eucalyptus* spp. The aim of this study was, therefore, to determine the identity of the *Mycosphaerella* sp. occurring on *S. cordatum* and to compare it with *M. molleriana* on eucalypts.

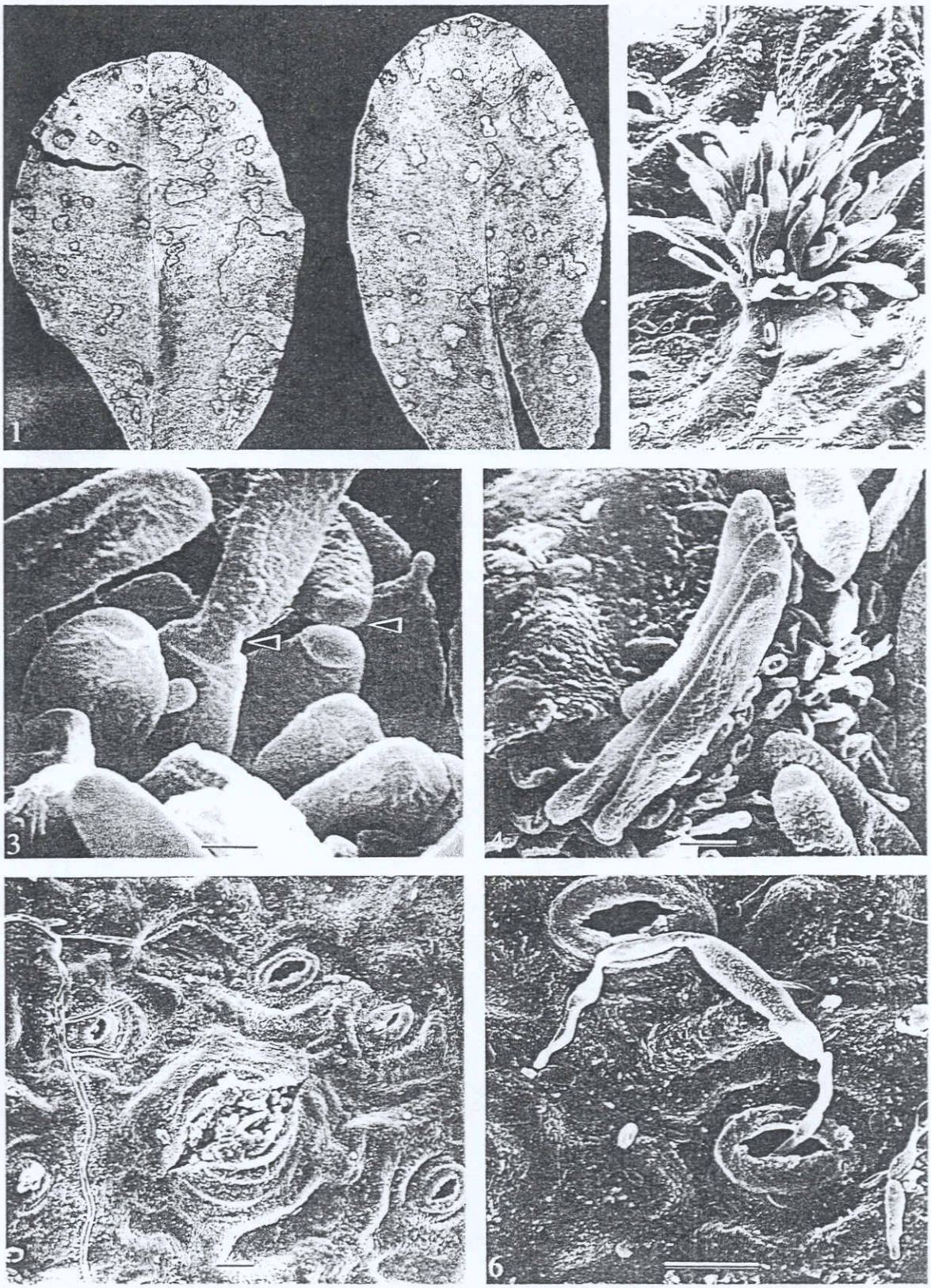
MATERIALS AND METHODS

Leaves of *S. cordatum* with MLB symptoms were collected in Kwambonambi (Northern Natal coast), near Barberton (Eastern Transvaal) and near Tzaneen (Northern Transvaal) and examined microscopically. Single-ascospore isolations were made from freshly collected leaves on malt-extract agar (10 g Merck malt extract, 20 g Merck agar, 1000 ml H_2O) (MEA), using the method described by Crous *et al.* (1991). Cultures obtained from single-ascospore isolations were transferred to carnation-leaf agar (CLA) (Fisher *et al.*, 1982), and incubated at 25 °C under a combination of near-ultraviolet and fluorescent light.

RESULTS AND DISCUSSION

The *Mycosphaerella* sp. occurring on *Syzygium* leaves caused distinct, light brown leaf spots, surrounded by a prominent, raised border (Fig. 1), not unlike those associated with *M. molleriana* on *Eucalyptus* spp. Furthermore, pseudothecia were also amphigenous, but predominantly hypogenous. The ascus dimensions of *M. molleriana* (30–68 × 9–18 µm) (Crous *et al.*, 1991) and the unknown *Mycosphaerella* sp. on *S. cordatum* (31–50 × 10–17 µm) also overlapped. However, the asci of the latter material differed slightly in shape, tending to be more ovoid to obclavate while those of *M. molleriana* tended to be ellipsoidal (Crous *et al.*, 1991).

Ascospores of the *Mycosphaerella* sp. on *S. cordatum* germinated with the germ-tube parallel to the long axis of the spore, similar to ascospore germination in *M. molleriana* (Park & Keane, 1982*a*). Ascospores, however, had thinner walls and germ-tubes were not as wide as those found in *M. molleriana*. In general, the size of the ascospores in *M. molleriana* (9–20 × 2·5–4·5 µm) (Crous *et al.*, 1991) and the unknown species (10–20 × 2·5–4 µm) were similar, making it difficult to distinguish between them on the basis of these structures. Ascospore shape in the unknown species tapered more prominently to the one end than observed for *M. molleriana*. The basal cells of the ascospores (as they are arranged in the



Figs 1–6. Symptoms and morphological characteristics of *M. marasasii* and its *Pseudocercospora* anamorph on *S. cordatum* leaves. **Fig. 1.** Leaf spots with necrotic centres. **Fig. 2.** Fascicle of conidiophores protruding from a stoma (Bar = 10 µm). **Fig. 3.** Conidia (arrows) attached to conidiophores (Bar = 2 µm). **Fig. 4.** Conidia showing slightly roughened surface (Bar = 3 µm). **Fig. 5.** Substomatal pseudothecium (Bar = 10 µm). **Fig. 6.** Ascospore with germ-tube infecting through a stoma (Bar = 10 µm).

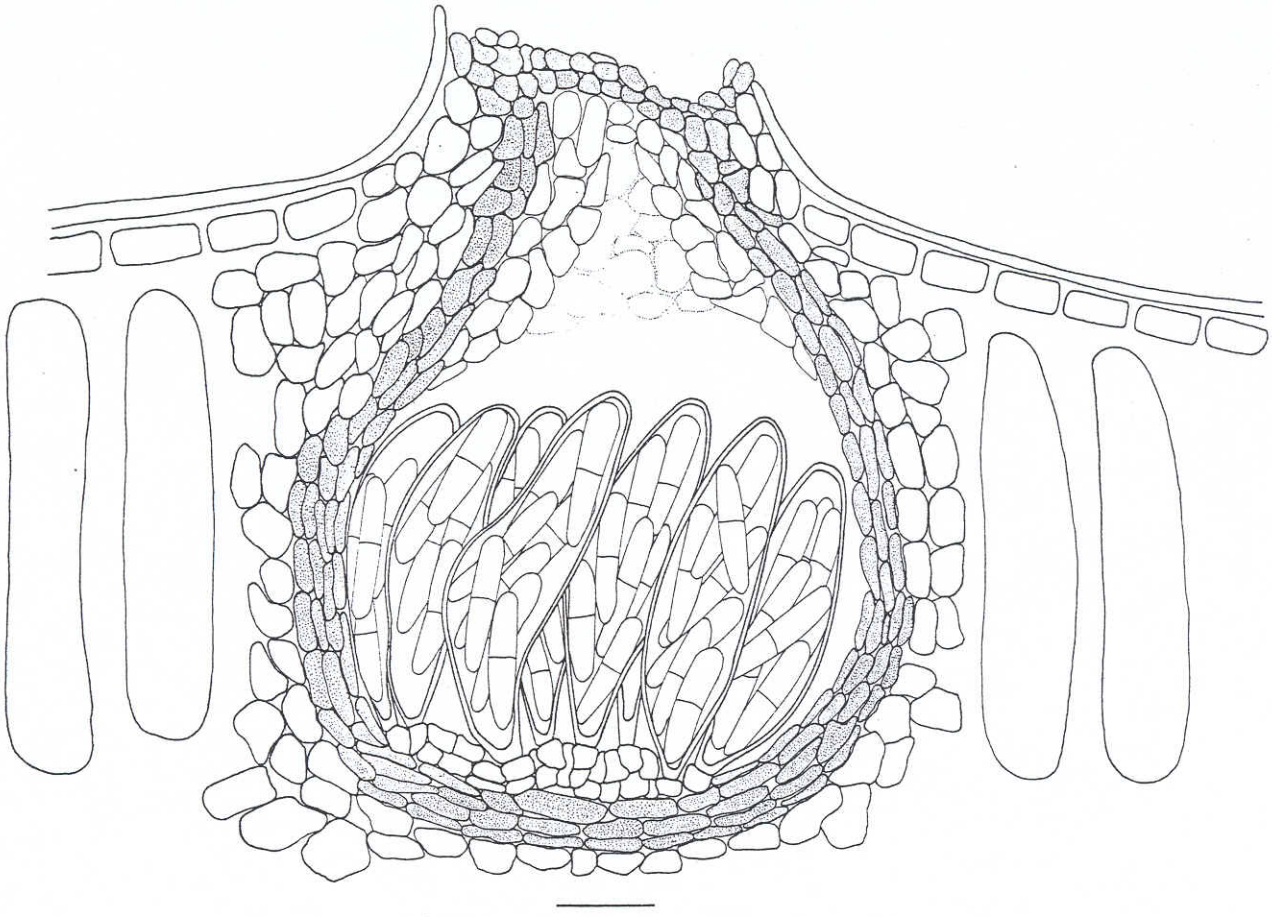


Fig. 7. Transverse section through a substomatal pseudothecium of *M. marasasii*. (Bar = 10 μm).

ascus) on the material from *S. cordatum* were slightly longer than the apical cells, and there was no constriction at the septum. This is in contrast to ascospores of *M. molleriana* which can be slightly constricted at the median septum (Crous *et al.*, 1991).

Single-ascospore isolations on MEA produced colonies which were a light grey colour. These were unlike the dark green to black cultures produced by *M. molleriana*. They also grew noticeably faster than those of *M. molleriana* on MEA.

Morphological characteristics of the *Mycosphaerella* sp. on *S. cordatum* were compared with, and found to be distinct from, those of all other *Mycosphaerella* spp. occurring on *Eucalyptus* (Park & Keane, 1982*a*, *b*, 1984). Characteristics of this material were also compared with *M. aequatoriensis* Petrak described from *Eugenia* in Ecuador (Petrak, 1948). The rationale for this comparison was the fact that, like *Syzygium* and *Eucalyptus*, *Eugenia* belongs to the Myrtaceae. Spores of *M. aequatoriensis* were described as clavate-oblong to more cylindrical with obtuse ends, being slightly attenuated at the one end, not or only slightly constricted at the septum, 12–23 × 3–4 μm in size, with paraphyses. These characteristics clearly distinguish *M. aequatoriensis* from our collection on *S. cordatum*.

Colonies derived from single ascospores of the *Mycosphaerella* sp. on *S. cordatum* produced conidiophores and conidia after 1 wk of incubation on CLA. These structures were identical to those of a cercosporoid fungus collected by P. van der Byl in 1912 on leaves of *S. cordatum* in South Africa (PREM 5138). We have subsequently also collected similar material on *S. cordatum* leaves in various parts of the country (PREM 50637–50639).

Conidia and conidiophores were lightly dematiaceous and scars on the conidiogenous cells were not thickened (Figs 2–4). Based on these facts, we have assigned the material to the genus *Pseudocercospora* Speg. (Deighton, 1976, 1979, 1987).

A number of species of *Cercospora sensu lato* have been described on various members of the Myrtaceae (Chupp, 1953; Pollack, 1987). Collections of cercosporoid fungi on *Syzygium* spp. lodged at the International Mycological Institute (IMI) have been examined. These include *Cercospora syzygii* Mandal (IMI 226656); a *Cercospora* sp. (IMI 136008); a *Stigmina* sp. (IMI 89987*b*) and a *Pseudocercospora* sp. (IMI 234833). None of these species or collections was found to be the same as the *Pseudocercospora* sp. occurring on *S. cordatum* in South Africa.

Pseudocercospora eucalyptorum Crous, Wingfield, Marasas & Sutton is a cercosporoid fungus occurring commonly on various *Eucalyptus* species in South Africa (Crous *et al.*, 1989). The *Pseudocercospora* sp. occurring on *S. cordatum* is distinct from *P. eucalyptorum*. The latter fungus has conidia with up to 6 septa, and 23–110 × 2·5–4 μm in size (Crous *et al.*, 1989), whereas conidia of the *Pseudocercospora* on *S. cordatum* are 1–4 septate and 20–90 × 1·8–2·5 μm in size. Furthermore, conidia

of *P. eucalyptorum* are olivaceous and slightly roughened, whereas those observed on *S. cordatum* leaves are medium brown and prominently verruculose.

From these comparisons, it is concluded that the *Mycosphaerella* sp. found on *S. cordatum* in South Africa represents a presently undescribed species. Similarly, the *Pseudocercospora* anamorph of this fungus does not appear to have been formally described.

Mycosphaerella marasasii Crous & Wingfield, sp. nov.

Etym.: Named after Professor Walter Friedrich Otto Marasas in honour of his contribution to South African mycology

Laesiones amphigenae, irregularia puncta variantia usque ad maiores folii maculas, pallide brunneae, cinctae margine tenui eminenti obscurius brunneo elevato. *Ascocarpi* amphigeni, praecipue hypogeni, nigri, aggregati vel separati, globosi, immersi poro apicali solum epidermidem penetrante usque erumpentiores eminentesque e pagina inferiore, 70–120 µm diam, ostiolo papillato; parietes atro-brunnei 3–5 stratis cellarum crassitunicatarum, complanascentes longius ab ostiolo. *Asci* aparaphysati, fasciculati, bitunicati, subsessiles, ovoidei usque obclavati, recti vel incurvi, octospori, 31–50 × 10–17 µm. *Ascosporae* 2–3 seriatae, obliquae, imbricatae, hyalinae, guttulatae vel aguttulatae, tenuitunicatae, rectae vel lenissime curvatae, fusiformes, uniseptatae, latissimae in media parte cellularum apicalium, attenuatae, prominentius ad extremum alterum, 10–20 × 2·5–4 µm.

Holotypus PREM 50635 in foliis vivis *Syzygium cordatum* Hochst. Tzaneen, N. Tvl., R.S.A., 26 Sept. 1989, M. J. Wingfield.

Lesions amphigenous, irregular spots to larger leaf blotches, light brown in colour, surrounded by a thin, prominent, darker brown, raised border (Fig. 1). *Ascocarps* amphigenous, predominantly hypogenous, black, aggregated or separate, globose, immersed with only the apical pore penetrating the epidermis, to more erumpent and protruding on the lower surface, 70–120 µm diam, with a papillate ostiole; walls dark brown, 3–5 layers of thick-walled cells, becoming flattened further away from the ostiole (Figs 5–8). *Asci* aparaphysate, fasciculate, bitunicate, subsessile, ovoid to obclavate, straight or incurved, 8-spored, 31–50 × 10–17 µm. *Ascospores* 2–3 seriate, oblique, overlapping, hyaline, guttulate, or aguttulate, thin-walled, straight or very slightly curved, fusiform, 1-septate, widest in the mid-section of the apical cells, tapering more prominently at one end than the other, 10–20 × 2·5–4 µm (Figs. 6, 8).

Specimens examined: on leaves of *Syzygium cordatum*, Tzaneen, N. Tvl, R.S.A., 26 Sept. 1989, M. J. Wingfield, PREM 50635 (holotype), PPRI 4023; *S. cordatum*, Sabie, E. Tvl, R.S.A., 2 Nov. 1989, P. W. Crous, PREM 50636 (paratype).

Pseudocercospora marasasii Crous & Wingfield, sp. nov.

Laesiones ut in descriptione *Mycosphaerellae marasasii*. *Conidiomata* amphigena, cinerea usque pallido-brunnea. *Mycelium* plerumque internum, stroma adest. *Conidiophora* verruculosa, in fasciculos aggregata per stomata emergentes, brunnea ad fusca, 0–3-septata, recta usque varie curvata vel semel geniculata, non ramosa, 6–22 × 3–7 µm. *Cellulae conidiogenae* laeves usque subasperae, ad apicem obtuse rotundatae, raro 1–3 percurrentes, 4·5–20 × 2–3·5 µm. *Conidia* holoblastica, verruculosa, medio- vel atro-brunnea, anguste cylindrica vel subattenuata ad basem, recta vel leniter curvata, basis

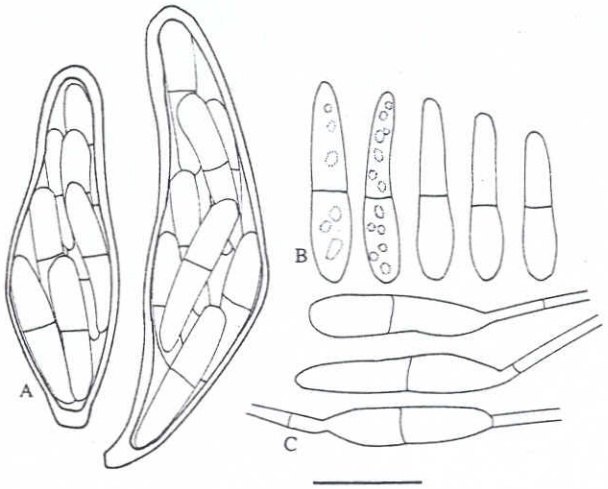


Fig. 8 (A–C). Asci and ascospores of *M. marasasii*. A, Ovoid to obclavate asci; B, fusoid ascospores; C, germinating ascospores (Bar = 10 µm).

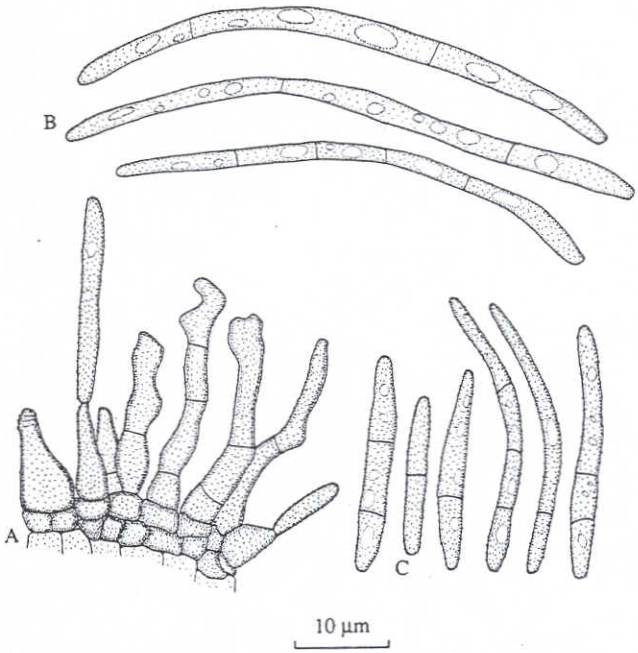


Fig. 9 (A–C). Conidiophores and conidia of *P. marasasii*. A, Conidiophores with percurrent as well as sympodial proliferation; B, conidia from sporulating cultures; C, conidia from leaves.

truncata usque longa obconice truncata, apex conicus usque obtusus, cicatrices conidicae indistinctae, 1–4-septata in foliis, 1–6-septata in cultura, 20–90 × 1·8–2·5 µm, etsi exceptiones usque ad 180 µm reperiuntur in cultura.

Holotypus PREM 50637 in foliis vivis *Syzygium cordatum* Hochst., Barberton, E. Tvl, R.S.A., 1 Feb. 1988, M. J. Wingfield.

Lesions as described for *M. marasasii*. *Conidiomata* amphigenous, grey to light brown in colour (Figs 2, 9). *Mycelium* mostly internal, stroma present. *Conidiophores* verruculose, aggregated in fascicles emerging through stomata, medium to dark brown, 0–3 septate, straight to variously curved or once geniculate, unbranched 6–22 × 3–7 µm. *Conidiogenous cells* smooth to slightly roughened, bluntly rounded at the apex,

rarely 1–3 percurrent, 4·5–20 × 2–3·5 µm (Fig. 3). *Conidia* holoblastic, verruculose, medium to dark brown, narrowly cylindrical or slightly attenuated at the base, straight or gently curved, base truncate to long, obconically truncate, apex conical to obtuse, conidial scars indistinct, 1–4 septate on leaves (Figs 4, 9), 1–6 septate in culture, 20–90 × 1·8–2·5 µm, although exceptions of up to 180 µm are found in culture.

Specimens examined: on living leaves of *Syzygium cordatum*, Barberton, E. Tvl, R.S.A., 1 Feb. 1988, M. J. Wingfield, PREM 50637 (holotype) PPRI 4024. Paratypes: *S. cordatum*, Barberton, E. Tvl, R.S.A., 22 Aug. 1912, P. A. van der Byl, PREM 5138; *S. cordatum*, Richards Bay, N. Natal, R.S.A., 5 Dec. 1988, M. J. Wingfield, PREM 50638; *S. cordatum*, Kwambonambi, N. Natal, R.S.A., 5 Dec. 1988, M. J. Wingfield, PREM 50639 (IMI 332125); dried cultures on carnation-leaf agar derived from a single ascospore of *Mycosphaerella marasasii* from a pseudothecium on leaves of *Syzygium cordatum*, Tzaneen, N. Tvl, R.S.A., 26 Sept. 1989, M. J. Wingfield, PREM 50640.

The possibility exists that pathogens of native Myrtaceae in South Africa could become adapted to infect *Eucalyptus* spp. which are grown as exotics in the country. The best example of such a situation is that of the native guava rust pathogen, *Puccinia psidii* Winter, that causes serious damage to commercially propagated exotic *Eucalyptus* spp. in Brazil (Dianese, Moraes & Silva, 1984; Ferreira, 1989). Although it was originally considered a possibility, this study has shown no evidence of *M. marasasii* or its anamorph on *Eucalyptus* spp. in South Africa. We have similarly not found the related *Eucalyptus* pathogens, *M. molleriana* or *P. eucalyptorum* on native *S. cordatum*.

We thank Mr H. J. van Tonder, Plant Protection Research Institute, Pretoria, for assistance with electron microscopy, Mr L. F. van Ryneveld, University of the Orange Free State, Bloemfontein, for correcting the Latin description and Dr B. C. Sutton, IMI, England, for his comments on the *Pseudocercospora* material. We are also thankful to IMI for the loan of specimens examined in this study.

(Received for publication 12 December 1990 and in revised form 3 March 1991)

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