

New species of *Mycosphaerella* occurring on *Eucalyptus* leaves in Indonesia and Africa

P.W. Crous and M.J. Wingfield

Abstract: Although Africa and Indonesia have not been particularly well surveyed for *Mycosphaerella* leaf spot fungi, several species are known to occur on *Eucalyptus* leaves in these areas. Three new species of *Mycosphaerella* with anamorph states are described from herbarium specimens and cultures in the present study. *Mycosphaerella suttoniae* and *Mycosphaerella heimioides* are described from *Eucalyptus* leaves from Indonesia. The former species is of particular interest, because its anamorph *Phaeophleospora epicoccoides* is the first species of *Phaeophleospora* linked to *Mycosphaerella*. *Mycosphaerella irregulariramosa* is described from *Eucalyptus saligna* growing in the Northern Province of South Africa. Both *M. irregulariramosa* and *M. heimioides* have *Pseudocercospora* anamorphs, and these are described as *Pseudocercospora irregulariramosa* and *Pseudocercospora heimioides*. Notes are also provided on the host range and geographic distribution of previously described species of *Mycosphaerella* on *Eucalyptus* leaves in other parts of Africa and in Indonesia.

Key words: *Eucalyptus*, *Kirramyces*, *Mycosphaerella*, *Phaeophleospora*, *Pseudocercospora*, systematics.

Résumé : Bien que l'Afrique et l'Indonésie aient été particulièrement bien explorées pour déceler les *Mycosphaerella*, champignons responsables de taches foliaires, on connaît plusieurs espèces qui poussent sur les feuilles d'*Eucalyptus* dans ces régions. Dans cette étude, les auteurs décrivent trois nouvelles espèces de *Mycosphaerella* avec leurs stades anamorphes provenant de spécimens d'herbiers et de cultures. Ils décrivent le *Mycosphaerella suttoniae* et le *Mycosphaerella heimioides* provenant de feuilles d'*Eucalyptus* originaires d'Indonésie. La première espèce présente un intérêt particulier, parce que son anamorphe, le *Phaeophleospora epicoccoides* est la première espèce de *Phaeophleospora* liées au *Mycosphaerella*. Ils décrivent le *Mycosphaerella irregulariramosa* provenant de l'*E. saligna* poussant dans la province du Nord-Ouest de l'Afrique du Sud. Le *M. irregulariramosa* ainsi que le *M. heimioides* possèdent des anamorphes de type *Pseudocercospora* qui sont alors décrits comme *Pseudocercospora irregulariramosa* et *Pseudocercospora heimioides*. Les auteurs présentent également des notes sur l'amplitude des hôtes et la distribution géographique d'espèces de *Mycosphaerella* déjà décrites sur feuilles d'*Eucalyptus*, dans d'autres parties de l'Afrique et de l'Indonésie.

Mots clés : *Eucalyptus*, *Kirramyces*, *Mycosphaerella*, *Phaeophleospora*, *Pseudocercospora*, systématique.
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Introduction

Eucalyptus L'Hér. plantations cover more than eight million hectares internationally, and thus represent a biomass resource of international importance (Turnbull 1991). In their centres of origin (primarily Australia), there are more than 600 species that form a major component of a unique ecosystem. *Eucalyptus* spp. have also been planted as exotics in plantations in various parts of the tropics and southern hemisphere.

Diseases pose a great threat to *Eucalyptus* spp., both in natural ecosystems and in plantations. Pathogens introduced into native eucalypt forests have the potential to cause epidemic disease situations. Dieback of Jarrah (*Eucalyptus marginata* Donn ex Sm.) in Western Australia, caused by

Phytophthora cinnamomi Rands, appears to represent such a situation (Zentmeyer 1980). Many pathogens have been recorded on exotic *Eucalyptus* spp., of which several have caused serious disease problems (Sankaran et al. 1995) enhanced by the clonal nature of the plantations.

Species of *Mycosphaerella* Johanson are well known as important pathogens of *Eucalyptus* spp. The so-called *Mycosphaerella* leaf blotch (MLB) disease is one of the important constraints to *Eucalyptus* propagation in various parts of the world (Lundquist and Purnell 1987; Carnegie et al. 1994). More than 22 species of *Mycosphaerella* have been associated with MLB (Carnegie and Keane 1994; Crous and Wingfield 1996), although very little is known about the relative importance of most of these. Detailed studies have also shown that more than one species of *Mycosphaerella* is commonly associated with diseases previously thought to be caused by *Mycosphaerella molleriana* (Thüm.) Lindau (Crous et al. 1991; Crous and Wingfield 1996).

In recent years, new characters have been defined that have made it possible to recognise distinct taxa among *Mycosphaerella* spp. associated with MLB. Because teleomorph structures on leaves are the dominant signs associated with *Mycosphaerella* infections, morphological characteristics associated with these structures have been the primary basis

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for the circumscription of these fungi. Many of these fungi also have unique anamorphs, although these can usually only be detected in axenic culture. Characteristics associated with ascospore germination and colony growth in culture are also useful and reliable taxonomic tools (Crous and Wingfield 1996).

Recent surveys of plantation-grown *Eucalyptus* spp. in Indonesia and South Africa have led to the description of several new species of *Mycosphaerella* (Crous and Alfenas 1995; Crous and Wingfield 1996). These studies have now been intensified to survey additional sites in the two countries. A number of new taxa were collected and are described here. Furthermore, data pertaining to the host range and geographic distribution of previously described species are also updated.

Materials and methods

Eucalyptus leaves with MLB symptoms were collected from plantations in Indonesia (Lake Toba area, northern Sumatra) and Africa (Kenya, South Africa, Tanzania, and Zambia). Lesions were excised from leaves, and single ascospore cultures were established on 2% malt extract agar (Biolab) (MEA) using the technique described by Crous et al. (1991). Germinating ascospores were examined after 24 h, their germination patterns were determined, and then they were transferred to MEA. Cultures were incubated for 2 weeks at 25°C in the dark and subcultured onto divided plates with one half containing carnation leaf agar (CLA) (Fisher et al. 1982; Crous et al. 1992) and the other MEA, incubated at 25°C under continuous near-ultraviolet light. Linear growth on agar for each culture was determined after 1 month (Crous and Wingfield 1996). Colony colors (top and bottom) were scored using the color charts of Rayner (1970). Wherever possible, 30 measurements were made of structures mounted in lactophenol, and the extremes are given in parentheses. Herbarium specimens were lodged at the National Collection of Fungi, Pretoria (PREM).

Results

Material of MLB disease collected from *Eucalyptus* spp. from Africa and Indonesia included three undescribed *Mycosphaerella* spp., which are dealt with in the taxonomy section below. In addition, materials collected from Kenya, Tanzania, and Zambia were colonized by several species known to occur in South Africa. In Kenya and Zambia, leaves of *Eucalyptus globulus* Labill. were colonized by *Mycosphaerella juvenis* Crous & M.J. Wingf. (PREM 54972, PREM 54973), with the same species also occurring on *Eucalyptus maidenii* F. Muell. in Tanzania (PREM 54971). Furthermore, Tanzanian collections of *E. maidenii* leaves were also frequently colonized by *Mycosphaerella marksii* Carnegie & Keane (PREM 54971). Other than *M. juvenis*, leaves of *E. globulus* from Zambia were also colonized by *Mycosphaerella africana* Crous & M.J. Wingf. and *Mycosphaerella lateralis* Crous & M.J. Wingf. (PREM 54973). The common species associated with the most serious leaf spotting on *E. globulus* in these countries was *M. juvenis*. This species is also the most common and serious pathogen of *Eucalyptus nitens* (Deane et Maid.) Maid. in South Africa. An examination of older herbarium specimens lodged at PREM led us to conclude that this species made *E. globulus* unsuitable for

afforestation in South Africa and made it possible to plant only certain provenances of *E. nitens* (Lundquist and Purnell 1987).

In their study of *Mycosphaerella* spp. occurring on eucalypts in Indonesia, Crous and Alfenas (1995) described *Mycosphaerella gracilis* Crous & Alfenas (anam. *Pseudocercospora gracilis* Crous & Alfenas) from leaf spots on *Eucalyptus urophylla* S.T. Blake. They also recorded *Mycosphaerella parkii* Crous et al. from leaf spots on *Eucalyptus grandis* Hill ex Maid. The latter species is well known from *Eucalyptus saligna* Sm. and *E. globulus* in Brazil. On fresh material obtained in the present study, several collections of MLB from *E. urophylla* were associated with *M. gracilis* (PREM 54977), while collections from *E. grandis* were commonly associated with *M. parkii* (PREM 54968). Ascospore morphology and germination of *M. gracilis* were the same as those observed for the type collection. Agar colonies were grey with a dark grey to black submerged mycelium, smooth and even edged, with fluffy grey-white aerial mycelium, and readily produced the anamorph *P. gracilis* (Crous et al. 1995a). Colonies of *M. parkii* were generally fast growing and olive-green with abundant aerial mycelium (Crous et al. 1995b), consistent with the type collection from Brazil. Most single-ascospore colonies produced the anamorph *Stenella parkii* Crous & Alfenas in culture. Although conidia were within the range described for the type collection, several of these also occurred in branched chains, a feature not observed in the type material. Furthermore, although pseudothecia of *M. parkii* are known to be amphigenous on leaf spots, several collections had pseudothecia that were either more prominently epiphyllous or hypophyllous.

Another well-known species from *Eucalyptus dunnii* Maid. and *E. grandis* in Brazil is *Mycosphaerella suberosa* Crous et al., which was also collected from a *Eucalyptus* sp. (PREM 54970) in Indonesia during this study. This is the first record of *M. suberosa* from outside South America.

Mycosphaerella marksii was recently described from Australia, where it occurs on several *Eucalyptus* spp. (Carnegie and Keane 1994). This species was subsequently recorded from leaves of *E. grandis* and *E. nitens* in South Africa (Crous and Wingfield 1996). It is characterized by epiphyllous pseudothecia occurring on light brown, irregular to sub-circular lesions with red-purple margins. Ascospores are in the range $11-18 \times 2.5-3.5 \mu\text{m}$, have asymmetrical apical cells, and germinate with germ tubes parallel to the long axis of the spore. In the present study, isolates corresponding to *M. marksii* were collected from *E. globulus* in Indonesia (PREM 54976).

Mycosphaerella heimii Crous has previously been known only from *Eucalyptus* in Madagascar. In the present study, *M. heimii* was commonly collected on *E. urophylla* leaves in Indonesia. Leaf spots were either irregular or subcircular, 2–20 mm in diameter, light brown, becoming dark brown towards the raised border, with red-brown to purple margins. Ascospores were ellipsoidal, not constricted at their septa, guttulate, and widest in the middle of the apical cell, $(9-10-11(-13) \times 2-2.5(-3) \mu\text{m}$. Single-ascospore colonies readily formed *Pseudocercospora heimii* Crous in culture. Conidia were within the range observed for the type

