Mycosphaerella nubilosa, a synonym of *M. molleriana*

P. W. CROUS
Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa

M. J. WINGFIELD
Department of Microbiology, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa

R. F. PARK
The University of Sydney, Plant Breeding Institute, P.O. Box 180, Castle Hill, N.S.W. 2154, Australia

Mycosphaerella leaf blotch is a serious disease of *Eucalyptus* in South Africa. Both *M. nubilosa* and *M. molleriana* have been reported to occur in this country. An examination of numerous collections has failed to show reliable differences between collections identified as either *M. nubilosa* or *M. molleriana*. Further studies have shown the type of *M. molleriana* to be incorrectly measured, and that the two species are conspecific, with *M. nubilosa* being a synonym of the earlier described *M. molleriana*.

Mycosphaerella molleriana (Thüm.) Lindau was first described as *Sphaerella molleriana* Thüm. from *Eucalyptus globulus* Labill. leaves in Portugal (von Thümen, 1881). Subsequently, a fungus identified as *M. molleriana* was found to cause serious defoliation of eucalypts in South Africa (Doidge, 1950; Doidge *et al*., 1953). More recently, *Mycosphaerella* leaf blotch was recognized as a serious disease of *E. nitens* (Deane et Maid.) Maid. and *E. globulus* Labill. in the Eastern Cape and Eastern Transvaal (Lundquist, 1985). The cause of this disease was also attributed to *M. molleriana*. Lundquist & Purnell (1987) later ascribed the severe leaf disease of *E. nitens* to *M. nubilosa* (Cke.) Hansf., and made no further reference to *M. molleriana*. This study was therefore undertaken to determine the correct identity of the fungal species responsible for *Mycosphaerella* leaf blotch in South Africa.

MATERIALS AND METHODS

Collections of many leaves displaying *Mycosphaerella* leaf blotch were made during extensive surveys of local eucalypt plantations between 1986 and 1988. The collections were lodged at the National Collection of Fungi, Pretoria (PREM 49381-49410). This material was examined in detail along with all previous collections of *Mycosphaerella* on *Eucalyptus* leaves filed at PREM. In addition, type material of *M. molleriana* and *M. nubilosa* were compared with the South African collections.

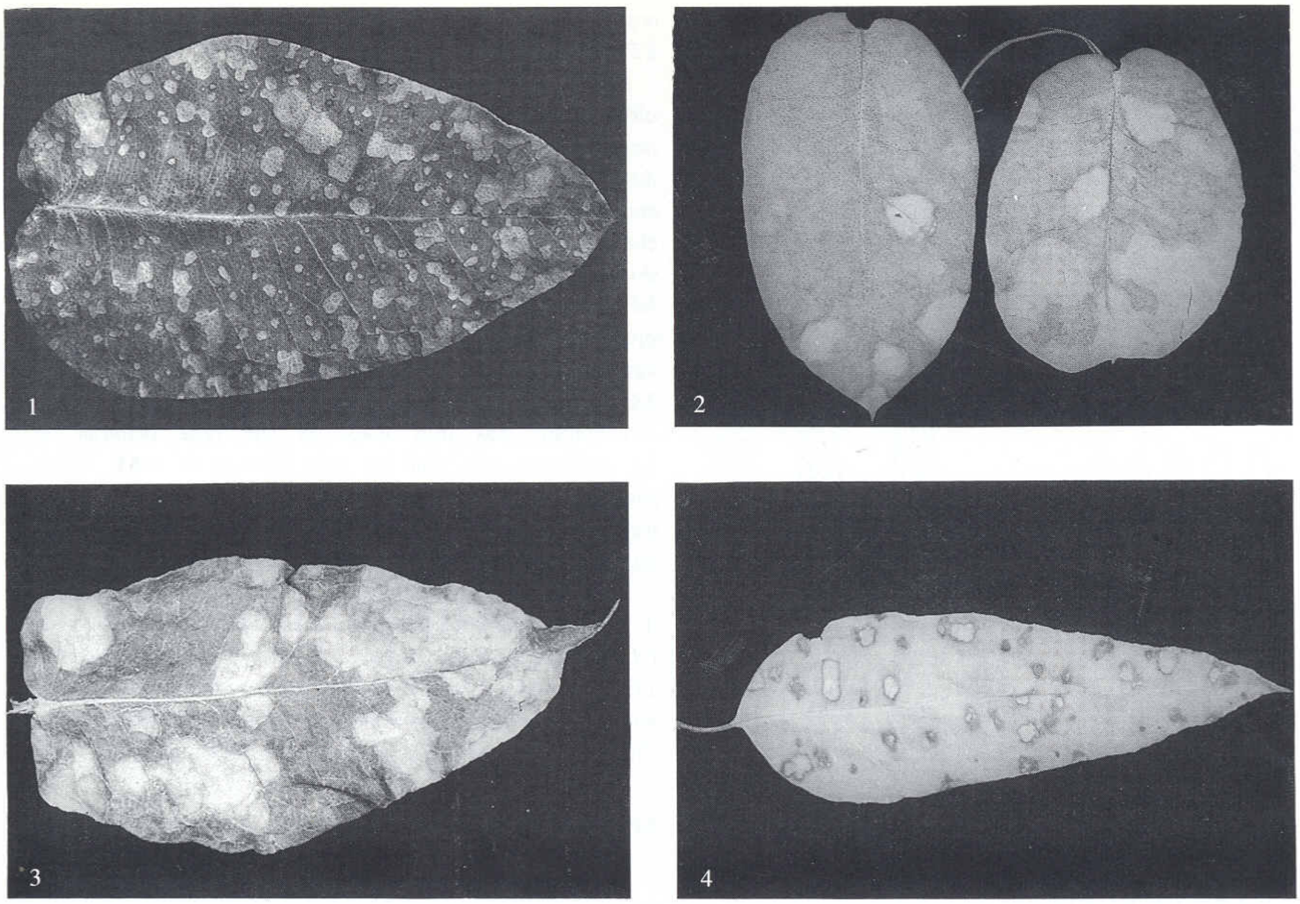
Single-ascospore isolations were made from freshly collected samples on malt-extract agar (10 g Merck malt extract, 20 g Merck agar, 1000 ml H_2O) (MEA). Initially, lesions were cut from freshly collected leaves, soaked in water for 1 h, blotted dry, and attached to the inside lids of the MEA plates. After 24 h at room temperature, lids were replaced and the plates incubated for a further 2 d in the dark at 25 °C. Germinating ascospores were then transferred to fresh MEA plates and incubated for 3 wks at 25° with 12 h dark/light under mixed near-ultraviolet and fluorescent lights.

Ascospore germination was studied by ejecting ascospores from freshly collected *E. nitens* leaves as described above. The MEA plates were incubated for a further 3 d in the dark at 25°, after which ascospores germinating on the agar surface were stained with lactophenol-cotton blue, covered with a glass coverslip and examined microscopically.

RESULTS AND DISCUSSION

Symptoms caused by the *Mycosphaerella* sp. in South Africa varied on different hosts. On the more susceptible species such as *E. nitens* and *E. globulus*, the fungus caused large spreading lesions (Figs 1–3) similar to those reported by Dick (1982) and Park & Keane (1982*a*). On *E. grandis* Hill: Maid., leaf spots were more discrete (Fig. 4), but on *E. grandis* × *nitens* hybrids, spots were generally larger. On species such as *E. macarthurii* Deane et Maid., *E. nova-anglica* Deane et Maid. and *E. smithii* R. T. Bak. spots were more regular and rounded. The extreme variation in symptom expression between and within host species indicated that this could not be used as a reliable taxonomic criterion. This is supported by the findings of a study by Park (1988), in which it was shown that symptoms caused by *M. nubilosa* on *E. globulus* varied from specks to large blight depending upon leaf age at the time of infection.

The dimensions of pseudothecia, asci and ascospores (Figs 5–7) from local *Mycosphaerella* collections varied between and within the different host species at different localities, but most closely fitted those ascribed to *M. nubilosa* (Table 1). Pseudothecia frequently occurred on the upper and lower leaf surfaces, which contrasts with the reports of Hansford (1956) who refers to those of *M. nubilosa* as occurring only on the lower surfaces. Park & Keane (1982*b*) also stated that the pseudothecia of *M. nubilosa* were hypophyllous, but considered that the distribution of pseudothecia may have been related to leaf anatomy. Ascospores were usually straight and tapered towards one of the rounded ends (Fig. 6). On most



Figs 1–4. Variation in symptom expression on *Eucalyptus* leaves. **Fig. 1.** Small fleck-like lesions dispersed over *E. nitens* leaf. **Figs 2, 3.** Confluent lesions on *E. nitens* resulting in twisting of leaf lamina. **Fig. 4.** *E. grandis* leaf with fleck-like lesions with purple borders.

Table 1. Comparison of pseudothecia, asci and ascospores of *M. molleriana*

	Measurements (μm)			
Species	Pseudothecia	Asci	Ascospores	Author
Sphaerella nubilosa	40–60	—	16 × 3	Cooke (1892)
Mycosphaerella nubilosa	100–156	50 × 18	12–14 × 2·5–3	Hansford (1956)
	up to 150	40–50 × 16–20	12–17 × 2·5 × 4·5	Dick (1982)
	101–148	42–57 × 8–15	11–16 × 3–4	Park & Keane (1982)
Mycosphaerella molleriana	—	30–40 × 12–15 (mm)	7–9 × 2·5 (mm)	von Thümen (1881)
	40–100	33–50 × 12–18	16–20 × 3–4	Type material
	163–248	34–43 × 6–7	10–20·5 × 3·4–4	Verwoerd & du Plessis (1931)
	75–90	30–40 × 12–15	10–13 × 2–3	Doidge (1948)
	40–150	30–68 × 9–18	9–20 × 2·5–4·5	Present study

hosts they showed no constriction at the septum, but in some cases asci contained a few ascospores slightly constricted at the septum. Some spores also tapered to both ends, although the majority tapered only to one end.

Ascospores from all South African *E. nitens* collections tested germinated in a manner typical of *M. nubilosa* (Park & Keane, 1982*a*) with germ-tubes growing parallel to the long axis of the spore. This removed any doubt that we were not dealing with the closely related *M. cryptica* (Cke.) Hansf., which germinates with tubes at right angles to the long axis of the spore (Park & Keane, 1982*a*). In addition, cultures were sterile, and did not produce the anamorph stage *Colletogloeum nubilosum* Ganapathi & Corbin of *M. cryptica* (Park & Keane, 1982*a*, 1984). Cooke & Harkness (1881) refer to the Coelomycete *Sphaeropsis mollerianum* Thüm., which was described from dead leaves of *Eucalyptus*. Although the name *S. mollerianum* suggests a possible anamorph of *M. molleriana*, no relationship was found in this study. We therefore accept that no anamorph has yet been found or described for species identified either as *M. nubilosa* or *M. molleriana*.

The present study leaves no doubt that only one species of *Mycosphaerella* is responsible for leaf blotch of *Eucalyptus* in South Africa, and that this species is the fungus currently known as *M. nubilosa*. The fact that the *Mycosphaerella* occurring on *Eucalyptus* leaves locally was known as *M. molleriana* for many years (Doidge, 1950; Doidge *et al.*,