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BRIEF ARTICLE

MYCOSPHAERELLA SUBEROSA ASSOCIATED WITH CORKY LEAF SPOTS ON *EUCALYPTUS* IN BRAZIL

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Species of *Mycosphaerella* Johnson are regarded as amongst the most important pathogens of *Eucalyptus* L'Hériter leaves worldwide (Dick, **1982**; Crous and Wingfield, **1991**). They are of particular concern in parts of the world where eucalypts are grown intensively in plantations often generated from cuttings (Lundquist and Purnell, **1987**; Crous and Wingfield, **1991**). This situation is true in Brazil where numerous species have been recorded both on eucalypts and on related myrtaceous hosts (Ferreira, **1989**; Corlett, **1991**).

Ferreira (**1989**) reported a species of *Mycosphaerella* to be associated with corky leaf spots (CLS) of *Eucalyptus* spp. in Brazil. In a separate study (Crous et al., **1993**), a new small-spored species of *Mycosphaerella, M. parkii* was described from small, circular, light brown leaf spots on *Eucalyptus grandis* Hill *ex* Maid. in plantations in the state of Espirito Santo. The symptoms associated with *M. parkii* were distinct from the CLS symptoms referred to by Ferreira (**1989**). Although reference was made to the *Mycosphaerella* sp. associated with the CLS disease (Crous et al., **1993**), ascospore morphology was found to be similar to *M. molleriana* and *M. delegatensis* (Park and Keane, **1984**; Crous et al., **1991**). It was thus felt that ascospore germination and cultural studies were required in order to sufficiently distinguish this material from other species (Crous et al., **1993**). The aim of the present study was to characterize the species of *Mycosphaerella* associated with CLS. It was compared with other known species of *Mycosphaerella* occurring on *Eucalyptus* and determined to be an undescribed species of *Mycosphaerella*.

Leaves of *Eucalyptus* spp. exhibiting CLS disease were collected at various locations in the Provinces Espirito Santo, Minas Gerais and Bahia. Lesions were excised, and single-ascospore isolations made on 2% malt extract agar (Biolab) (MEA), using the method described by Crous et al. (**1991**). Cultures were incubated on MEA and carnation-leaf agar (CLA) (Crous et al., **1992**) at 25 C under near ultraviolet light.

On leaf material pseudothecia varied from superficial to subepidermal with papillate ostioles. Ascospores were 10–17 × 3–6 μm, similar to those of *M. delegatensis* and *M. molleriana* (Table I). Ascospores could, however, be distinguished by being slightly wider than those of *M. molleriana*, and shorter than those of *M. delegatensis* (Table I). Ascospores were also found to darken during germination, and not germinate in a manner characteristic for either *M. delegatensis* or *M. molleriana* (Park and Keane, **1984**; Crous et al., **1991**).

Ascospores that become dematiaceous during germination have been reported for only one *Mycosphaerella* sp. occurring on *Eucalyptus* leaves, namely *M. parva*. Furthermore, the latter is a small-spored species which germinates via straight germ tubes (Park and Keane, **1982**). The association with corky leaf spots, ascospores that

TABLE I

DIMENSIONS OF ASCOMATA, ASCI AND ASCOSPORES OF *MYCOSPHAERELLA* SPP. OCCURRING ON *EUCALYPTUS*

	Dimensions (μm)					
Species	Ascomata (diam)	Asci, μm (length × width)	Ascospores, μm (length × width)	Ascospore germination	Anamorph	References
M. cryptica (Cke) Hansf.	75–117	36–57 × 8–13	9–13 × 2–4	Perpendicular to spore	*Colletogloeum nubilosum* Ganapathi & Corbin	Park and Keane, 1982
M. delegatensis Park & Keane	70–112	36–69 × 12–19	16–25 × 3–5	Single germ tube[b]	*Stagonospora delegatensis* Park & Keane	Park and Keane, 1984
M. didymelloides Petrak	80–150	38–50 × 7–8	7.5–10 × 3–3.5	—	—	Corlett, 1991
M. eucalypti (Wakef.) Hansf.	150	100 × 24	45–50 × 6	—	—	Hansford, 1957
M. heimii Bouriquet	64	42 × 9	10 × 2.5	—	—	Bouriquet, 1946
M. martinae Hansf.	100	40–50 × 10–12	11–13 × 2.5–3	—	—	Hansford, 1956
M. molleriana (Thüm.) Lindau	40–150	30–68 × 9–18	9–20 × 2.5–4.5	Parallel to spore	—	Crous et al., 1991
M. molleriana var. *megalospora* da Camara	—	50–60 × 18–20	20–25 × 6–8	—	—	Corlett, 1991
M. parkii Crous, Wingfield, Ferreira & Alfenas	90	22–40 × 6–11	8–14 × 2.5–3	Parallel to spore	—	Crous et al., 1992
M. parva Park & Keane	42–91	29–48 × 6–13	7–10 × 1–3	Straight germ tubes[b]	—	Park and Keane, 1982
M. suberosa[a]	100–145	35–60 × 10–14	10–17 × 3–6	Multiple germ tubes[b]	—	Present study
M. swartii Park & Keane	88–177	47–78 × 13–17	20–27 × 4–6	—	*Sonderhenia eucalyptorum* (Hansf.) Swart & Walker	Park and Keane, 1984; Swart and Walker, 1988
M. walkeri Park & Keane	96–157	47–81 × 13–17	20–27 × 4–6	—	*Sonderhenia eucalypticola* (Hansf.) Swart & Walker	Swart and Walker, 1988

[a] A new species.

[b] Ascospores darken at germination.

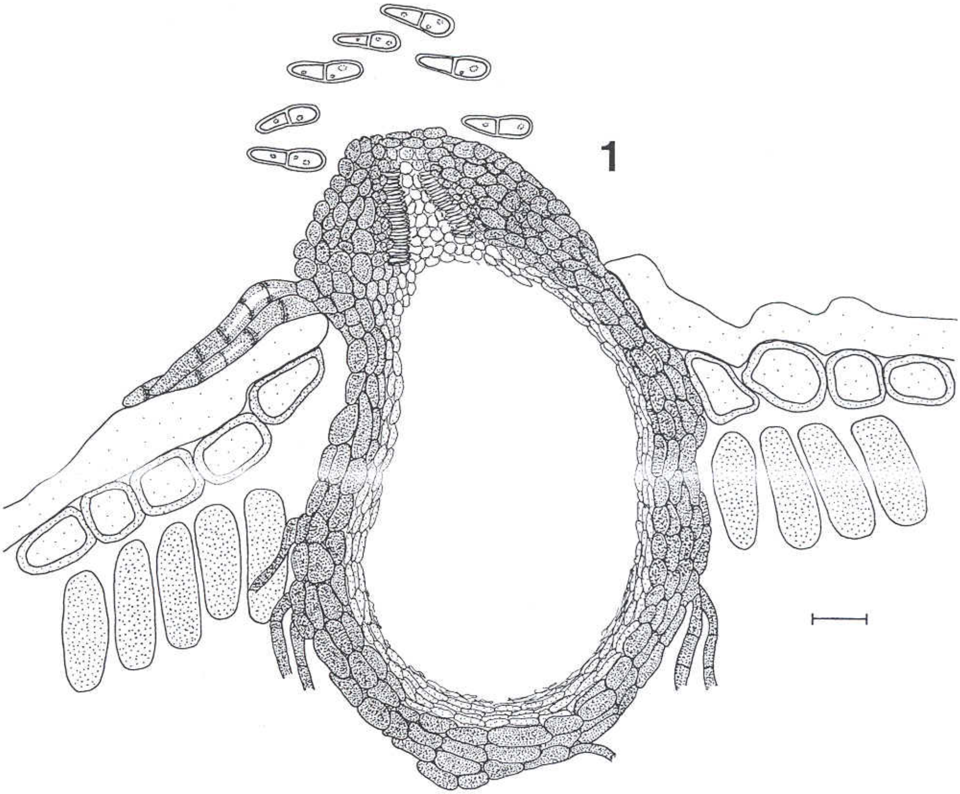

FIG. 1. Vertical section through an erumpent, subepidermal pseudothecium of *M. suberosa* with ascospores. Bar = 10 μm.

are hyaline to light brown in color, that darken and produce multiple germ tubes at germination, as well as ascospore dimensions and subepidermal to superficial, erumpent pseudothecia suggests that the present collection represents a new species of *Mycosphaerella* which is described as follows:

Mycosphaerella suberosa Crous, Ferreira, Alfenas *et* Wingfield, *sp. nov.* FIGS. 1–6

Laesiones amphigenae, 0.5–1.5 cm diam, mediobrunneae vel fuscae, suberosae marginibus irregularibus, saepe cinctae rubro-purpureis marginibus in superioribus et levioribus superficiebus laesionum. Pseudothecia praecipue hypophylla, solitaria vel aggregata, nigra, superficialia vel subepidermalia, globosa, glabra, 100–145 μm lata, 130 μm alta; ostiolum apicale papillatum usque ad 30 μm diam; paries constans ex cellis medio-brunneis, 3–4 stata texturae angularis, subhymenium constans ex 3–5 stratis cellarum hyalinarum. Asci in fasciculati, bitunicati, aparaphysati, subsessiles, octospori, ellipsoidei vel obclavati, recti vel curvati, 20–38 per pseudothecium, 35–60 × 10–14 μm. Ascosporae 2–3 seriatae vel irregulariter dispositae, obliquae, superpositae, recte ellipsoideae, obtusae ad utrumque finem, hyalinae vel spadiceae, leves, uniseptatae, guttulatae, exigue constrictae ad medium septum, latissimae in cella superiore media, attenuatae prominentius ad finem alterum quam ad alterum, 10–17 × 3–6 ($\bar{x}$ = 14 × 4.5) μm. Nullum anamorphum evenit aut in textura hospitus aut in cultura.

SPECIMEN TYPICUM in foliis vivis *Eucalypti dunnii* Maid., Santa Catarina, Espirito Santo, Brazil, Aug. 1992, M. J. Wingfield, HOLOTYPUS, PREM 51082.

ETYMOLOGY. *Suberosus* = corky, referring to the characteristic leaf spots.

Lesions amphigenous, 0.5–1.5 cm diam, medium to dark brown, corky with irregular margins, frequently surrounded by a red-purple border on the upper, smoother lesion surface. Pseudothecia predominantly hypophyllous, single or aggregated, black, superficial to subepidermal (FIGS. 1, 2), globose, glabrous, 100–145 μm wide, 130 μm high; apical papillate ostiole up to 30 μm diam; wall consisting of medium brown cells, three to four layers of *textura an-*

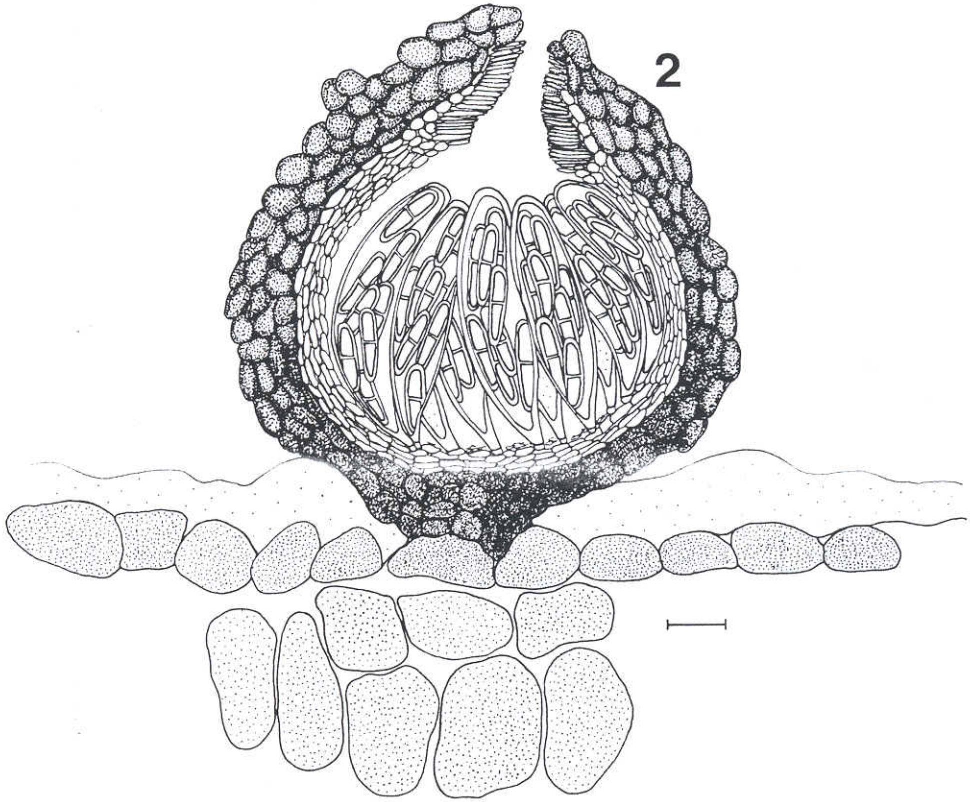

FIG. 2. Vertical section through a superficial pseudothecium of *M. suberosa* with asci and ascospores. Bar = 10 μm.

gularis, subhymenium consisting of three to five layers of hyaline cells. Asci in fasicles, bitunicate, aparaphysate, subsessile, eight-spored, ellipsoid to obclavate, straight or curved, 20–38 per pseudothecium, 35–60 × 10–14 μm (FIGS. 3, 6). Ascospores two to three seriate or irregularly arranged, oblique, overlapping, straight ellipsoidal, obtuse at each end, hyaline to light brown, smooth, 1-septate, guttulate, slightly constricted at the median septum, widest in the middle of the upper cell, tapering more prominently at one end than the other, 10–17 × 3–6 ($\bar{x} = 14 \times 4.5$) μm (FIGS. 4, 6). Ascospores germinated after 24 h on MEA, becoming darkly pigmented, and having several germ tubes emanating from each of the two cells (FIG. 5). Germ tubes formed randomly from the ascospores, were extensively branched, and frequently dichotomously branched at their growing apices. Resulting colonies grew slowly (less than 5 mm diam in 1 month), were dull black in color, hard in texture, with almost no aerial mycelium. Cultures remained sterile on MEA, but formed pseudothecia sparsely on CLA after 3–4 months. No anamorph occurred either on the host tissue or in culture.

SPECIMENS EXAMINED. BRAZIL. ESPIRITO SANTO: Santa Catarina, on *E. dunnii,* Aug. 1991, *M. J. Wingfield* (HOLOTYPE, PREM 51082) (*ex* type culture CBS 436.92). BAHIA: Concieçao da Barra, Picadao, on *E. grandis,* 27 Apr. 1992, *A. C. Alfenas* (PREM 51088); Teixeira de Freitas, on *E. grandis,* 12 Apr. 1991, *A. M. Moreira* (PREM 51083). MINAS GERAIS: Santa Barbara, on *E. grandis,* 25 Oct. 1991, *F. A. Ferreira* (PREM 51085); Santa Barbara, on *E. grandis,* 1991, *F. A. Ferreira* (PREM 51087); Dendrologia, Viçosa, on E. molluccana, 11 Dec. 1991, *F. A. Ferreira* (PREM 51086).

Subepidermal pseudothecia of *M. parkii* were also frequently associated with the larger, aggregated, pseudothecia of *M. suberosa* on CLS. Germinating ascospores of *M. parkii* remained hyaline, and germinated with one or two germ tubes,