

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'Hér. 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 Espírito 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 *Myco-*

sphaerella 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 Espírito 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*

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

^a A new species.

^b Ascospores darken at germination.

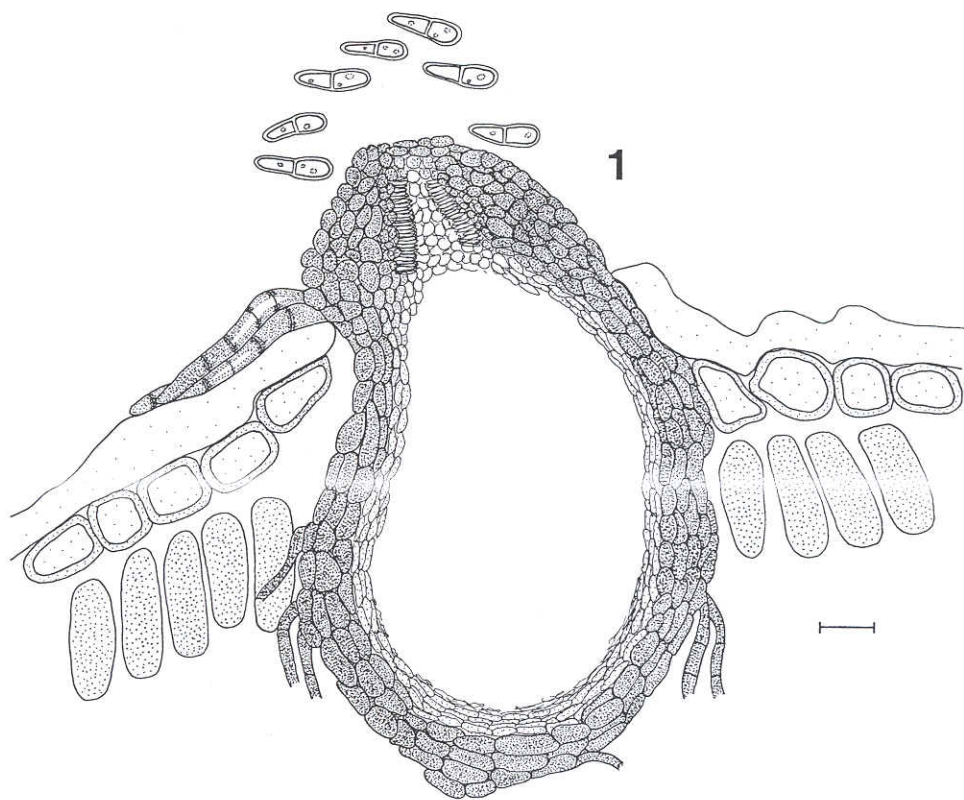


FIG. 1. Vertical section through an erumpent, subepidermal pseudothecium of *M. suberosa* with ascospores. Bar = 10 μm .

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, medio-brunneae 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 stratae texturae angularis, subhymenium constans ex 3–5 stratis cellularum hyalinarum. Asci in fasciculati, bitunicati, paraphysati, subsessiles, octospori, ellipsoidei vel obclavati, recti vel curvati, 20–38 per pseudothecium, 35–60 \times 10–14 μm . Ascospores 2–3 seriatas vel irregulariter

dispositas, obliquas, superpositas, recte ellipsoideas, obtusae ad utrumque finem, hyalinae vel spadiceae, leves, uniseptatae, guttulae, exigue constrictae ad medium septum, latissimae in cella superiore media, attenuatae prominentius ad finem alterum quam ad alterum, 10–17 \times 3–6 (\bar{x} = 14 \times 4.5) μm . Nullum anamorphum evenit aut in textura hospitis aut in cultura.

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

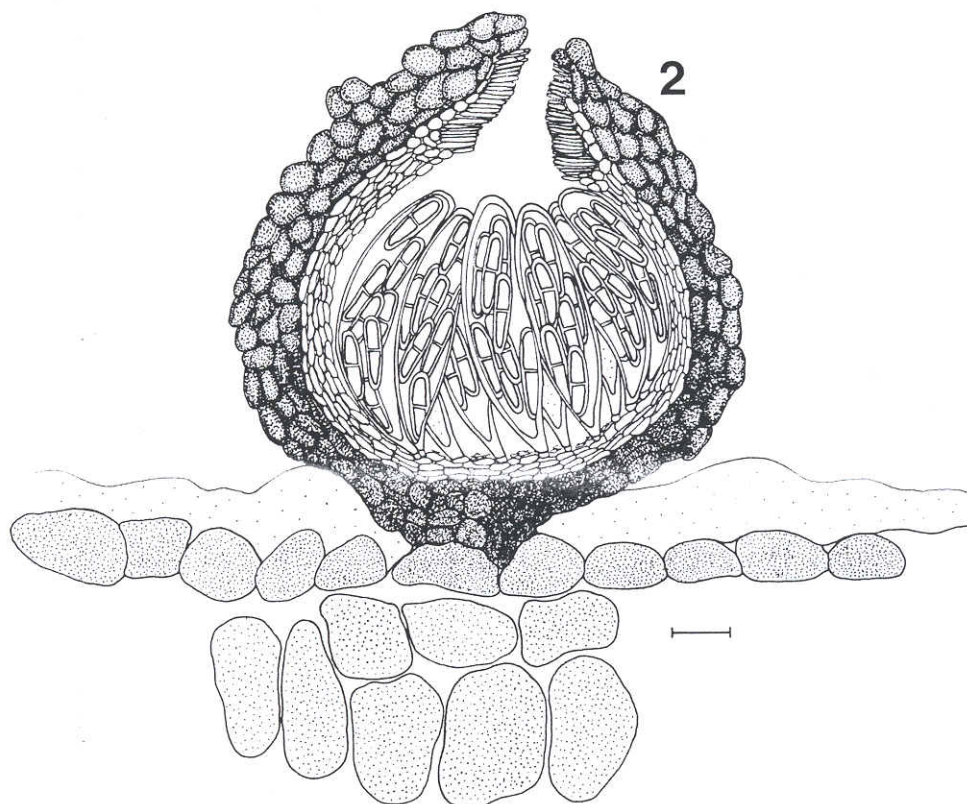


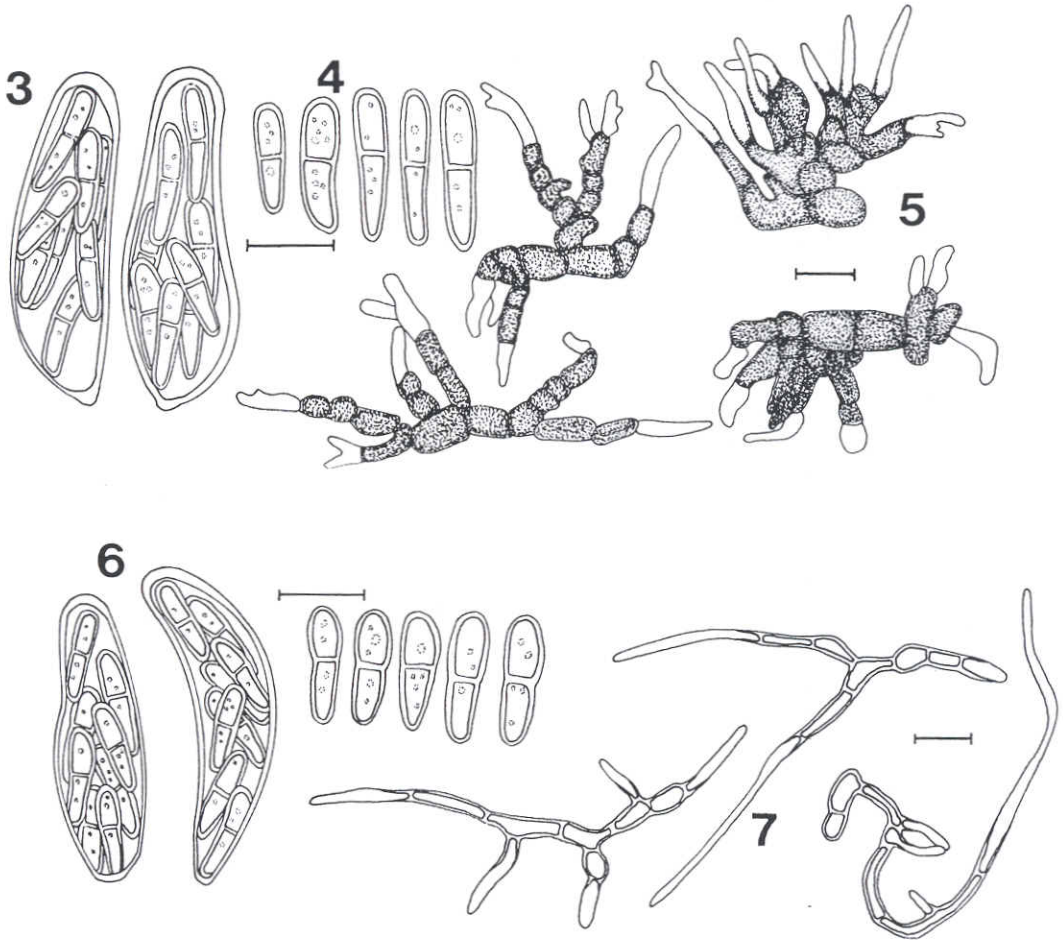
FIG. 2. Vertical section through a superficial pseudothecium of *M. suberosa* with asci and ascospores. Bar = 10 μ m.

gularis, subhymenium consisting of three to five layers of hyaline cells. Asci in fascicles, bitunicate, paraphysate, sessile, eight-spored, ellipsoid to obclavate, straight or curved, 20–38 per pseudothecium, $35\text{--}60 \times 10\text{--}14 \mu\text{m}$ (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, $10\text{--}17 \times 3\text{--}6$ ($\bar{x} = 14 \times 4.5$) 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. ESPÍRITO SANTO: Santa Catarina, on *E. dunnii*, Aug. 1991, *M. J. Wingfield* (HOLOTYPE, PREM 51082) (ex type culture CBS 436.92). BAHIA: Conceição 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,



FIGS. 3–7. Asci and ascospores of *Mycosphaerella* spp. Bars = 10 μ m. 3–6. *Mycosphaerella suberosa*. 3. Ellipsoidal asci. 4. Median septate ascospores. 5. Darkened ascospores germinating with multiple germ tubes (PREM 51082). 6. Asci and ascospores (PREM 51088). 7. Germinating ascospores of *M. parkii*.

primarily parallel to the long axis of the spore (FIG. 7). Germ tubes branched much less than those of *M. suberosa* (FIG. 5). Colonies were fast growing (more than 1.5 cm in 1 month), olivaceous-green in color, had smooth margins, were softer in texture than those of *M. suberosa*, and had a moderate degree of aerial mycelium. Ascospores in specimen PREM 51087 measured $7\text{--}10 \times 2\text{--}3.5 \mu\text{m}$, with asci being $20\text{--}33 \times 8\text{--}10 \mu\text{m}$. Ascospore germination and dimensions, as well as cultural characteristics were consistent with those of *M. parkii* (Crous et al., 1993).

On many of the leaves collected, lesions of *M. suberosa* occurred mixed with those of *M. parkii*. Although subepidermal pseudothecia of *M. parkii* were commonly collected in association with

the subepidermal to superficial pseudothecia of *M. suberosa* on corky lesions, pseudothecia of *M. suberosa* were never observed on the light brown lesions typical of *M. parkii*. Although both species have been isolated separately from prominent leaf spots of *Eucalyptus* spp. in Brazil, little is known about their virulence and possible association on *Eucalyptus* leaves.

Key Words: corky leaf spots, *Eucalyptus*, *Mycosphaerella suberosa*, pathology, systematics

LITERATURE CITED

- Bouriquet, G. 1946. Les maladies des plantes cultivées à Madagascar. *Encycl. Mycol.* 12: 418–419.
Corlett, M. 1991. An annotated list of the published

- names in *Mycosphaerella* and *Sphaerella*. *Mycol. Mem.* 18: 1-328.
- Crous, P. W., A. J. L. Phillips, and M. J. Wingfield. 1992. Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. *Mycologia* 84: 497-504.
- , and M. J. Wingfield. 1991. *Eucalyptus* leaf pathogens in South Africa: a national perspective. Pp. 749-759. In: *Proceedings of the IUFRO symposium for intensive forestry. The role of eucalypts*. Ed., A. P. G. Shönau. Southern African Institute of Forestry, Pretoria, South Africa.
- , ———, F. A. Ferreira, and A. Alfenas. 1993. *Mycosphaerella parkii* and *Phyllosticta eucalyptorum*, two new species from *Eucalyptus* leaves in Brazil. *Mycol. Res.* (In press)
- , ———, and R. F. Park. 1991. *Mycosphaerella nubilosa*, a synonym of *M. molleriana*. *Mycol. Res.* 95: 628-632.
- Dick, M. 1982. Leaf-inhabiting fungi of eucalypts in New Zealand. *New Zealand Forest Serv. Rep.* 1594: 525-537.
- Ferreira, F. A. 1989. *Patologia florestal. Principais doenças florestais no Brasil*. Sociedade de Investigações Florestais, Viçosa, Brazil. 570 pp.
- Hansford, C. G. 1956. Australian fungi. 3. New species and revisions (cont'd). *Proc. Linn. Soc. New South Wales* 81: 23-51.
- , 1957. Australian fungi. 4. New species and revisions (cont'd). *Proc. Linn. Soc. New South Wales* 82: 209-229.
- Lundquist, J. E., and R. C. Purnell. 1987. Effects of *Mycosphaerella* leaf spots on growth of *Eucalyptus nitens*. *Pl. Dis.* 71: 1025-1029.
- Park, R. F., and P. J. Keane. 1982. Leaf diseases of *Eucalyptus* associated with *Mycosphaerella* species. *Trans. Brit. Mycol. Soc.* 79: 101-115.
- , and ———. 1984. Further *Mycosphaerella* species causing leaf diseases of *Eucalyptus*. *Trans. Brit. Mycol. Soc.* 83: 93-105.
- Swart, H. J., and J. Walker. 1988. Australian leaf-inhabiting fungi. 28. *Hendersonia* on *Eucalyptus*. *Trans. Brit. Mycol. Soc.* 90: 633-641.