Mycosphaerella nubilosa, a synonym of *M. molleriana*

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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.

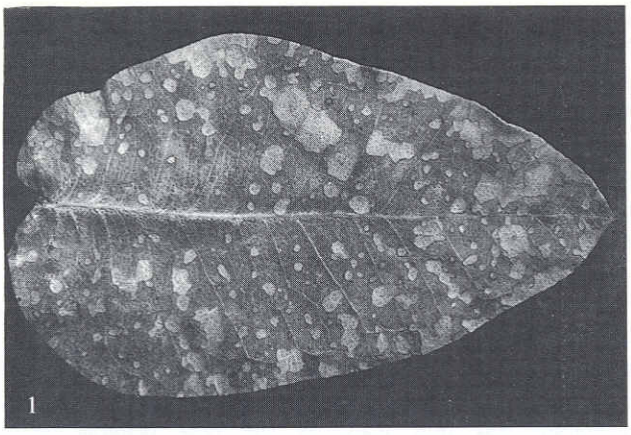
Single-ascospore isolations were made from freshly collected samples on malt-extract agar (10 g Merck malt extract, 20 g Merck agar, 1000 ml H_2O) (MEA). Initially, lesions were cut from freshly collected leaves, soaked in water for 1 h, blotted dry, and attached to the inside lids of the MEA plates. 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.

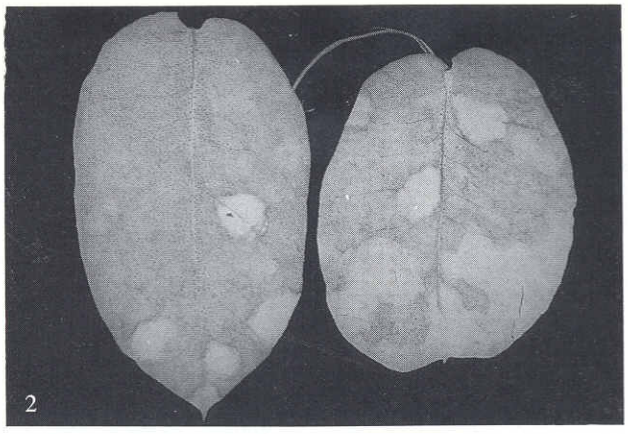
Ascospore germination was studied by ejecting ascospores from freshly collected *E. nitens* leaves as described above. The MEA plates were incubated for a further 3 d in the dark at 25°, after which ascospores germinating on the agar surface were stained with lactophenol-cotton blue, covered with a glass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

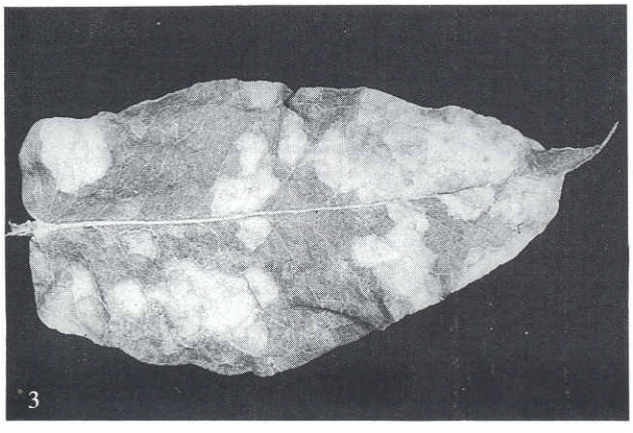
RESULTS AND DISCUSSION

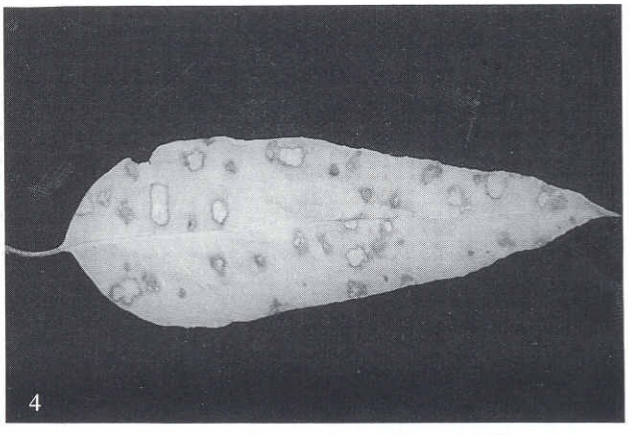
Symptoms caused by the *Mycosphaerella* sp. in South Africa varied on different hosts. On the more susceptible species such as *E. nitens* and *E. globulus*, the fungus caused large spreading lesions (Figs 1–3) similar to those reported by Dick (1982) and Park & Keane (1982*a*). On *E. grandis* Hill: 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.

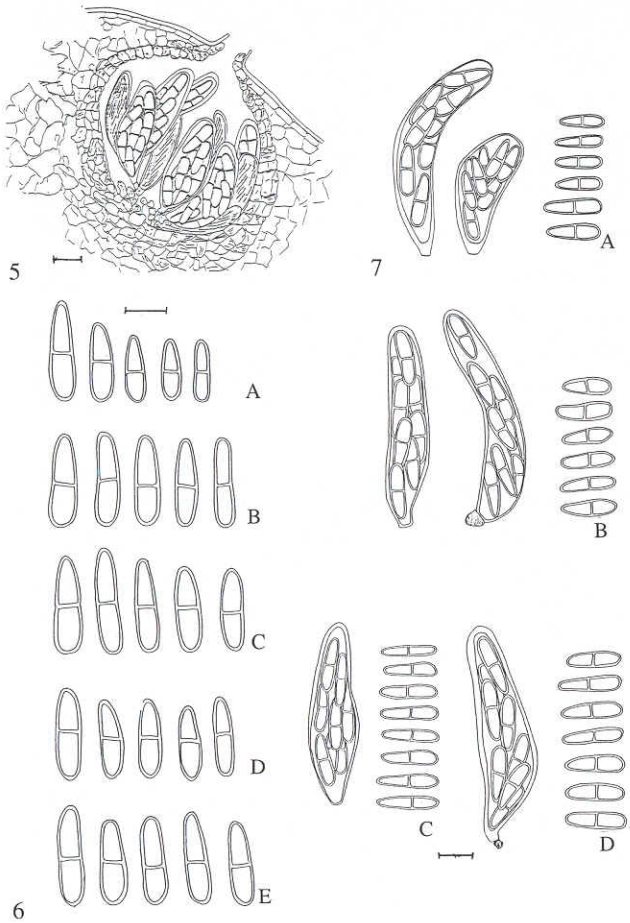
Table 1. Comparison of pseudothecia, asci and ascospores of *M. molleriana*

	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.*,



Figs 5–7. Pseudothecium, asci and ascospores of *M. molleriana*. **Fig. 5.** Transverse section through pseudothecium (BRIP 3094). **Fig. 6**(A–E). Variation in dimensions of ascospores. A, type of *M. nubilosa*, K. 584; B, PREM 4900; C, PREM 49902; D, PREM 49365; E, PREM 13431. **Fig. 7**(A–D). Variation in the dimensions and shape of asci and ascospores. A, PREM 49388; B, BRIP 3092; C, *M. molleriana* type K.; D, BRIP 3094. Bars = 10 µm.

1953; Lundquist, 1985; Lundquist & Baxter, 1985), led us to compare this species with *M. nubilosa*. Saccardo (1913) refers to *Sphaerella molleriana* var. *megalospora* Da Camara, described from *Eucalyptus* leaves in Portugal. The morphological characteristics of this species, with ascospores being 2–3 seriate, claviform, hyaline, barely to not constricted at median septum, and 20–25 × 6–8 µm in size, suggests that it can be a synonym of one of the larger-spored, more recently described *Mycosphaerella* spp. of *Eucalyptus*. Since no type material has been found, further collections are required to redescribe this species adequately, and it will not be further discussed here.

Sphaerella molleriana was described by von Thümen in 1881, and placed in the genus *Mycosphaerella* by Lindau in 1897 (Park & Keane, 1984). The description given by von Thümen (1881) is as follows: *'peritheciis amphigenis sed plerumque hypophyllis, dense gregariis, punctiformibus, conico-prominulis, semi-immersis, nitido-atris, minutis in macula irregularia, arescendo sordide fuscidula, obscuriore anguste cincta; ascis fasciculatis, late clavatis, utrinque angustatis, hyalinis, 30–40 mm long., 12–15 mm crass., subsessilibus; sporis fusoideis, utrinque acutatis, medio septatis, hyalinis, octo, bi-tristichis, 7–9 mm long., 2·5 mm crass.'*

Park & Keane (1984) examined the isotype (and various other collections) of *M. molleriana*, and concluded that it resembled *M. nubilosa* in general morphology. These authors distinguished the two species mainly upon disease symptoms and the protruding ascocarp pores, but indicated that these characters are probably not reliable. This study has clearly shown the extreme variation in symptoms caused by *M. nubilosa* in South Africa, and furthermore, the degree of protrusion of pseudothecia in local collections was also variable. Therefore, we questioned the distinction between *M. nubilosa* and *M. molleriana* on the basis of these criteria. An examination was thus made of the type material of *M. molleriana* (K?), filed by von Thümen in 1881, which subsequently showed that ascospores had been incorrectly measured. Re-measurement of the spores showed that their size and shape agreed with that of *M. nubilosa* (Table 1).

This study has shown that on the basis of available material, it is impossible to distinguish between *M. molleriana* and *M. nubilosa*. Since Cooke (1892) described *Sphaerella nubilosa* 11 years after Von Thümen's (1881) description of *S. molleriana*, the name *Mycosphaerella molleriana* has precedence. The following synonymy is therefore proposed:

Mycosphaerella molleriana (Thüm.) Lindau in Engler & Prantl., *Die Nat. Pflanzenfam.* **1**: 424 (1897).
= *Sphaerella molleriana* Thüm., *Instituto Coimbra* **28**: 31 (1881).
= *M. nubilosa* (Cke.) Hansford, *Proc. Linn. Soc. N.S.W.* **81**: 36 (1965).
= *Sphaerella nubilosa* Cooke, *Grevillea* **19**: 61 (1892).

The following amendment to the description of *M. molleriana* is provided to include observations and measurements made in this study.

Lesions vary from being pin spots or flecks to small round or irregular spots that coalesce to form larger leaf blotches, pale brown in colour, surrounded by a thin brown line, forming a ridge in some cases; *Pseudothecia* amphigenous, predominantly hypophyllous, black, in dense clusters, punctiform, globous, glabrous, immersed with only the apical pore penetrating the epidermis, to more erumpent and protruding, 40–150 µm diam. (Fig. 5); *Asci* aparaphysate, in a basal rosette, ellipsoidal to subsaccate, straight or narrowed at the top, subsessile, 8-spored, rounded and slightly thickened at the apex, 30–68 × 9–18 µm (Fig. 7a–d); *Ascospores* 2–3 seriate, oblique, overlapping, ellipsoidal with rounded ends, usually straight in the ascus, hyaline, smooth, 1-septate, not constricted to very slightly constricted, widest at septum or in middle of the one cell, tapering more prominent to the one end than the other, 9–20 × 2·5–4·5 µm (Fig. 6a–e).

Specimens examined:
M. nubilosa
BRIP file: on *E. maidenii* F. Muell., Chilanga, Zimbabwe, Mar. 1960, A. Angus, BRIP? ex IMI 85693; Mumbwa, Zimbabwe, Apr. 1963, J. C. Hoyle, BRIP 3092 ex IMI 110432; *Eucalyptus* sp., Liston, N.S.W., Aust. Apr. 1981, J. B. Heaton, BRIP 13431.
DAR file: on *E. goniocalyx* (?) F. Muell. ex. Miq., Brown Mountain (N.S.W.?), Apr. 1959, L. Fraser, DAR 4962; *E. globulus*, East Lindfield

(N.S.W.?), Oct. 1958, L. Fraser, DAR 4965; *E. globulus*, Penrith (N.S.W.), Sept. 1963, D. Morton, DAR 12122; *Eucalyptus* sp., W. Lindfield (N.S.W.), Mar. 1964, H. Phipps, DAR 12810; *E. globulus*, Windsor (N.S.W.?), May 1966, J. G. Stone, DAR 15422; *E. globulus*, Sylvania (N.S.W.?), Mar. 1967, A. L. Bertus, DAR 16258; *E. bicostata* Maid. *et al.*, Castle Hill (N.S.W.?), Mar. 1971, Castle Hill Nursery, DAR 21836; *E. bicostata*, Bilpin (N.S.W.), June 1971, O. M. Williams, DAR 22071; *E. globulus*, Glenhaven (N.S.W.?), Mar. 1973, A. L. Bertus, DAR 24131; *E. cladocalyx* (?) F. Muell., Mosman (N.S.W.?), Feb. 1974, K. Lewis, DAR 24181; *E. cladocalyx* (?), (N.S.W.?), Feb. 1974, L. Cornell, DAR 24206; *E. stjohnii*, R. T. Bak., Gymea bay (?), May 1974, R. O. Avery, DAR 24289; *E. globulus*, Glenhaven (N.S.W.?), July 1974, A. L. Bertus, DAR 24291; *E. globulus*, Nova Nova (Vic.), Oct. 1977, P. Fagg, DAR 30251; *E. globulus* subsp. *globulus*, Nowa Nowa (Vic.), Aug. 1979, R. F. Park, DAR 41955.

IMI file: on *E. globulus* subsp. *globulus*, Nova Nova (Vic.), Sept. 1979, R. F. Park, IMI 259398.

K file: on *Eucalyptus* sp., Melbourne (Vic.), 1891, Martin, 584(K), type of *M. nubilosa*; *Eucalyptus* sp., Brisbane, Bailey, Nov. 1911, C. T. White (K?).

PDD, DSIR file: on *E. gigantea* Hook, f., Raurimu nursery, Wellington, Apr. 1955, J. W. Gilmour, PDD 14466; *E. gigantea*, Atiamuri, Auckland, May 1972, J. M. Dingley, PDD 29976.

PREM file: on *E. maidenii*, Berlin, Cape, Jan. 1929, forester, PREM 23664; *E. maidenii*, Jessievale, E. Tvl., Mar. 1931, forester, PREM 26107; *E. globulus*, Cedara, Natal, Feb. 1931, forester, PREM 26117; *E. globulus*, Cedara, Natal, Feb. 1931, forester, PREM 26118; *E. maidenii*, Cedara, Natal, Feb. 1931, forester, PREM 26119; *E. stuartiana*, F. Muell., Cedara, Natal, Feb. 1931, forester, PREM 26120; *E. saligna* Sm., Pietermaritzburg, Natal, Feb. 1931, forester, PREM 26123; *E. tereticornis* Sm., Pietermaritzburg, Natal, Feb. 1931, forester, PREM 26124; *E. maidenii*, Merrivale, Natal, Feb. 1931, forester, PREM 26126; *E. globulus*, Hilton, Natal, Feb. 1931, forester, PREM 26127; *E. globulus*, Hilton, Natal, Feb. 1931, forester, PREM 26128; *E. globulus*, Merrivale, Natal, Feb. 1931, forester, PREM 26129; *E. globulus*, Merrivale, Natal, Feb. 1932, forester, PREM 26130; *E. globulus*, Rietspruit, Natal, Feb. 1931, forester, PREM 26131; *Eucalyptus* sp., Harding, Natal, Jan 1937, C. G. Wilding, PREM 28787; *E. globulus*, Krugersdorp, Tvl., Aug. 1940, forester, PREM 32202; *Eucalyptus* sp., Tzaneen, N. Tvl., Oct. 1963, W. F. O. Marasas, PREM 42650; *E. grandis*, Sabie, E. Tvl., May 1986, M. J. Wingfield, PREM 48997; *E. nitens*, Hogsback, E. Cape, Apr. 1986, M. J. Wingfield, PREM 48998; *E. nitens*, Grabouw, Cape, May 1987, M. J. Wingfield, PREM 48999; *E. maidenii*, Natal Midlands, Apr. 1986, M. J. Wingfield, PREM 49019; *E. nitens*, Natal Midlands, Apr. 1986, M. J. Wingfield, PREM 49020; *E. globulus*, Natal Midlands, Apr. 1986, M. J. Wingfield, PREM 49021; *E. nitens*, Sabie, E. Tvl., Feb. 1988, P. W. Crous, PREM 49381; *E. nitens*, Knysna, S. Cape, Feb. 1988, P. W. Crous, PREM 49382; *E. globulus*, Franschhoek, Cape, Feb. 1988, P. W. Crous, PREM 49383; *E. grandis*, Helvetia, E. Tvl., Mar. 1988, P. W. Crous, PREM 49384; *E. macarthurii*, Helvetia, E. Tvl., Mar. 1988, P. W. Crous, PREM 49385; *E. globulus*, Frankfort, E. Tvl., Mar. 1988, P. W. Crous. PREM 49386; *E. grandis* × *nitens*, White River, E. Tvl., Mar. 1988, P. W. Crous, PREM 49387; *E. nitens*, White River, E. Tvl., Mar. 1988, P. W. Crous, PREM 49388; *E. smithii*, E. Tvl., Mar. 1988, P. W. Crous, PREM 49389; *E. viminalis* Labill., Helvetia, E. Tvl., Mar. 1988, P W. Crous, PREM 49390; *E. bicostata*, Helvetia, E. Tvl., Mar. 1988, P. W. Crous, PREM 49391; *E. grandis*, Sabie, E. Tvl., Apr. 1988, P W. Crous, PREM 49392; *E. saligna*, Helvetia, E. Tvl., Mar. 1988, P. W. Crous, PREM 49393; *E. dalrympleana*, Helvetia, E. Tvl., Mar. 1988, P. W. Crous, PREM 49394; *E. nitens*, Helvetia, E. Tvl., Mar. 1988, P. W. Crous, PREM 49395; *E. viminalis*, Seven Oaks, Natal, July 1986, P. W. Crous, PREM 49396; *E. nitens*, Greenpoint, Natal, July 1986, P. W. Crous, PREM 49397; *E. macarthurii*, Seven Oaks, Natal, July 1986, P. W. Crous, PREM 49398; *E. dalrympleana*, Seven Oaks, Natal, July 1986, P. W. Crous, PREM 49399; *E. nova-anglica*, Seven Oaks, Natal, July 1986, P. W. Crous, PREM 49400; *E. bicostata*, Seven Oaks, Natal, July 1988, P. W. Crous, PREM 49401; *E. globulus*, Seven Oaks, Natal, July 1986, P. W. Crous, PREM 49402; *E. maidenii*, Seven Oaks, Natal, July 1986, P. W. Crous, PREM 49403; *E. globulus*, Stellenbosch, Cape, June 1986, P. W. Crous, PREM 49404; *E. globulus*, Stellenbosch, Cape, Nov. 1987, P. W. Crous, PREM 49405; *E. melliodora*, Stellenbosch, Cape, Nov. 1987, P. W. Crous, PREM 49406; *E. camaldulensis*, Stellenbosch, Cape, Nov. 1987, P. W. Crous, PREM 49407; *E. nitens*, Seven Oaks, Natal, July 1986, P. W. Crous, PREM 49408; *E. maidenii*, Stellenbosch, Cape, Nov. 1987, P. W. Crous, PREM 49409; *E. nitens*, Grabouw, Cape, May 1988, P. W. Crous, PREM 49410; *E. macarthurii*, Kalmoesfontein, E. Tvl., May 1986, J. E. Lundquist, PREM 49893; *E. bicostata*, Bergplaas, E. Tvl., July 1986, A. J. Urban, PREM 49894; *E. nitens*, Edelgesteente, Piet Retief, E. Tvl., Aug. 1984, J. E. Lundquist, PREM 49899; *E. globulus*, (?), Brooklands, E. Tvl., 1984, J. E. Lundquist, PREM 49900; *E. grandis*, Edelgesteente, E. Tvl., Aug. 1984, PREM 49901; *E. grandis*, Berlin, E. Tvl., Aug. 1984, J. E. Lundquist, PREM 49902; *E. maidenii*, Berlin, E. Tvl., Aug. 1984, J. E. Lundquist, PREM 49903; *E. nitens*, De Villiers plt., Amsterdam, E. Tvl., Aug. 1985, J. E. Lundquist, PREM 50442; *Eucalyptus* sp., Berlin, E. Tvl., Aug. 1984, J. E. Lundquist, PREM 50443.

M. molleriana

BRIP file: *E. tereticornis*, Hombrum, T.P.N.G., July, 1956, D. E. Shaw, BRIP 3094 ex IMI 74249.

K file: *E. globulus*, Lusitania, July 1879, Ad. Fr. Moller, K?, type of *M. molleriana*.

Incorrectly identified specimens:

E. cladocalyx var. *nana*, Kenmore, Qld., Dec. 1970, J. L. Alcorn, BRIP 3090 ex IMI 161737 [*M. cryptica*]; *E. tereticornis*, Maroochydore, Dec. 1971, J. L. Alcorn, BRIP 3093 [*M. cryptica*]; *Eucalyptus* sp., Bourke (N.S.W.), Sept. 1971, D. A. Campbell, DAR 44680 [*Clypeophysalospora latitans* (Sacc.) Swart]; *E. gigantea*, Atiamuri, South Auckland, June 1971, J. M. Dingley, PDD 29359 [*M. cryptica*]; *Eucalyptus* sp., Western Australia, May 1916, F. Stowards, K 396 [*Plectosphaera eucalypti* (Cke. & Mass.) Swart]; *Eucalyptus* sp., Nelspruit, E. Tvl., Mar. 1960, H. Schüepp, K (?) [*Harknessia* sp., *Seimatosporium* sp.]; *E. gomphocephala* (?) A. DC., Western Australia, F. Stowards, K 485 [*Seimatosporium* sp.]; *Eucalyptus* sp., Melbourne, Aust. (?) Martin, K 589 [*M. cryptica*]; *E. maculata*, (?) Natal, Feb. 1931, forester, PREM 26121 [*Seimatosporium eucalypti* (Mc Alp.) Swart].

Undetermined species:

E. tessellaris F. Muell., Toorbul, Qld., Mar. 1973, J. L. Alcorn, BRIP 8821; *E. crebra* F. Muell., Mt Coot-tha, (Qld.?) Mar. 1973, J. L. Alcorn, BRIP 8914 [also *Pseudocercospora eucalyptorum* Crous, Wingf., Maras. & Sutton]; *E. regnans* F. Muell., Atiamuri, South Auckland, June 1971, F. J. Newhook, PDD 29360; *E. gigantea*, Atiamuri, South Auckland, May 1972, J. Corbin, PDD 29975; *E. regnans*, Auckland, Jan. 1974, A. N. Ganapathy, PDD 37678; *E. gigantea* (?), Auckland, Jan. 1974, A. N. Ganapathy, PDD 37679; *E. gigantea*, Auckland, Feb. 1977, A. N. Ganapathi, PDD 37680; *E. gigantea*, Auckland, Sept. 1975, C. J. A. Barber, PDD 36303; *E. regnans*, Tokoroa, Matamata (?), Feb. 1973, PDD 36037 [also *Trimmatospora excentricum* Sutton & Ganapathi]; *E. corynocalyx*, Cedara, Natal, Feb. 1931, forester, PREM 26122; *E. globulus*, Pietermaritzburg, Natal, Feb. 1931, forester, PREM 26125; *E. diversicolor*, Kruisfontein, Knysna, Apr. 1944, E. M. Laughton, PREM 34301a.

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Involvement of Endogenous Glycogen in Fruiting of *Volvariella volvacea* and *Pleurotus sajor-caju*

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Intracellular glycogen was extracted and assayed from the fruit bodies of *Volvariella volvacea* and *Pleurotus sajor-caju* at different developmental stages. In both mushrooms, glycogen stored in stipe during the initial primordial stage was consumed for fruiting morphogenesis while glycogen in pileus was first broken down for the differentiation and formation of lamellae but later synthesized and accumulated during hymenial cell differentiation. Glycogen was then packed into basidiospores. As glycogen level in vegetative mycelium was high when fruit bodies emerged in *V. volvacea*, and numerous fruit-body primordia initiated directly on the explanted stipes of *P. sajor-caju* containing high glycogen content, these suggested that the level of endogenous glycogen is correlated with initiation of fruit bodies in *V. volvacea* and *P. sajor-caju* as in *Coprinus cinereus*.

Agarics store various polysaccharides as carbon and nutrient reserve (Manners & Sturgeon, 1981). In *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray, glycogen level is correlated with fruiting morphogenesis and fruiting competence (Jirjis & Moore, 1976; Moore *et al.*, 1979; Chiu & Moore, 1988; Brunt & Moore, 1989). Although *Volvariella volvacea* (Bull.: Fr.) Sing. and *Pleurotus sajor-caju* (Fr.) Sing. have been cultivated for many years, their fruiting mechanisms are not well studied. The present study examines whether glycogen level is correlated with the fruiting morphogenetic pathway of these two organisms.

Volvariella volvacea strain V42-18 and *Pleurotus sajor-caju* strain Pl-27 were supplied by Professor S. T. Chang. They were kept on CM (Raper & Miles, 1958), containing (g/l) glucose, 20; yeast extract, 2; peptone, 2; KH_2PO_4, 0·46; K_2HPO_4, 1; $MgSO_4$, 0·5; agar, 15. CM plates were incubated in darkness at 37 °C for *V. volvacea* and at 25° for *P. sajor-caju*, respectively. A fully colonized plate culture was mixed with 400 g of cotton-waste compost: 39·6% cotton waste, 0·4% $CaCO_3$ and 60·0% water, in an autoclavable bag to make a spawn culture for *V. volvacea* and a fruiting culture for *P. sajor-caju*, respectively. For the former, the fully colonized spawn culture was mixed with a tray of 4 kg of composted, pasteurized cotton waste compost to form the fruiting culture. Incubation of the fruiting cultures was carried out in darkness until the substrate was completely colonized by the mycelium. Ventilation was introduced by electric fans to the fruiting tray of *V. volvacea* and by cutting open the fruiting bags of *P. sajor-caju*. In addition, a photoperiod of 12 h dark/12 h light was introduced at this stage with the incubation temperature lowered to 32° for *V. volvacea* and 22° for *P. sajor-caju*, respectively. Within 3 days, fruit-body primordia were produced. For *V. volvacea*, 26 fruit bodies and 2 samples of vegetative mycelia of the primordial stage (size: 0·2 cm in diam) (the developmental stages are based on criteria described by Li, 1982), 4 fruit bodies and 4 samples of vegetative mycelia of the button stage (size: 0·5 cm in diam), 4 fruit bodies and 5 samples of vegetative mycelia of the elongation

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