**Mycosphaerella species associated with Eucalyptus in south-western Australia: new species, new records and a key**

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*Mycosphaerella ambiphylla* sp. nov. (anamorph: *Phaeophleospora*) and *Mycosphaerella aurantia* sp. nov., are described from diseased *Eucalyptus globulus* leaves. In addition, a new fungal record in Australia, *M. mexicana*, and two new records for Western Australia, *M. gregaria* and *M. parva*, are discussed. A key is provided to *Mycosphaerella* species on *E. globulus* in Western Australia.

**INTRODUCTION**

*Mycosphaerella* leaf blotch is a widespread disease occurring in natural *Eucalyptus* forests and in plantations (Crous 1998). It poses an economic threat to *Eucalyptus* plantations. For example, outbreaks of *Mycosphaerella* leaf blotch led to the cessation of *Eucalyptus globulus* plantings in South Africa in the 1930s (Crous 1998). *Mycosphaerella* leaf blotch causes loss in photosynthetic area and can lead to defoliation, resulting in reduced growth rates and wood volume. It may also cause poor tree form. Defoliation levels of 25% led to reductions in wood volume of *Eucalyptus nitens* infected with *Mycosphaerella* in South Africa (*Lundquist & Purnell* 1987). *Carnegie et al.* (1994) showed that *Mycosphaerella* leaf blotch causes a negative effect on growth rate in *E. globulus* and more recently *Carnegie et al.* (1998) report that levels of diseased leaf area as low as 10% result in a 17% reduction in height of *E. globulus* in plantations.

There are almost 30 species of *Mycosphaerella* associated with diseased eucalypt foliage worldwide (Crous 1998). The origin, distribution, and impact of many of these species are poorly understood. Many are only recorded outside of Australia in *Eucalyptus* plantations established as exotics (Crous 1998) but the origin of these *Mycosphaerella* species is unknown. In Australia, *M. cryptica* and *M. nubilosa* are the most serious disease-causing species (Park & Keane 1982, Carnegie *et al.* 1998, Park 1988a) whereas in southern Africa, *M. juvenis*, which is not recorded elsewhere, is responsible for most disease (Crous 1998). Recent papers have extended the known geographic and host range of some *Mycosphaerella* species associated with diseased *Eucalyptus* foliage (Carnegie, Keane & Podger 1997, Crous *et al.* 1998, Maxwell *et al.* 2000, Maxwell, Hardy & Dell 2001). Further intensive surveys of plantation and native eucalypt forest are required in order to determine the full range and possible origin of *Mycosphaerella* species associated with *Eucalyptus*.

Investigations into *Mycosphaerella* on eucalypts in Australia have emphasised the south-eastern region with little consideration given to the south-western region. The 1994 survey of *Carnegie et al.* (1997) was the first to describe species of *Mycosphaerella* associated with disease on *Eucalyptus* in Western Australia (WA). They identified *M. cryptica* associated with *E. globulus*, *E. marginata* and *E. patens*; and *M. marksii* and *M. suberosa* associated with *E. globulus*. The former two fungi are common in eastern Australia and *M. suberosa* was previously known only from *Eucalyptus* in Brazil, Colombia (*Crous et al.* 1993, Crous 1998) and Indonesia (*Crous & Wingfield* 1997). More recently, Maxwell *et al.* (2000, 2001) reported *M. lateralis* and *M. nubilosa* from *E. globulus* during preliminary surveys of plantations in WA. This extension in the known range of *Mycosphaerella* species underscores the need for a comprehensive survey of *Mycosphaerella* on *Eucalyptus* plantations in WA.

During the period March to June 2000, the *E. globulus* estate in south-western Australia was systematically sampled for leaf pathogens. The results of this survey for *Mycosphaerella* are reported here.

**MATERIALS AND METHODS**

**Collection of samples**

Diseased *Eucalyptus globulus* leaf material was collected from 30 plantations from Esperance in the south-east...
of WA to Albany in the south, inland to Manjimup and north to Bunbury (Fig. 1). Single ascospore isolations were made from lesions on 50 leaves selected randomly from each of two 100 m transects at opposite ends of each plantation. Fungi were isolated, cultured and identified.

Species identification

Ascospores were discharged from mature lesions as described by Crous (1998), except that the Petri-dishes with adherent lesions were inverted in order to favour the attachment of actively discharged spores to the agar. Ascospore germination patterns were measured, drawn and recorded after 24–48 h of incubation at 20 °C, from a piece of agar that had been transferred to a slide and viewed under an Olympus BH2 light microscope.

Under a dissecting microscope (×70), single ascospore germinants were transferred to 90 mm Petri-dishes containing 20 ml of 2% Difco Malt Extract Agar (MEA) and maintained in pure culture. These plates containing 20 ml of 2% Difco Malt Extract Agar spore germinants were transferred to 90 mm Petri-dishes Leaf Agar (CLA; Fisher et al. from each species were sub-cultured onto Carnation fruiting structures. In addition, representative isolates were made from cultures on MEA and CLA after 4, 8 and 12 wk growth under nuv, mounted under acidified glycerol blue (0.05% aniline blue (Gurr) in 50% glycerol blue (0.05% aniline blue (Gurr) in 50% acidified (0.1 % HCl) glycerol) and investigated under an Olympus BH2 light microscope for the formation of anamorph states. Other features recorded include the formation of pigment and crystals in the agar.

Leaf symptoms were recorded. Ascomata were described from squash mounts and hand sections of lesions from which ascospores had recently discharged. In order to relate germination patterns to ascoma characteristics, hand-sections were made from the area of lesion corresponding to that below the spores on the Petri-plate. Sectioned ascomata were mounted, stained with acidified glycerol blue and investigated under an Olympus BH2 light microscope on normal or phase contrast settings (×100–1000). Thirty measurements were made of ascus, ascospore and conidium dimensions under phase-contrast. From this, the 95% confidence intervals were calculated and are presented with extremes in parentheses. All drawings were made with the aid of an Olympus drawing tube.

DNA extraction

Myelia for DNA extraction were freeze-dried, then ground to a fine powder under liquid nitrogen. Freeze-dried mycelia-powder was added to the 0.2 ml mark of a 1.5 ml microcentrifuge tube. Then 300 µl of extraction buffer (Raeder & Broda 1985) was added and mixed with the homogenate by gentle inversion. This solution was incubated at 65 °C for 2 h and then centrifuged by a Beckman Microfuge at 13 200 g for 10 min. The resulting supernatant was transferred into sterile 1.5 ml microcentrifuge tubes and the DNA purified by a silica binding method (Bresa-Clean™) according to the manufacturer’s recommendations.

Sequencing ITS region of ribosomal DNA

The ITS region of the ribosomal DNA was amplified using the ITS 1 and ITS 4 primers described in White et al. (1990). All PCR reactions were performed aseptically in 50 µl volumes containing 2 ng genomic DNA, 0.3 µM primer, 2 mM MgCl₂ (Biotech International; Perth, Australia), 1.1 U Tth polymerase (Biotech International), polymerisation buffer (Biotech International). Thermal cycle reactions were performed on the GeneAmp PCR system according to the following conditions. An initial denaturation step of 94 °C for 10 min, followed by 30 cycles of: denaturation at 94 °C for 30 s; annealing at 53 °C for 30 s; and extension at 72 °C for 2 min. This was followed by a 7 min extension cycle at 72 °C.

The PCR products were cleaned using the Bresa-Clean™ method. Forward and reverse primers (ITS 1 and 4; White et al. 1990) were used to sequence double stranded ITS fragments with the ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit according to the manufacturer’s instructions. Sequences were edited and aligned using SeqEd™ (v1.0.3, Applied Biosystems Inc.), and these compared with those

![Fig. 1. Location of Eucalyptus globulus plantations (*) surveyed for Mycosphaerella species in south-western Australia.](image-url)
on the GenBank® database through a BLAST search. Sequences were submitted to GenBank® and those accession numbers are recorded here.

**TAXONOMY**

The ITS sequences of the two new species described below do not match any sequence lodged with GenBank® as of October 2002.

**Mycosphaerella aurantia** A. Maxwell, sp. nov.  
(Figs 2–8, 14–15)

*Etym.: aurantius*, Latin for orange-coloured (Stearn 1973), named for the orange-grey colouring of the culture surface on 2% MEA.

Laesiones amphibigenae, bruneae, semi-circulares, 1–8 mm diam. Ascomata amphigena, dispersa, nigra, globosa, 87–105 × 83–102 μm, ostiolata; parietes brunei, e 3–5 stratis texturae angularis compositi. Asci bitunicati, fasciculati, obovoidi ad ellipsoidei, recti ad incurvati, 8-sporei, (22–)30–49(–85) × (8–)11–13(–16) μm. Ascosporae bi- vel triseriatae, imbri-catae, hyalinae, guttulatae, fusiformi-ellipsoideae extremitatibus rotundatis, non constrictae, per medium 1-septatae, ad extremitatem basalem angustatae, (9–)11–12(–15) × 2–2.5 (–3) μm. Ascosporae germinatio ab extremitatibus ambobus ad axem longum sporiae parallela; ascosporae hyalinae sed post 24 horas parum constrictae et subtiliter verruculosae, tum post 36 horas ramulos laterales facientes. Culturae post octo hebdomadum in 2% MEA ad 25 °C in tenebris, pagina supera brunneo-aaurantia, 7C6 (1.5YR: 5.7: 6.7), infera cinereo-brunea, 7F3 (5R: 3.3: 0.9). Crystalla rufa in agario crescentes. Velocitas incrementi post mensam unam sub 25 °C 16–24 mm. Anamorphasum non visum.

Figs 2–13. *Mycosphaerella aurantia* (holotype). Fig. 2. Lesion on adaxial surface of leaf. Fig. 3. Lesion on abaxial surface of leaf. Fig. 4. Distribution of ascomata on lesion. Fig. 5. Surface of culture on MEA showing distinctive apricot colour formed after 8 wk. Fig. 6. Reverse surface of culture on MEA. Figs 7–13. *Mycosphaerella ambiphylla* (holotype). Fig. 7. Adaxial surface of juvenile leaf showing corky appearance of lesions. Fig. 8. Abaxial surface of juvenile leaf showing lesion form. Fig. 9. Adaxial surface of adult leaf showing corky appearance of lesions. Fig. 10. Abaxial surface of adult leaf showing corky appearance of lesions. Fig. 11. Lesion showing distribution of the ascomata. Fig. 12. Surface of culture on MEA. Fig. 13. Reverse surface of culture on MEA. Bars: Figs 2–3, 5–10, 12–13 = 10 mm; Fig. 4 = 5 mm; Fig. 11 = 2 mm.
**Typus:** Australia: Western Australia; Bunbury, Summerlea plantation of Western Australian Chip and Pulp (WACAP) 115’ 37’ E, 33’ 40’ S, on Eucalyptus globulus, 1 May 2000. A. Maxwell (PERTH 05849543 – holotypus, MURU0001 – isotypus ex-type culture CBS 110500); Albany, Callistemon plantation of Integrated Tree Cropping (ITC), on E. globulus, 11 April 2000 A. Maxwell (MURU0002 – paratypus). GenBank sequence ex-type AY 150331.

**Lesions:** amphigenous, brown, sub-circular, 1–8 mm diam. Ascomata amphigenous, sparse, black, globose, 87–105 x 83–102 μm, ostiolate, walls brown comprising 3–5 layers of textura angularis. Asci bitunicate, fusoid-ellipsoid, ends rounded, medianly 1-septate not constricted, tapering toward basal end, (9–)11–12–(15) x 2–2.5(–3) μm. Ascospore germination from both ends parallel to the long axis of the spore, remaining hyaline but becoming slightly constricted and finely verruculose at 24 h then forming lateral branches after 36 h. Culture colour on 2% MEA after 8 wk at 25°C in the dark, surface brownish orange, 7C6 (1.5YR: 5.7: 6.7); reverse greyish brown, 7F3 (5R: 3.3: 0.9). Red crystals form in agar. Growth rate 16–24 mm after 1 month at 25°C. Anamorph not seen.

**Habit:** Host Eucalyptus globulus. Occurring on juvenile leaves only. Found throughout the south-west of Australia. Isolated alone or with Mycosphaerella cryptica, M. nubilosa, M. parva or M. gregaria on the same lesion.

**Notes:** This species can be differentiated from other similar Mycosphaerella species on the basis of a combination of characteristics. It is most clearly different from other species of Mycosphaerella isolated from Eucalyptus in culture, as its upper surface becomes greyish orange on 2% MEA. The spores are similar to those of M. cryptica in morphology except that they are smaller and not (or only rarely) constricted. The germination pattern of this species differs from that of M. cryptica, as do the cultural characteristics. Mycosphaerella aurantia is most similar to M. tasmaniiensis and the M. heimii complex. However, it differs from the former as it has thick-walled not thin-walled ascospores and it does not form a Mycovellosiella anamorph in culture. M. aurantia is unlike M. heimii in ascospore shape, culture colour and it does not produce a Pseudocercospora anamorph on 2% MEA in culture under nuv. The new species also differs from the other small-spored species because it has slightly larger ascospores, and in M. keniensis the ascospores do not become constricted upon germination; M. parva has constricted ascospores which darken and become prominently verruculose upon germination; M. hemioides germinates perpendicularly to the ascospore and forms a Pseudocercospora anamorph on 2% MEA under nuv.
Mycosphaerella ambiphylla A. Maxwell, sp. nov. (Figs 9–13, 16–20)

Etym.: ambiphyllus, named for the formation of ascomata on both surfaces of the leaf, ambi (Latin) ‘both’ and phyllus (Latin) ‘leaf’.

Anamorph: Phaeophleospora sp.

Lesions elevatae, parum suberese, amphigenes, atro-rufubrunneae marginibus rufis, irregularaes ad circulares, 1–8 mm diam. Ascomata amphigena, dispersa, nigra, globosa, (60–)86–96–(110) × (60–)88–100–120 μm; parietes brunnei, e 2–3 stratis texturae angularis compositi. Asci bitunicati, fasciculati, obovoidei ad ellipsoides, recti ad incurvati, 8-spored, (30–)55.5–64.5–(80) × (7–)9–11–(16) μm. Ascosporae bi-vel tri-seriatae, imbricatae, guttulatae, fusiformes ad fusiformi-ellipsoidae apice obtuso, ad basin truncatae, per medianum 1-septatae, parum constrictae parietibus crassis, (3–)3.5–4.5–5–(6) μm. Ascospore germination post 24 horas ab extrematibus ambiphyllos ad axem longum sporae paralelae; sporae 3-septatae et gradatim constrictae, subhyalinae, parietibus laevibus. Velocitas incrementi post unam mensam ad 25 °C 35–45 mm. Culturae in 2% MEA pagina supera olivacea, 3C6 (6.5Y: 6.8: 6.8), infera olivacea, 3F6 (8.5Y: 3.4: 2.7). Pycnidia in 2% MEA et CLA post octo aceo-flava, 3C6 (6.5Y: 6.8: 6.8), infera olivacea, 3F6 (8.5Y: 3.4: 2.7). Reverse olive, 3F6 (8.5Y: 3.4: 2.7).

Habit: Host Eucalyptus globulus. Occurring on adult and juvenile leaves. Occurring alone or with Mycosphaerella cryptica, M. nubilosa, M. parva or M. suberosa on the same leaf. Isolated alone or along with M. cryptica, M. nubilosa, M. parva, or M. suberosa from a single lesion.

Notes: Lesions of Mycosphaerella ambiphylla are similar to those of M. suberosa in that they are suberised, although not to the same degree. In culture, M. ambiphylla is flat, olive-yellow and comparatively fast growing (40 mm month⁻¹) whereas M. suberosa is compact, raised, black and very slow growing (2–5 mm month⁻¹). Also, M. suberosa ascospores germinate from several germ-tubes after 24 h, and become dark, verruculose and distorted (type E; Crous 1998) whereas M. ambiphylla has only one germination tube at each end of the ascospore (type C; Crous 1998). The ascospores of M. molleriana most resemble those of M. molleriana, M. nubilosa and M. vespa in terms of size, morphology and germination pattern. All of these species germinate from both ends with slight constriction of the septum. In M. ambiphylla, the ascogonae are amphigenous, the ascospores are thick-walled and slightly larger and wider (14–15 × 4.5–5 μm), whilst in M. molleriana the ascogonae are mostly hypophyllums, the ascospores are thin-walled and slightly smaller and narrower (12–14 × 3–3.5 μm) in length (Crous 1998). Importantly, M. molleriana forms a Colletotrichum anamorph, whereas M. ambiphylla forms a Phaeophleospora anamorph. M. ambiphylla occurs on juvenile and adult leaves, is fast growing (35–45 mm month⁻¹) and readily forms the Phaeophleospora anamorph in culture, whereas M. nubilosa occurs almost exclusively on juvenile leaves, is slow growing (10 mm month⁻¹) and does not form an anamorph in culture. Mycosphaerella ambiphylla is most clearly differentated from the recently described M. vespa on the basis of the anamorph formed. The former develops a Phaeophleospora anamorph whereas the latter develops a Coniothyrium anamorph (Milgat et al. 2001). In addition M. ambiphylla is faster growing (35–45 mm month⁻¹) compared to 20–35 mm month⁻¹) and forms ascomata on both surfaces of the leaf, as opposed to M. vespa, which is hypophyllous (Carnegie & Keane 1998).

Phaeophleospora accommodates pycnidial fungi forming brown, rough-walled (holoblastic), cylindrical conidia with obtuse apices and truncate bases with a
Coniothyrium ovatum

The specimens of *Mycosphaerella mexicana* isolated in the present survey agree with that of the type description (Crous 1998) except for the following small differences: *Asci* were shorter and wider in the present study (52–60 × 16.5–19 (μm)) than in the type description (50–80 × 10–15 (μm)). Cultural features are not described in the type description; therefore these features are described here. *Culture colour* on 2% MEA surface olive grey, 3F2 (−: 3.5: 0.2); reverse olive grey, 3F2 (but ‘darker’) (−: 3.5: 0.2). *Myelia* a dense, aerial form. *Growth rate* 12–18 mm month⁻¹.

**Habit:** Host Eucalyptus globulus. Observed on older juvenile leaves, occurring alone or with a combination of *Myosphaerella cryptica*, *M. marksii*, *M. nubilosa* or *M. parva* on the same lesion.

**Specimens examined:** Australia: Western Australia: Manjimup, Darling View plantation (WACAP), 116° 00'E, 33° 10'S, on Eucalyptus globulus, 2 May 2000, A. Maxwell (PERTH 05849632, MURU0006, MURU0007, MURU0008). Culture CBS 110502.

### Mycosphaerella gregaria

The specimens of *Mycosphaerella gregaria* from the present study agreed with the type description of Carnegie & Keane (1997) except for the following small differences. *Asci* were smaller in the present study (28–32 × 5.5–7 (μm)) than in the type (37.5–47.5 × 6.5–8.5 (μm)). *Ascospores* were smaller in the present study (9.5–11 × 2–2.5 (μm)) than in the type description (10.5–15.5 × 2.5–3.5 (μm)). *Culture colour* is described in the type but not with reference to standardized colour charts. This study on 2% MEA, surface greyish rose, 11B6 (10RP: 5.5: 8.5) becoming olive brown, 4E4 (5Y: 4.8: 3.1) towards margin. Reverse, brownish grey, 4F2 (−: 3.5: 0.3). Forms sclerotia, and unlike the type description, does not form a red or red-brown pigment in the agar.

**Habit:** Host Eucalyptus globulus. Observed on older juvenile and leaves intermediate between their juvenile and adult phase. Widespread in south-western Australia. Occurring alone or with one or a combination of *Mycosphaerella cryptica*, *M. marksii*, *M. nubilosa* or *M. parva* on the same leaf.

### Table 1. Comparison of the pycnidial and conidial dimensions of *Coniothyrium* species associated with *Eucalyptus* species and the *Phaeophleospora* anamorph of *Mycosphaerella ambiphylla* from *Eucalyptus globulus*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pycnidia diam (μm)</th>
<th>Conidia (length × width μm)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. ambadi</td>
<td>Not given</td>
<td>6–7 × 3.5–4.5</td>
<td>Sutton (1974)</td>
</tr>
<tr>
<td>C. eucalypticola</td>
<td>Very small</td>
<td>8.5–10 × 6–7.5</td>
<td>Sutton (1980)</td>
</tr>
<tr>
<td>C. kalangarensis</td>
<td>To 250</td>
<td>4–7 × 2.5–5</td>
<td>Sutton (1980)</td>
</tr>
<tr>
<td>C. ovatum</td>
<td>32–75</td>
<td>(7.5–)9(–12) × (2.5–)3(–5)</td>
<td>Milgate et al. (2001)</td>
</tr>
<tr>
<td>M. ambiphylla</td>
<td>80–300</td>
<td>(5–)10–15 × (3–)3.5–4.5(–5)</td>
<td>This study</td>
</tr>
</tbody>
</table>

Marginal frill. The conidia are produced from brown, ampulliform, lageniform or short cylindrical, rough-walled cells with several proliferations. In the neotype designation for the type species, the conidiogenous cells of *Phaeophleospora eugeniae* are described as percurrent (Crous, Ferreira & Sutton 1997). However, sympodial conidiogenesis is not precluded for this genus. Similar genera to *Phaeophleospora* are *Microsphaeropsis*, *Colletogloeopsis*, *Readeriella*, and *Coniothyrium*. *Microsphaeropsis* conidia lack ornamentation and the conidiogenous cells are enteroblastic, ‘phialidic’ and hyaline (Sutton 1980). This differs from the present anamorph, which forms finely verruculose holoblastic conidia, from percurrent or sympodially proliferating, finely verruculose brown conidiogenous cells. In *Readeriella*, conidiogenesis is exclusively percurrent and the conidia produced are deltoid, thick-walled with three lateral obtuse projections (Sutton 1980) unlike the thin-walled, cylindrical to elliptical conidia of the present anamorph. *Colletogloeopsis* differs from the anamorph under consideration in that it forms thick-walled spores in an acervulus, not in a pycnidium. The anamorph of *M. ambiphylla* differs from the genus *Coniothyrium* in the following respects: the conidia of *Coniothyrium* are verruculose and the conidiogenous cells are hyaline and smooth-walled (Sutton 1980) whereas in this anamorph both the conidia and the conidiogenous cells are pale brown and finely verruculose; also, conidiogenesis in *Coniothyrium* is characterised by percurrent proliferation only. In contrast, conidiogenesis in the present anamorph is both percurrent and sympodial.

Presently, all of the fungi accommodated within *Phaeophleospora* have septe conidia. The conidia of *M. ambiphylla* are aseptate, therefore this species is clearly different from previously described species within the genus *Phaeophleospora*. However, in a recent re-examination of the holotype of *Coniothyrium ovatum*, Milgate *et al.* (2001) disagree with the original description of the conidiogenous cells as hyaline and smooth-walled (Swart 1986), finding that they were brown and verruculose; i.e. similar to *Phaeophleospora*. Therefore, a comparison is made between *C. ambadi*, *C. eucalypticola*, *C. kalangarensis*, *C. ovatum* and *M. ambiphylla* (Table 1). It is evident that the conidia and pycnidia of *M. ambiphylla* are larger than those of the four *Coniothyrium* species on eucalypts.
Specimens examined: Australia: Western Australia: Bunbury, Summerlea plantation (WACAP), 116° 37’ E, 33° 40’ S, on Eucalyptus globulus, 1 May 2000, A. Maxwell (PERTH 05849551); Manjimup, Channeleybearup plantation (WACAP), on E. globulus, 16 Feb. 2000, A. Maxwell (MURU0009); Busselton, Reid plantation (WACAP), on E. globulus, 2 May 2000, A. Maxwell (MURU0010); Esperance, Chips plantation (ITC) on E. globulus, 15 Dec. 2000, A. Maxwell (MURU0011, culture CBS 110501).


The specimens of Mycosphaerella parva from the present study agree with the type description of Park and Keane (1982) except for the following small differences. Ascomata narrower size range in the present study (56–68 μm), than in the type (42–91 μm) diam. Asci were smaller in the present study (30–38 × 8.5–10.5 μm) than in the type (29–48.5 × 6–13 μm). Cultural features are not given in the type description. This study: Culture colour on 2% MEA: surface, olive, 3D5 (5.5Y: 5.9: 4.1); reverse, goose-turd, 3F3 (7.5Y: 3.5: 0.6).

Habit: Host Eucalyptus globulus. Observed on older juvenile leaves. Widespread in south-western Australia. Occurring alone or with one or a combination of Mycosphaerella cryptica, M. gregaria, M. marksii, M. nubilosa or M. mexicana on the same lesion.

Specimens examined: Australia: Western Australia: Bunbury, Darling View plantation (WACAP), 116° 00’ E, 33° 10’ S, on Eucalyptus globulus, 2 May 2000, A. Maxwell (PERTH 05849586; MURU0012); Manjimup, Woodraka plantation (WACAP) 116° 05’ E, 34° 30’ S, 29 Feb. 2000, A. Maxwell (MURU0013, culture CBS 110503).

Other Mycosphaerella species isolated in this survey: M. nubilosa, M. cryptica, M. marksii, M. suberosa and M. lateralis. These have been previously recorded in WA and are therefore not discussed in this paper. However, they are included in the following key for the identification of Mycosphaerella species present on E. globulus in WA.

Germination patterns described in the key refer to the typical pattern seen after 24 h on 2% MEA at 20 °C, and reference letter where given, is according to the scheme of Crous (1998). Cultural feature such as surface colour, pigment formation and anamorph formed refer to growth on 2% MEA after 2 months under mv. Growth rates refer to growth rate on 20 ml of 2% MEA in 90 mm plates at 25 °C.

DISCUSSION

This survey has identified two new species of Mycosphaerella (M. ambiphylla and M. aurantia) and extended the known geographic range of three other species (M. gregaria, M. mexicana and M. parva). A new anamorph is described linked to M. ambiphylla. The occurrence of two new species and three new disease records in WA is significant for the plantation-eucalypt industry worldwide. The finding of two new species brings with it the need to quantify the disease impact of these on eucalypt plantations, and the extension of the range of three species has quarantine implications.

The recognition of two new Mycosphaerella species increases the number of Mycosphaerella species associated with eucalypts to 31. This includes the 27 species recognised in Crous (1998) and the newly described M. vespa (Carnegie & Keane 1998) and M. intermedia (Dick & Dobbie 2001).

M. ambiphylla and M. aurantia were the only Mycosphaerella species present on some lesions, suggesting that they are primary pathogens. However, they frequently occurred in association with other Mycosphaerella species. The role of these new species in causing disease needs to be examined. Epidemiological and pathogenicity studies have been conducted on M. cryptica and M. nubilosa (Park 1988a, b), some limited infection work conducted on M. parva (Park & Keane 1982) and M. vespa (Milgate et al. 2001), but not on any of the remaining 27 species occurring on eucalypts. Infection studies and pathogenicity tests need to be conducted with these little understood species in order to understand their role in the disease syndrome.

Quarantine issues are raised by the extension of the geographic range of M. gregaria, M. parva and M. mexicana. The origin of species formerly known only outside of Australia is of particular relevance. Mycosphaerella mexicana, isolated in this study, was previously known only from Mexico (Crous 1998). The known geographic range of other species of Mycosphaerella such as M. suberosa have also recently been extended, from South America (Crous et al. 1993, Crous 1998) and Indonesia (Crous & Wingfield 1997), to now include Western Australia (Carnegie et al. 1997). The biogeography of these and many other species occurring on eucalypts is not well known. It may be that these species occur on a range of hosts scattered across many continents. When eucalypts are established in new areas, inoculum on host trees already present in these areas may then infect these newly established trees. Alternatively, inoculum may travel with eucalypt seed or seedlings into the new areas of establishment. A third alternative is that spores are able to travel vast distances in wind currents from their centre of origin and infect hosts where they occur in new areas. It is important to determine how these pathogens are spreading in order to inform quarantine policy decisions.

The distribution of different Mycosphaerella species may be determined through more extensive disease surveys on eucalypts and adjacent myrtaceous hosts in areas where plantations occur. The centre of origin of a given Mycosphaerella species may be determined from population level studies using molecular markers. Work is currently underway comparing the population of M. nubilosa in WA with that in eastern Australia. Further work of this nature needs to be made to investigate the likely origin of other Mycosphaerella species recently isolated in WA and elsewhere.
### Key to Mycosphaerella species occurring on Eucalyptus globulus in Western Australia

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lesions corky, more prominent on one side of the leaf than the other; ascomata in concentric rings</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Lesions not corky, not more prominent on one side of the leaf than the other; ascomata not in concentric rings</td>
<td>2</td>
</tr>
<tr>
<td>2(1)</td>
<td>Ascospore germination from each end parallel to the long axis of the spore, spores becoming constricted at the median septum, not verruculose, not darkening or distorting, type C; cultures fast growing (40 mm month⁻¹); Phaeoepileospora anamorph</td>
<td>ambiphyla</td>
</tr>
<tr>
<td></td>
<td>Ascospore germination parallel or perpendicular long axis from one or both ends, constricting at the median septum, ascospores and the adjacent germ tube cells becoming darker and verruculose, slow growing (&lt; 20 mm month⁻¹)</td>
<td>3</td>
</tr>
<tr>
<td>3(2)</td>
<td>Ascospores (6–)8–9–11) μm; germination from one end perpendicular to the long axis of the spore, sometimes both ends, spores distorting, ascospores and the adjacent germ tube cells becoming slightly darker and verruculose, type N or L</td>
<td>parva</td>
</tr>
<tr>
<td></td>
<td>Ascospores (11)12–18(20) μm; germination from one or both ends, ascospores distorting or constricting at the median septum, ascospores and the adjacent germ tube cells becoming markedly dark and verruculose, type E or H</td>
<td>4</td>
</tr>
<tr>
<td>4(3)</td>
<td>Ascospores (11–)13–15–16 μm; ascospore germination from several germ tubes, ascospores becoming markedly distorted; cultures very slow growing (&lt; 5 mm month⁻¹); culture surface black; mycelia raised in folded mounds and also deeply embedding into and distorting the agar</td>
<td>suberosa</td>
</tr>
<tr>
<td></td>
<td>Ascospores (15–)17–18–20 μm; ascospore germination from each end parallel to the long axis of the spore, ascospores becoming constricted at the median septum but not markedly distorted; cultures slow growing (&lt; 18 mm month⁻¹); culture surface dark olivaceous grey; mycelia not raised and folding, not deeply embedding or distorting the agar</td>
<td>mexicana</td>
</tr>
<tr>
<td>5(1)</td>
<td>Ascomata amphigenous or epiphyllous</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Ascomata hypophyllous</td>
<td>15</td>
</tr>
<tr>
<td>6(5)</td>
<td>Ascomata amphigenous</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Ascomata epiphyllous</td>
<td>13</td>
</tr>
<tr>
<td>7(6)</td>
<td>Ascomata densely distributed over the lesion; ascospore germination from one end, perpendicular to the long axis of the spore, not distorting or constricting, type A; in culture forms red-brown diffusible pigment; Colletogloeopsis anamorph</td>
<td>cryptica</td>
</tr>
<tr>
<td></td>
<td>Ascomata not densely distributed over the lesion; ascospore germination parallel or perpendicular, not type A; not producing a red-brown diffusible pigment on MEA; not forming a Colletogloeopsis anamorph on MEA</td>
<td>8</td>
</tr>
<tr>
<td>8(7)</td>
<td>Ascospore germination parallel or perpendicular, ascospores becoming dark and verruculose, constricted at the median septum or distorted</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Ascospore germination parallel to the long axis of the spore, not becoming dark or verruculose, slightly constricted at the median septum but not distorted</td>
<td>10</td>
</tr>
<tr>
<td>9(8)</td>
<td>Ascospores (6–)8–9–11) μm; germination from one end perpendicular to the long axis of the spore, sometimes both ends; ascospores distorting, spores and adjacent germ tube cells becoming slightly darker and verruculose, type N or L</td>
<td>parva</td>
</tr>
<tr>
<td></td>
<td>Ascospores (15–)17–18–20 μm; ascospore germination from each end parallel to the long axis of the spore, ascospores constricting at the median septum but not distorting, spores and adjacent germ tube cells becoming markedly darker and verruculose, type H</td>
<td>mexicana</td>
</tr>
<tr>
<td>10(8)</td>
<td>Lesions often forming along leaf margins; ascomata aggregated in clumps of 3–10; cultures forming sclerotia &amp; red-pink patches on MEA</td>
<td>gregaria</td>
</tr>
<tr>
<td></td>
<td>Lesions may or may not form along leaf margins; ascomata not aggregated in clumps of 3–10; not forming sclerotia or red-pink patches on MEA</td>
<td>11</td>
</tr>
<tr>
<td>11(10)</td>
<td>Cultures fast growing (40 mm month⁻¹); culture surface olive grey; Phaeoepileospora anamorph</td>
<td>ambiphyla</td>
</tr>
<tr>
<td></td>
<td>Medium growth (15–30 mm month⁻¹), culture surface pale olive brown or orange grey; not forming Phaeoepileospora anamorph</td>
<td>12</td>
</tr>
<tr>
<td>12(11)</td>
<td>Culture surface pale olive brown; Dissoconium anamorph</td>
<td>lateralis</td>
</tr>
<tr>
<td></td>
<td>Culture surface orange grey; no anamorph</td>
<td>aurantia</td>
</tr>
<tr>
<td>13(6)</td>
<td>Ascomata sparse (1–20 per lesion); ascospores (15)17–18(20) μm, constricted at the median septum, slightly olivaceous and verruculose; ascospore germination from each end parallel to the long axis of the spore, ascospores and adjacent germ tube cells becoming darker and more verruculose</td>
<td>mexicana</td>
</tr>
<tr>
<td></td>
<td>Ascomata not sparse (&gt; 20 per lesion); ascospores &lt;15 μm, not constricted at the median septum, not pigmented or verruculose; ascospore germination from each end parallel to the long axis of the spore but not darkening or becoming verruculose</td>
<td>14</td>
</tr>
<tr>
<td>14(13)</td>
<td>Ascospores with an asymmetrical apical cell; on germination ascospores not becoming constricted at the median septum, not developing lateral branches; culture surface olivaceous grey; no anamorph</td>
<td>marksi</td>
</tr>
</tbody>
</table>
Ascospores with or without an asymmetrical apical cell; on germination ascospores becoming constricted at the median septum and developing lateral branches; culture surface olivaceous grey;

\[
\text{Dissoconium anamorph} \quad \text{15(5) Ascospores (11)12–14(16) \, \mu m; germination from each end parallel to the long axis of the ascospore, becoming constricted at the median septum, not becoming verruculose or distorted} \quad \text{nobilosa}
\]

\[
\text{Ascospores (6)8–9(11) \, \mu m; germination from one end sometimes each end, perpendicular to the long axis of the ascospore, becoming constricted at the median septum, verruculose and distorted} \quad \text{parva}
\]

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Park, R. F. (1988b) Effect of certain host, inoculum, and environmental factors on infection of \( \text{Eucalyptus} \) species by two \( \text{Mycosphaerella} \) species. \textit{Transactions of the British Mycological Society} 90: 221–228.


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