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Taxonomy, phylogeny and population biology of *Mycosphaerella* species occurring on *Eucalyptus*. A literature review

1.0 INTRODUCTION

Species of *Eucalyptus sensu stricto* (excluding *Corymbia* and *Angophora*) are native to Australia, Indonesia, Papua New Guinea and the Philippines where they grow in natural forests (Ladiges 1997, Potts & Pederick 2000, Turnbull 2000). From these natural environments, various *Eucalyptus* spp. have been selected and planted as non-natives in many tropical and sub-tropical countries where they are among the favoured tree species for commercial forestry (Poynton 1979, Turnbull 2000). Commercial plantations of *Eucalyptus* spp. are second only to *Pinus* spp. in their usage and productivity worldwide and several million hectares of *Eucalyptus* spp. and their hybrids are grown in intensively managed plantations (Old *et al*. 2003). *Eucalyptus* spp. offer the advantage of desirable wood qualities and relatively short rotation periods in commercial forestry programmes where rotations range from 5−15 years with appropriate silvicultural and site practices (Zobel 1993, Turnbull 2000).

Although *Eucalyptus* spp. are favoured commercial forestry species, they are threatened by many pests and diseases (Elliott *et al*. 1998, Keane *et al*. 2000). There are many native and non-native fungal pathogens that can infect the roots, stems and leaves of *Eucalyptus* trees (Park *et al*. 2000, Old & Davison 2000, Old *et al*. 2003). Consequently there are many pathogens that can infect and cause disease on *Eucalyptus* trees simultaneously. It is important, therefore, to identify and understand the biology of such pathogens in order to develop effective management strategies for commercial *Eucalyptus* forestry.

Some of the most important *Eucalyptus* leaf diseases are caused by species of *Mycosphaerella* Johanson. More than 100 species of this genus are recognised for causing leaf diseases on *Eucalyptus* spp. (Crous 1998, Crous *et al*. 2004a, Crous *et al*. 2006). These leaf diseases are collectively known as Mycosphaerella Leaf Disease (MLD). *Mycosphaerella* spp. have been identified from both natural *Eucalyptus* stands and commercial *Eucalyptus* plantations where they cause a range of symptoms including leaf spots, leaf withering, twig cankers, premature defoliation, multi-leadered stems, seedling blight and in severe cases death of young trees (Park & Keane 1982b, Crous 1998). The majority of *Eucalyptus* spp. become

infected by *Mycosphaerella* spp. during their juvenile leaf phase and the leaf spots caused by *Mycosphaerella* spp. reduce the photosynthetic capacity of *Eucalyptus* leaves, leading to premature leaf defoliation and overall growth stunting (Park & Keane 1982b, Lundquist & Purnell 1987, Carnegie & Ades 2003, Pinkard & Mohammed 2006).

Mycosphaerella is one of the largest ascomycete genera. Over 3000 taxa are currently accommodated in this genus, with the majority of species recognised as saprobes or pathogens of woody and herbaceous hosts (von Arx 1983, Corlett 1991, Aptroot 2006). The sexual stage of *Mycosphaerella* is morphologically conserved and species of the genus are difficult to culture, thus making species identification particularly complex (Crous 1998, Crous *et al*. 2004a). There are, however, approximately 30 anamorph genera that are associated with *Mycosphaerella* (Crous *et al*. 2000, 2004a, 2006). These anamorph states are morphologically variable and provide greater information for species delineation (Crous & Wingfield 1996, Crous *et al* 2000, 2006, Verkley & Priest 2000). Ultimately, results from DNA sequence analyses is the most effective method used in *Mycosphaerella* species identification. Therefore, morphological characteristics, combined with recent advances in DNA-based technologies have served to elucidate species concepts within *Mycosphaerella* and aid in competent species identification.

An extensive body of research on *Mycosphaerella* has been published in recent years. This literature review, therefore, serves to critically analyse the existing research on *Mycosphaerella* and place it into context with the particular aims of this thesis, which are to study those species of *Mycosphaerella* involved in MLD of *Eucalyptus*.

2.0 MYCOSPHAERELLA LEAF DISEASE (MLD)

2.1 DISEASE EPIDEMIOLOGY

An understanding of the epidemiology of MLD provides valuable information for the management of this disease. Epidemiological knowledge, such as the infection process and disease development of *Mycosphaerella* spp. occurring on *Eucalyptus* spp., is based largely on studies of *Mycosphaerella cryptica* (Cooke) Hansf. and *Mycosphaerella nubilosa* (Cooke) Hansf., two of the most important *Eucalyptus* leaf pathogens (Park & Keane 1982a, b, Park 1988a, b). The infection process and disease development of these *Mycosphaerella* spp. on *Eucalyptus* can be considered in terms of three phases namely, spore deposition and infection, fungal growth and formation of reproductive structures and finally spore liberation.

2.1.1 *Spore deposition and infection*

Both the ascospores and conidia of *Mycosphaerella* spp. can be involved in disease development on *Eucalyptus* leaves. However, ascospores act as the primary source of inoculum in many species (Beresford 1978, Park & Keane 1987). The primary inoculum source is from attached infected leaves or from fallen overwintered leaf litter, which is the general trend for many ascomycete leaf-inhabiting fungi (Luley & McNabb 1989, Patton & Spear 1983, Park & Keane 1987). Ascogonia or conidiomata of *Mycosphaerella* spp. have been shown to remain viable for a period of several months, providing sufficient inoculum for successive infection cycles (Cheah & Hartill 1987, Park & Keane 1987, Park 1988b). In certain *Mycosphaerella* spp. such as *Mycosphaerella citri* Whiteside, the cause of citrus greasy spot, it is known that ascospore production can occur throughout the entire year ensuring a continual inoculum source (Mondal & Timmer 2002).

Infection of *Eucalyptus* leaves by *Mycosphaerella* spp. predominantly occurs during the vegetative period of the host during the summer and autumn months (Ganapathi 1979, Cheah & Hartill 1987). Park (1988a), showed that the young expanding leaves, less than 46 days old, of *E. globulus*, were particularly susceptible to *M. nubilosa*, while Ganapathi (1979), showed that leaves of *Eucalyptus delegatensis* were most susceptible to infection during the first 21 days after they unfold. However, as the leaves of *Eucalyptus* spp. age they become more resistant to infection as a result of the deposition of resistant compounds such as lignin (Park 1988a).

Infection of the leaf surface by the spores may either be direct or indirect. Germ tubes of *M. cryptica* ascospores are able to penetrate both directly, through the cuticle, or indirectly through stomata (Park & Keane 1982b, Park 1988a). During direct penetration, a protoappresorium is formed alongside the ascospore or at the end of the germ tube (Ganapathi 1979, Park & Keane 1982b). The protoappresorium forms an infection peg that penetrates the cuticle allowing the spore to form invasive hyphae that grow between the cuticle and epidermal cells. Branching hyphae are subsequently formed that grow intercellularly throughout the epidermal layer (Park 1988a). In the case of indirect penetration, infection occurs through the stomata where the germ tubes produce hyphal swellings within the stomatal pores and substomatal cavities (Park & Keane 1982b, Niyo *et al*. 1986). Conidia of *Mycosphaerella lateralis* Crous & M.J. Wingf. have been shown to germinate on both adaxial

and abaxial leaf surfaces, but only penetrate the leaf through stomata on the abaxial leaf surface and do not produce hyphal swellings or appresoria (Jackson *et al*. 2004). Crous *et al*. (1989c), showed from growth room inoculations how conidia of *Phaeophleospora epicoccoides* (Cooke & Massee) Crous, F.A. Ferreira & B. Sutton (as *Phaeoseptoria eucalypti* Hansf.) were able to penetrate leaves of *Eucalyptus* spp., from the subgenus *Symphyomyrtus*, through leaf stomata. Due to the larger number of stomata on abaxial leaf surfaces there is an increase in infection and subsequent pseudothecial development on abaxial leaf surfaces (Niyo *et al*. 1986).

Levels of moisture in the environment affect the ability of the fungal spores to infect host material. Higher levels of infection, and consequently disease development, have been noted for spores of *Mycosphaerella populorum* G.E. Thomps., on *Populus*, after periods of rainfall and leaf wetting (Luley & McNabb 1989). Conidial germination of *Mycosphaerella fijiensis* var. *difformis* J.L. Mulder & R.H. Stover decreased as levels of relative humidity decreased and maximum germ tube development was observed in the presence of free water (Jacome *et al*. 1991). Temperature also affects the ability of spores to infect the leaf surface. It has been shown that ascospores and conidia of *M. fijiensis* var. *difformis* germinate at temperatures ranging from 20–35 °C with maximum germination occurring at 25 °C (Jacome *et al*. 1991).

Once ascospores or conidia are deposited onto the leaf surface they germinate and form germ tubes. Germination of ascospores and conidia generally occurs in surface moisture, although it has been shown that spores of some *Mycosphaerella* spp. occurring on *Eucalyptus*, such as *M. nubilosa* and *M. cryptica*, can survive periods of desiccation on the leaf surface and still remain viable and infective (Beresford 1978, Park 1988a).

2.1.2 *Fungal growth and formation of reproductive structures*

Upon entry into the leaf, fungal hyphae grow along the vascular bundles and colonize the leaf tissue, becoming established throughout the leaf. Following chlorosis, hyphae grow intercellularly throughout the spongy and palisade mesophyll and eventually aggregate in the substomatal cavities as has been shown in *M. cryptica* and *M. nubilosa* occurring on *Eucalyptus* (Park & Keane 1982b). These hyphal aggregates then develop into immature ascomata with trichogynes (Park & Keane 1982b).

Ganapathi (1979) described the development of pseudothecial ascomata of *M. cryptica* (as *M. nubilosa*) in detail. His studies showed that the ascocarp initials comprise a group of

cells. This developing ascoma has the appearance of a stroma with the presence of developing trichogynes, which grow toward the stromatal apex. During ascogonial development, the stroma matures and breaks through the host surface. The developing trichogynes grow through the top of the stroma and are fertilised by spermatia of the genus *Asteromella* Pass. & Thüm. Spermatia are formed in a gelatinous matrix that seaps from the ostiole onto the leaf surface (Ganapathi & Corbin 1979). After fertilisation, ascogonia mature and through successive developmental steps form asci and ascospores. Mature ascogonia of *Mycosphaerella* spp. generally have large thick, elongated cells impregnated with melanin that form the outer layers of the ascocarp wall (Niyo *et al.* 1986). Although cells making up the inner ascocarp walls generally contain lower melanin levels than those of the outer ascogonial walls, similar cellular organelles are observed in both cell types (Niyo *et a*l. 1986).

2.1.3 *Spore liberation*

Liberation of ascospores is dependant on moisture. For example, in *M. cryptica* and *M. nubilosa*, ascospores are discharged when the relative humidity is greater than 95 % and are not discharged when the relative humidity is below 90 % (Beresford 1978, Park & Keane 1982b, Cheah & Hartill 1987). Cheah & Hartill (1987) found that ascospores of *M. cryptica* are discharged after rainfall and that discharge continues for up to two hours after rainfall has ceased. They also suggest that longer periods of light rainfall will lead to more ascospores being discharged because this allows for ascospore maturation in the asci. Discharge of ascospores in this case will continue in the presence of sufficient moisture and relative humidity until the asci within the pseudothecia are exhausted of ascospores (Cheah & Hartill 1987). Mondal *et al*. (2003), observed that ascospore release of *M. citri* under field conditions occurred 1 to 2 hours after rainfall had begun, with peak ascospore release occurring 6 to 8 hours after the onset of rainfall. Furthermore, ascospore release continued for up to 16 hours after rainfall. Dew may also serve as a stimulus for ascospore release, but fewer ascospores are found to be released under these conditions as has been shown in *M. populorum* on mixed hybrids of *Populus* and suggested for *M. citri* on *Citrus* (Luley & McNabb 1989, Mondal *et al*. 2003).

Park & Keane (1982b), found that the optimum temperature for ascospore discharge in *M. nubilosa* and *M. cryptica* was 25 °C and 20 °C, respectively, and that they may be ejected up to a distance of 12–15 mm above the pseudothecia. This would allow the spores to be wind dispersed. Ascospores are likely to be dispersed by wind for considerable distances as has

been suggested for ascospores of *M. citri* that may be wind dispersed for a distance of up to 80 meters (Mondal *et al*. 2003). However, conidia of *Mycosphaerella* spp. would not be dispersed for long distances, for example, conidia of *M. cryptica* are usually produced in a gelatinous matrix on the leaf surface and as such would are splash-dispersed over short distances within the same tree (Beresford 1978, Cheah & Hartill 1987).

3.0 SYMPTOMATOLOGY

Infection of *Eucalyptus* leaves by *Mycosphaerella* spp. results in the formation of various symptoms. These symptoms include leaf spots, leaf blotches, leaf blight, leaf withering, lamina distortion, twig and stem cankers, tip die-back, growth stunting, multi-leadered trees and in severe cases death of the sapling or tree (Dick 1982, Crous *et al*. 1989b, Wingfield *et al*. 1996, Crous 1998, Park *et al*. 2000).

Infection by several *Mycosphaerella* spp. occurs during the juvenile phase of tree growth, resulting in severely infected and spotted leaves. Due to the leaf spotting, there is a loss in the photosynthetic capability of the leaves and they are prematurely shed as has been shown for *M. nubilosa* on *E. globulus* (Pinkard & Mohammed 2006). Furthermore, almost complete defoliation of juvenile and intermediate leaves of *E. globulus* subsp. *globulus* by *M. nubilosa* has been reported from Australia (Park & Keane 1982b). Through inoculation of potted *Eucalyptus camaldulensis* with spore suspensions of *P. epicoccoides* (teleomorph: *Mycosphaerella suttonii* Crous & M.J. Wingf.), Crous *et al*. (1989c) were able to show how this fungus causes premature defoliation after 12 weeks.

The most common symptom of *Mycosphaerella* infection is the development of leaf spots on *Eucalyptus* leaves. Leaf spots may be of varying shapes, for example circular to irregular (*M. ambiphylla* A. Maxwell, *M. cryptica*, *M. vespa* Carnegie & Keane, *Coniothyrium ovatum* H.J. Swart) (Dick 1982, Crous *et al*. 1988, Carnegie & Keane 1998, Maxwell *et al*. 2003), irregular (*M. nubilosa*) (Dick 1982), round or slightly irregular (*M. parkii* Crous, M.J. Wingf., F.A. Ferreira & Alfenas) (Crous & Alfenas 1995, Crous *et al*. 1993b), small and discrete (*M. heimii* Crous) (Crous & Swart 1995), sub-circular to irregular (*M. suttonii*) (Crous & Wingfield 1997), sub-circular (*M. aurantia* A. Maxwell, *M. irregulariramosa* Crous & M.J. Wingf.) (Crous & Wingfield 1997, Maxwell *et al*. 2003), sub-circular to confluent (*Pseudocercospora eucalyptorum* Crous, M.J. Wingf., Marasas & B. Sutton) (Crous *et al*. 1989d) and leaf spots may be absent (*M. heimioides* Crous & M.J. Wingf.) (Crous & Wingfield 1997, Crous 1998). Several species of *Mycosphaerella* have also

been shown to have an endophytic growth phase, for instance *M. endophytica* Crous & H. Smith on *Eucalyptus* (Crous 1998), and *M. punctiformis* (Pers.) Starbäck on *Quercus* (Verkley *et al*. 2004a). It has, for instance, been shown that *M. punctiformis* is able to colonise and grow endophytically in living and dead leaves of *Quercus* and is only detected by the presence of spermagonia in senescent *Quercus* leaves (Verkley *et al*. 2004a).

Leaf spots may vary in colour on leaf surfaces. They may be brown (*M. ambiphylla*) (Maxwell *et al*. 2003), dark brown with a yellow-red margin (*M. suberosa* Crous, F.A. Ferreira, Alfenas & M.J. Wingf.) (Dick & Dobbie 2001), yellow to brown (*M. nubilosa*) (Crous *et al*. 1989a, Crous 1998), grey to pale brown (*M. tasmaniensis* Crous & M.J. Wingf., *P. eucalyptorum*) (Crous *et al*. 1989a, Crous *et al*. 1998), pale brown to red-brown (*M. vespa*) (Carnegie & Keane 1998), dark purple to black (*C. ovatum*) (Crous *et al*. 1988, Crous *et al*. 1989a), purple to brownish (*P. epicoccoides*) (Crous *et al*. 1998) pale brown surrounded by a red-purple margin (*M. suttonii*) (Crous & Wingfield 1997) or rust-brown (*M. intermedia* M.A. Dick & Dobbie) (Dick & Dobbie 2001).

Leaf lesions on *Eucalyptus* caused by *Mycosphaerella* spp. may have distinct lesion margins and lesion zones of various colours that are usually darker than the centre of the lesions. Lesions of *M. intermedia* form lesions with raised, dark brown margins that are surrounded by a red-purple zone (Dick & Dobbie 2001). Lesions of *P. epicoccoides* form lesions that are surrounded by a distinct purple discolouration (Crous *et al*. 1989a). Lesions of *M. ambiphylla* on the other hand are known to be suberized with red margins (Maxwell *et al*. 2003). Lesions may also be surrounded by red-purple borders (*M. suberosa*) (Crous *et al*. 1993a). *Eucalyptus* leaves may in certain instances become prominently buckled with lesion development as has been shown for *M. cryptica* (Park & Keane 1982b, Crous 1998).

Aggressive *Mycosphaerella* spp. may move from the leaf onto young *Eucalyptus* stems and cause cankers. *Mycosphaerella cryptica* has been observed to cause stem cankers on young branches and shoots of *E. obliqua* and *E. globulus* subsp. *globulus* (Park & Keane 1982b). However, these cankers were only caused by the acervuli of *Colletogloeopsis nubilosum* (Ganap. & Corbin) Crous & M.J. Wingf., the anamorph of *M. cryptica* (Park & Keane 1982b). Dick (1982) reported the occurrence of cankers on young petioles, shoots and twigs caused by *M. cryptica*. Such cankers eventually lead to twig girdling and die-back of the young stem and also the thinning of crowns and the death of young tree tops (Dick 1982). Cankers also result in growth stunting, multi-leadered trees and a bushy appearance of the young *Eucalyptus* tree (Dick 1982).

4.0 TELEOMORPH CONCEPTS

Mycosphaerella is one of the largest ascomycete genera. More than 3000 taxa, that are characterised as parasites or saprobes of various vascular and woody hosts, are currently accommodated within *Mycosphaerella* (von Arx 1983, Corlett 1991, Corlett 1995, Aptroot 2006). Morphologically, *Mycosphaerella* is characterised by the formation of small, spherical, ostiolate ascomata, 8-spored, bitunicate asci without filamentous paraphyses and 2-celled, hyaline ascospores without appendages, but that can have a mucous sheath (von Arx 1983, Crous *et al*. 2000). The spermatial state of *Mycosphaerella* spp. is widely accepted to be accommodated within *Asteromella*. This genus is characterised by the formation of hyaline rod-shaped, ellipsoidal, cylindrical or allantoid spermatia formed on phialides that line the inner walls of *Mycosphaerella* spermagonia (von Arx 1983, Crous & Wingfield 1996, Verkley *et al*. 2004a).

Barr (1972) separated *Mycosphaerella* into two sub-genera based on ascus shape and anamorph associations. Each sub-genus was further separated into sections based on ascospore type and habitat. Sub-genus *Mycosphaerella* was characterised by oblong, elongate or clavate asci occurring in broad fascicles, arising from the basal layer of small cells. This sub-genus was further divided into the sections *Mycosphaerella* (saprobic ascomata, obovate, oblong to ellipsoidal ascospores with rounded apices), *Macula* M.E. Barr (parasitic ascomata, leaf spots exhibiting marginal zones, obovate, oblong to ellipsoidal ascospores with rounded apices), *Caterva* M.E. Barr (saprobic species with fusoid ascospores that have pointed ends), *Longispora* M.E. Barr (saprobic species, fusoid to elongate ascospores with a 6 : 1 or more length to width ratio) and *Plaga* M.E. Barr (parasitic species, lesions exhibiting marginal zone, fusoid ascospores with pointed ends) (Barr 1972). The sub-genus *Didymellina* (Höhn.) M.E. Barr was characterised by asci that are saccate, ovoid, oblong, few in fascicles, arising from an arched basal cushion of hyaline cells, ascospores mostly crowded in the ascus. Sub-genus *Didymellina* was further sub-divided in sections *Didymellina* (parasitic species forming leaf spots), *Cymadothea* (F.A. Wolf) Arx (multiloculate ascomata, obovate ascospores, conidial state *Polythrincium* Kunze, parasitic species causing leaf blotches), *Stigmina* M.E. Barr (uniloculate ascomata, obovate ascospores, conidial state *Stigmina*, parasitic species causing leaf blotches), *Tassiana* M.E. Barr (saprobic ascomata, obovate ascomata) and *Fusispora* M.E. Barr (saprobic ascomata, fusoid ascospores with pointed ends) (Barr 1972). Von Arx (1983) disagreed with Barr's (1972) circumscription of *Mycosphaerella* because the

delimiting characteristics were too divergent. This was further substantiated by Crous (1998), who found that the delimitation of Barr (1972) did not agree with the observed anamorph/teleomorph connections within *Mycosphaerella*.

Crous *et al*. (2000) separated *Mycosphaerella* into six sections based on morphological characteristics of the asci, ascospores and anamorph associations. Sections accepted by Crous *et al*. (2000) include the sections *Mycosphaerella* (cylindrical uniseriate asci, inequilateral, uniseriate, thin-walled, small ascospores with rounded apices, *Ramularia* Unger anamorphs), *Tassiana* (pyriform asci, thick-walled, equilateral, large ascospores that are constricted at the septum and have rounded apices, *Cladosporium* Link anamorphs), *Caterva* (cylindrical asci, thin-walled, inequilateral, medium sized ascospores with more or less pointed ends, *Asteromella* spermatial states), *Longispora* (cylindrical asci, aggregated, thin-walled, long, equilateral ascospores rarely constricted at the median septum, with rounded apices and pointed bases, *Phleospora* Wallr. or *Septoria* Sacc. anamorphs), *Fusispora* (pyriform asci, thin-walled, equilateral, fusiform ascospores that are rarely constricted at the septum and that are pointed at both ends, unknown anamorphs) and section *Plaga* (endophytic species, obovoid to ellipsoidal or cylindrical asci, small to medium fusiform to obovoid ascospores with rounded apices, anamorphs: *Colletogloeopsis* Crous & M.J. Wingf., *Mycovellosiella* Rangel, *Phaeophleospora* Rangel, *Pseudocercospora* Speg., *Pseudocercosporella* Deighton, *Sonderhenia* H.J. Swart & J. Walker, *Stenella* Syd. and *Uwebraunia* Crous & M.J. Wingf.) (Crous *et al*. 2000). According to this circumscription, those species of *Mycosphaerella* occurring on *Eucalyptus* can, therefore, be accommodated in section *Plaga*.

Braun *et al*. (2003) separated section *Tassiana* of *Mycosphaerella* into a new and separate teleomorph genus *Davidiella* Crous & U. Braun. Through a phylogenetic study of the Internal Transcribed Spacer (ITS) and Small Subunit (SSU) gene regions it was evident that *Mycosphaerella* spp. with *Cladosporium* anamorphs grouped sister to the larger *Mycosphaerella sensu stricto* clade. Due to this, *Davidiella* (*Davidiellaceae*, *sensu* Schoch *et al*. 2006), with the type species *Davidiella allicina* (Fr.) Aptroot (Aptroot 2006), was erected for those species of *Mycosphaerella* with *Cladosporium* anamorphs. Morphologically, *Davidiella* produces ascomata that are identical to *Mycosphaerella* section *Tassiana sensu* Barr (1972), but has distinct *Cladosporium* anamorphs (Braun *et al*. 2003).

Mycosphaerella is morphologically very similar to another ascomycete genus, *Didymella* Sacc. *Didymella* can, however, be distinguished from *Mycosphaerella* based on characters of the ascospores, presence of filamentous paraphyses and anamorph associations

(von Arx 1983). *Sphaerulina* Sacc. and *Microcyclus* Sacc., Syd. & P. Syd. were also once considered closely related to *Mycosphaerella*, but they have been classified into a different family (von Arx 1983). *Sphaerulina* is polyphyletic, however, and some taxa with 3-septate ascospores are presently accommodated in *Mycosphaerella* (Crous *et al*. 2003). Sivanesan (1984) placed *Mycosphaerella* within the order *Dothideales* and in the family *Dothideaceae*. However, Sutton & Hennebert (1994) placed *Mycosphaerella* within the order *Dothideales* and the family *Mycosphaerellaceae*. Despite many revisions and divisions within *Mycosphaerella*, the teleomorph remains morphologically conserved (Crous *et al*. 2000). Due to the conserved nature of the teleomorph, few morphological features are phylogenetically informative for species delimitation within *Mycosphaerella*. Through extensive phylogenetic analyses using DNA sequence data from four nuclear gene regions combined with parsimony and bayesian phylogenetic inferences, Schoch *et al*. (2006) have shown that *Mycosphaerella* does not group within the *Dothideales* but is rather accommodated within the *Capnodiales* (*Dothideomycetes*). Schoch *et al*. (2006) further proposed the Ascomycete sub-class *Dothideomycetidae*, containing the orders *Dothideales*, *Capnodiales* and *Myriangiales*. Following this classification, the taxonomic position of *Mycosphaerella* is as follows:

Phylum: *Ascomycota*
Class: *Dothideomycetes*
Sub-class: *Dothideomycetidae*
Order: *Capnodiales*
Family: *Mycosphaerellaceae*
Genus: *Mycosphaerella*

5.0 ANAMORPH ASSOCIATIONS OF *MYCOSPHAERELLA* SPP. OCCURRING ON *EUCALYPTUS* LEAVES

The use of anamorph morphology in mycological classification is, in many cases, more useful than that of the teleomorph. This is due to the diverse and heterogeneous nature of the anamorphs in comparison to the relatively conserved morphology of the teleomorph states. Crous & Mourichon (2002) stated that the anamorph states of *Mycosphaerella* spp. were not phylogenetically informative, but did acknowledge that anamorph morphology was still the most informative feature to be used to distinguish among species.

Anamorph genera of *Mycosphaerella* are morphologically diverse and are classified in both the coelomycetes and hyphomycetes (von Arx 1983, Crous & Wingfield 1996, Crous 1998). Barr (1972) noted that the anamorph genera of *Mycosphaerella* were morphologically variable and accepted eleven anamorph genera based on conidial septation, pigmentation and the formation of conidial chains. Von Arx (1983), studying the conidiomatal structure, type of scars on the conidiogenous cells and position on the host of various species representing anamorphs of *Mycosphaerella* eventually accepted 23 anamorph form genera. Sutton & Hennebert (1994) studied the mode of conidiogenesis of *Mycosphaerella* anamorphs and eventually also accepted 23 anamorph genera for *Mycosphaerella*. Crous *et al*. (2000) also recognised 23 anamorph genera of *Mycosphaerella* and separated these genera based on characters of the mycelium (presence or absence of superficial mycelium), conidiophores (arrangement, branching, pigmentation), conidiogenous cells (placement, proliferation and scar type) and conidia (formation, shape, septation, wall and pigmentation).

Von Arx (1983) noted that some species of *Mycosphaerella* that have known anamorphs are indistinguishable from species without anamorphs. He, therefore, believed that anamorphs should not be used to distinguish between genera, sub-genera or sections of *Mycosphaerella*. Braun (1990), disagreed with the view of von Arx (1983) and stated that anamorph characters that should be used for generic separation include characteristics of the caespituli (fasciculate/synnematous), nature of the conidial scars (thickened, conspicuous/unthickened, obscure) and conidial formation. Braun (1990) did, however, propose that anamorph genera that have colourless or coloured conidiophores or conidia should not be merged. Crous (1998) evaluated morphological features of *Mycosphaerella* spp. occurring on *Eucalyptus* trees using multiple correspondence analysis (MCA), and found that the species of *Mycosphaerella* grouped according to their anamorph associations. He, therefore, suggested that species of *Mycosphaerella* should be separated into groups according to their anamorph affiliations. Stewart *et al*. (1999) were able to effectively separate the cercosporoid genera *Cercospora* Fres., *Pseudocercospora* and *Passalora* Fr. through sequence data from the ITS region and therefore agreed with the view of Crous (1998).

Mycosphaerella has been linked to approximately 30 different anamorph genera including coelomycetes and hyphomycetes. However, when considering those *Mycosphaerella* spp. occurring on *Eucalyptus* leaves, only 14 anamorph genera are recognised (Crous 1998, Crous *et al*. 2006). For the purpose of this review only those *Mycosphaerella* anamorph genera occurring on *Eucalyptus* will be discussed further.

5.1 *Colletogloeopsis*

Ganapathi & Corbin (1979) described *Colletogloeum nubilosum* as the anamorph of *M. cryptica* (which they referred to as *M. nubilosa*) from *E. delegatensis* in New Zealand. They found it difficult to place *C. nubilosum* because the conidiogenous cells were annellidic and the conidia were aseptate and produced sympodially. They noted that the anamorph genera *Pollaccia* E. Bald. & Cif. and *Colletogloeum* Petr. were the genera most appropriate in which to place *C. nubilosum*. They thus placed *C. nubilosum* in *Colletogloeum* because the sexual state of *Pollaccia* was *Venturia* Sacc. and no sexual states had been found for other *Colletogloeum* species. They noted that *C. nubilosum* was distinct from other species of *Colletogloeum* in that it has aseptate conidia.

Crous & Wingfield (1997) re-evaluated the species of *Colletogloeum* on *Eucalyptus* and found that these species did not agree with the morphological description of the type of *Colletogloeum* and that these species formed a separate group that were characterised by brown, verruculose, thick-walled, aseptate conidia, from sympodially or percurrently proliferating brown, verruculose conidiogenous cells (Crous & Wingfield 1997). Crous & Wingfield (1997) therefore, established a new genus, *Colletogloeopsis* for anamorphs of *M. cryptica* (*Colletogloeopsis nubilosum*) and *Mycosphaerella molleriana* (Thüm) Lindau (*Colletogloeopsis molleriana* Crous & M.J. Wingf.). Recently, Cortinas *et al*. (2006a) emended the description of *Colletogloeopsis* to also accommodate pycnidial anamorphs. Since the description of *Colletogloeopsis*, several new *Colletogloeopsis* spp. have been identified from *Eucalyptus* leaves including *Colletogloeopsis stellenboschiana* Crous and the *Colletogloeopsis* anamorph of *Mycosphaerella pseudocryptica* Crous (Crous *et al*. 2006). In total, six *Mycosphaerella* spp. occurring on *Eucalyptus* are known to have *Colletogloeopsis* anamorphs, namely *Mycosphaerella* sp. (*C. stellenboschiana*), *Mycosphaerella* sp. [*C. zuluense* (M.J. Wingf., Crous & T.A. Coutinho) M.N. Cortinas, M.J. Wingf., & Crous], *Mycosphaerella* sp. (*C. gauchensis* M.N. Cortinas, Crous & M.J. Wingf.), *M. cryptica* (*C. nubilosum*), *M. molleriana* (*C. molleriana*) and *M. pseudocryptica* (*Colletogloeopsis* sp.) (Crous 1998, Cortinas *et al*. 2006a, b, Crous *et al*. 2006).

5.2 *Coniothyrium*

Sutton (1975) described *Coniothyrium kallangurense* B. Sutton & Alcorn from leaves of *Eucalyptus microcordyis* in Queensland, Australia. He noted that two other *Coniothyrium* spp. from *Eucalyptus* (*C. eucalypticola* B. Sutton and *C. ahmadii* B. Sutton) differed from *C. kalangurense* in having thick-walled, ornamented conidia. Crous *et al*. (1988) identified *C. ovatum* on *E. cladocalyx* and *Eucalyptus lehmannii* in South Africa. *C. ovatum* was responsible for leaf spots on these species and produced substomatal black pycnidia that formed long cirri with slightly verrucose dark brown conidia that are obovate with truncate bases (Crous *et al*. 1989a). They further stated that *C. ovatum* had a limited distribution and as such posed little threat due to the ability of *Eucalyptus* trees to outgrow the pathogen (Crous *et al*. 1988, Crous *et al*. 1989a). Milgate *et al*. (2001) isolated *M. vespa* from *Eucalyptus* leaves in Tasmania and found it to produce an anamorph resembling *C. ovatum*.

Coniothyrium zuluense M.J. Wingf., Crous & T.A. Coutinho, known to cause stem lesions on *Eucalyptus*, was thought to reside within the *Pleosporales* of the *Leptosphaeriaceae*. However, by employing DNA sequence data from the 18S and ITS gene regions of the rRNA operon, Cortinas *et al*. (2006a) found that *C. zuluense* does not group within the *Pleosporales* and rather in *Mycosphaerella* close to *Mycosphaerella* spp. producing *Colletogloeopsis* anamorphs. Therefore, *C. zuluense* was transferred to *Colletogloeopsis* as *Colletogloeopsis zuluense* and the generic circumscription of *Colletogloeopsis* was emended to include *Coniothyrium*-like species that produce pycnidia (Cortinas *et al*. 2006a).

5.3 *Davisoniella*

Swart (1988) described the coelomycete genus *Davisoniella* H.J. Swart that is characterised by the production of stromatic, subepidermal conidiomata and holoblastic, percurrent conidiogenous cells that arise from the inner walls of the conidiomatal locule and conidia that are oval, brown, verruculose, rounded apices, truncate bases with marginal frills. Swart (1988) further described one species, namely *Davisoniella eucalypti* H.J. Swart, from leaves of *Eucalyptus marginata* to be accommodated within this genus. Only one *Mycosphaerella* species known from *Eucalyptus* has been identified as having a *Davisoniella* anamorphic state. *Mycosphaerella davisoniellae* Crous was described from *Eucalyptus marginata* leaves in western Australia (Crous *et al*. 2006). The anamorph of *M. davisioniellae*, *Davisoniella eucalypti*, produces subcylindrical, ampulliform or doliiform conidiogenous cells that proliferate percurrently (Swart 1988, Crous *et al*. 2006). Conidia of *D. eucalypti* are known to be solitary, aseptate, verruculose, thick-walled, oval with truncate to subtruncate bases and

distinct basal frills (Crous *et al*. 2006). Phylogenetically and morphologically, *Davisoniella* spp. are related to *Colletogloeopsis* spp., but can be distinguished from the latter by producing unilocular to multilocular conidiomata on the leaf surfaces (Crous *et al*. 2006).

5.4 *Dissoconium*

De Hoog *et al*. (1983) established the genus *Dissoconium* de Hoog, Oorschot & Hijwegen based on the type species *Dissoconium aciculare* de Hoog, Oorschot & Hijwegen. This fungus produces conidia that are sub-hyaline, thin-walled, continuous or with a median septum, has one-celled conidia which are obovoidal, two-celled conidia which are constricted at the septum, rounded apical cells and inflated and often broader basal cells. De Hoog *et al*. (1991) described the new species *Dissoconium dekkeri* de Hoog & Hijwegen, which is a presumed mycoparasite on *Erysiphaceae* spp. Crous *et al*. (1999) sequenced the ITS region of species of *Mycosphaerella* and *Dissoconium* and found that *M. lateralis* (anamorph: *Uwebraunia lateralis* Crous & M.J. Wingf.) grouped in a clade with *Dissoconium* spp., and reduced *U. lateralis* to synonymy under *Dissoconium dekkeri*. Other *Mycosphaerella* spp. occurring on *Eucalyptus* known to produce *Dissoconium* anamorphs include *Mycosphaerella communis* Crous & J.P. Mansilla (anamorph: *Dissoconium commune* Crous & J.P. Mansilla) (Crous *et al*. 2004a). The status of *Uwebraunia*, which is morphologically indistinguishable from *Dissoconium*, is presently unclear.

5.5 *Mycovellosiella*

Species of *Mycovellosiella* produce superficial hyphae on the host plant, with intercalary conidiogenous cells with lateral nodes, conspicuous conidial scars and conidiophores that are produced terminally and laterally with conidia that are either formed singly or in chains (von Arx 1983, Crous & Braun 2003). Braun (1993, 1995) transferred some species of *Ramularia*, *Cerocosporella* Sacc. and *Cercospora* to *Mycovellosiella* due to their formation of superficial mycelia, coloured conidiophores and conidia and the production of secondary mycelium. *Mycovellosiella tasmaniensis* Crous & M.J. Wingf. (teleomorph: *M. tasmaniensis*) was described from leaves of *E. nitens* in Tasmania and represented the only species of *Mycovellosiella* to be described from *Eucalyptus* (Crous *et al*. 1998). *Mycovellosiella tasmaniensis* is characterised by septate conidiophores that arise from superficial mycelia with terminal, mono to polyblastic conidiogenous cells that proliferate sympodially and

forming catenate conidia that occur in chains and conidia that have flattened, darkened, refractive, thickened loci (Crous *et al*. 1998).

Upon further evaluation of *Mycovellosiella* spp., Crous & Braun (2003) found that the development of secondary superficial mycelia with solitary conidiophores is a variable character and stated that the development of creeping superficial hyphae should not be used to distinguish between cercosporoid genera. Therefore, Crous & Braun (2003) found that there was no true character to separate *Mycovellosiella* from *Passalora* and synonomised *Mycovellosiella* under *Passalora sensu lato*.

5.6 *Passalora*

Passalora is a cercosporoid hyphomycete anamorph genus of *Mycosphaerella* and has the type species *Passalora bacilligera* Fr. & Mont. This fungus is characterised by coloured conidiophores and ellipsoidal to fusiform, obclavate to subcylindrical, 1–3-septate, pigmented conidia that are formed singly (Crous & Braun 2003). Conidia in *Passalora* are ellipsoid-ovoid, broadly fusiform, clavate, obclavate, subcylindrical, colourless to pigmented, broad with few septa (Braun 1995). The conidiophores are loosely fasciculate (Braun 1995). Stewart *et al*. (1999) found that from sequence data analysis, isolates of *Passalora* grouped separate from *Cercospora* and *Pseudocercospora*, thus agreeing with the generic circumscription of *Passalora*. Crous & Braun (2003) reduced *Berteromyces* Cif. and *Oreophyllum* Cif. to synonymy with *Passalora*. Two species of *Passalora* are known from *Eucalyptus*, namely *Passalora zambiae* Crous & T. Coutinho (teleomorph: *Mycosphaerella* sp.) and *Passalora eucalypti* (Crous & Alfenas) Crous & U. Braun (teleomorph: *Mycosphaerella* sp.) (Crous *et al*. 2004a). *Passalora zambiae* produces medium brown, smooth, branched or unbranched, 0–2-septate conidiophores (Crous *et al*. 2004a). Furthermore, conidiogenous cells are terminal and intercalary, tapering to truncate apices and proliferating sympodially to produce catenulate conidia (Crous *et al*. 2004a).

5.7 *Phaeophleospora*

Phaeophleospora is a coelomycete genus of *Mycosphaerella*. There have been many revisions of the taxonomy of this genus and its close morphological similarity to other coelomycete genera. Due to this similarity, many species have in the past been incorrectly accommodated in various other coelomycete genera. *Phaeoseptoria eucalypti* Hansf. was described by

Hansford (1956) from *E. grandis* in Sydney, Australia. However, Walker (1962) studied other collections of *P. eucalypti* and found that Hansfords' (1956) original description made no mention of the characteristic annellations seen around the conidiogenous loci. Walker (1962), therefore, amended Hansfords' (1956) description and included morphological characters such as annellations and roughened conidia. Dick (1982) recorded *P. eucalypti* from *Eucalyptus saligna* in New Zealand where it is responsible for causing purple leaf lesions on *Eucalyptus* spp. *Phaeoseptoria eucalypti* was subsequently also collected in South Africa from several *Eucalyptus* spp. (Crous *et al*. 1988). Walker (1962), noted that *Phaeoseptoria* resembles another coelomycete genus *Hendersonia* Berk., and Swart & Walker (1988), considered *Hendersonia grandispora* McAlpine to be congeneric with *P. eucalypti*.

Walker *et al*. (1992) examined the generic circumscription of *P. eucalypti* and found that *P. eucalypti* was congeneric with another well-known *Eucalyptus* pathogen, *Cercospora epicoccoides* Cooke & Massee. The type of *H. grandispora* was also examined and found that it too was incorrectly identified as a species of *Hendersonia*, and, instead was morphologically identical to several collections of *P. eucalypti*. Walker *et al*. (1992), also examined the type species of another *Phaeoseptoria* species from *Eucalyptus*, namely *Phaeoseptoria luzonensis* T. Koboyashi and found that this taxon also did not represent a distinct species and that it too was congeneric with *P. eucalypti*. Therefore, *C. epicoccoides*, *H. grandispora* and *P. luzonensis* were all found to be congeneric with *P. eucalypti* (Walker *et al*. 1992).

Walker *et al*. (1992), also found that several other *Eucalyptus* leaf fungi [*Septoria pulcherrima* Gadgil & M.A. Dick, *S. normae* Heatler, *S. ceuthosporoides* (Cooke & Harkn.) Sacc., *S. mortolensis* Penz. & Sacc. and *S. eucalypti* G. Winter & Roum.] were morphologically identical to *Cercospora eucalypti* Cooke & Massee apud Cooke. They further erected a new genus *Kirramyces* J. Walker, B. Sutton & Pascoe in which they placed *Kirramyces epicoccoides* (Cooke & Massee) J. Walker, B. Sutton & Pascoe (= *Cercospora epicoccoides*, *Hendersonia grandispora*, *Phaeoseptoria eucalypti*, *Phaeoseptoria luzonensis*) and *Kirramyces eucalypti* (Cooke & Massee) J. Walker, B. Sutton & Pascoe [= *Cercospora eucalypti*, *Pseudocercospora eucalypti* (Cooke & Massee) Gou & Liu, *Septoria pulcherrima*, *Stagonospora pulcherrima* (Gadgil & M.A. Dick) H.J. Swart]. Wingfield *et al*. (1996) described a new species of *Kirramyces*, *Kirramyces destructans* M.J. Wingf. & Crous causing a serious leaf disease on *Eucalyptus* in Indonesia. Crous *et al*. (1997), found that *Phaeophleospora* is morphologically similar to *Kirramyces*, and that *Kirramyces* only differed from *Phaeophleospora* by the production of more conidial septa. Crous *et al*. (1997),

therefore synonomised *Kirramyces* under the older name of *Phaeophleospora*. Genera that are morphologically similar to *Phaeophleospora* include *Microsphaeropsis* Höhn., *Colletogloeopsis*, *Readeriella* Syd. and *Coniothyrium* (Maxwell *et al*. 2003). Several species of *Mycosphaerella* occurring on *Eucalyptus* are known to have *Phaeophleospora* anamorphs namely *M. suttonii* (anamorph: *P. epicoccoides*), *M. toledana* Crous & G. Bills (anamorph: *P. toledana* Crous & G. Bills) *Mycosphaerella* spp. [anamorphs: *P. eucalypti*, *P. destructans* (M.J. Wingf. & Crous) Crous, F.A. Ferreira & B. Sutton] (Crous 1998, Crous *et al.* 2004a).

5.8 *Pseudocercospora*

Pseudocercospora is a morphologically variable genus (Crous *et al*. 2000). This genus accommodates synnematal counterparts of *Cercospora* that are characterised by unthickened conidial scars and pigmented conidia with percurrent and sympodial conidiogenous cell growth (von Arx 1983, Crous *et al*. 1989d, Sutton & Hennebert 1994, Crous *et al*. 1999, Crous *et al*. 2000). Sutton & Hennebert (1994) stated that *Pseudocercospora* is closer to the annellidic coelomycetes than to the sympodial coelomycetes due to the unthickened conidial scars observed for *Pseudocercospora* spp.

There are several cercosporoid genera that are closely allied to *Pseudocercospora*. Sutton & Hennebert (1994) noted that due to the variation in conidiomatal structure of certain species, *Pseudocercospora* is also closely related to *Phleospora*. By sequencing the ITS region of species of *Pseudocercospora sensu stricto* and *Paracercospora* Deighton, Stewart *et al*. (1999) found that *Paracercospora* isolates grouped with isolates from *Pseudocercospora* and, therefore, suggested that *Paracercospora* should be reduced to synonymy with *Pseudocercospora*. *Pseudocercosporella* is also closely allied to *Pseudocercospora*. *Pseudocercosporella* is characterised by the production of hyaline conidia and hyaline or almost hyaline conidiogenous cells. Beilharz *et al*. (2002) described *Pseudocercospora warcupii* Beilharz, Pascoe & Parbery, which represents a species intermediate between *Pseudocercospora* and *Pseudocercosporella* in producing both pigmented structures characteristic of *Pseudocercospora* and hyaline structures characteristic of *Pseudocercosporella*. The hyphomycete genus *Cercostigmina* U. Braun is closely allied to *Pseudocercospora* and it has been found through DNA sequencing studies that some species of *Cercostigmina* cluster with species of *Pseudocercospora*, suggesting that *Cercostigmina* should be reduced to synonymy with *Pseudocercospora* (Crous & Braun 2003). Crous *et al*. (2006) also showed that *Stigmina* Sacc. and *Phaeoisariopsis* Ferraris are synonyms of

Pseudocercospora, and thus reduced them to synonymy under *Pseudocercospora*, which was especially conserved for this purpose.

It has been hypothesized that *Pseudocercospora* represents a monophyletic lineage within *Mycosphaerella* (Stewart *et al*. 1999, Ávila *et al*. 2005). However, by employing DNA sequence data from four nuclear gene regions, Hunter *et al*. (2006) found that *Pseudocercospora* is not monophyletic and instead is polyphyletic within *Mycosphaerella*. This has been substantiated by the observation that *Pseudocercospora* has evolved more than once within *Mycosphaerella* (Crous & Braun 2003, Ayala-Escobar *et al*. 2006, Crous *et al*. 2006). Furthermore it appears that species of *Pseudocercospora* have speciated relatively recently due to the short branch lengths of *Pseudocercospora* spp. observed in phylogenetic studies (Ávila *et al*. 2005).

There are currently many *Mycosphaerella* spp. known from *Eucalyptus* leaves that are known to form *Pseudocercospora* anamorphs (Crous 1998, Crous *et al*. 2004a, 2006). Perhaps the most pathogenic *Pseudocercospora* species is *P. eucalyptorum*, which is a cosmopolitan cercosporoid fungus occurring on *Eucalyptus* leaves. This species has a wide *Eucalyptus* host range in South Africa, but is most prevalent on *E. nitens* (Crous *et al.* 1989a, d).

5.9 *Pseudocercosporella*

Von Arx (1983) stated that the conidiogenous structures of *Pseudocercosporella* and *Cercoseptoria* Petr. were similar, and that the type species of *Pseudocercosporella*, *P. ipomoeae* Sawada ex Deighton, should be classified in *Cercoseptoria*. Braun (1990) disagreed with von Arx (1983) and preferred to retain *Pseudocercosporella* apart from *Cercoseptoria* because the two genera possessed species with pigmented and colourless structures. *Pseudocercosporella* is characterised by unthickened, inconspicuous conidial scars and solitary conidia (Braun 1990). Braun (1992) transferred species of *Ramularia* to *Pseudocercosporella* due to the presence of solitary conidia and conidial scars that are inconspicuous and unthickened. Two species of *Mycosphaerella* occurring on *Eucalyptus* leaves are known to produce *Pseudocercosporella* anamorphs, namely *Mycosphaerella endophytica* (anamorph: *P. endophytica* Crous & H. Smith) and *Mycosphaerella pseudoendophytica* Crous & G.C. Hunter (anamorph: *Pseudocercosporella* sp.) (Crous 1998, Crous *et al*. 2006).

5.10 *Readeriella*

Readeriella is a coelomycete genus characterized by the formation of spherical, dark brown immersed pycnidia with annellidic, lagenifrom to ampulliform, aseptate, hyaline, unbranched, 1–3 annelate conidiogenous cells formed on the inner pycnidial walls and conidia that are holoblastic, pale brown, smooth-walled, aseptate, truncate having a basal marginal frill and projecting laterally with 3 rounded points (Sutton 1971). *Readeriella* has the type species *Readeriella mirabilis* Syd. & P. Syd., which was described from leaves of *Eucalyptus capitellata* and *E. regnans* (Sutton 1971). *Readeriella mirabilis* has also been identified from leaves of *Eucalyptus cinerea* and *Eucalyptus nicholii* that are used for ornamental foliage in Australia (Barber *et al*. 2003). *Readeriella readeriellophora* Crous & J.P. Mansilla (teleomorph: *M. readeriellophora* Crous & J.P. Mansilla) was described from *E. globulus* leaves in Spain (Crous *et al*. 2004a). Another *Readeriella* sp., *Readeriella novozelandiae* Crous was described from *Eucalyptus botryoides* leaves in New Zealand (Crous *et al*. 2004a). *Readeriella novozelandiae* was found to be morphologically similar to *R. mirabilis* but could be distinguished from the latter species by having smaller conidia (Crous *et al*. 2004a). All *Readeriella* species that are currently known have been described from *Eucalyptus*.

5.11 *Sonderhenia*

Sonderhenia accommodates pycnidial anamorphs of *Mycosphaerella* and is characterised by percurrently proliferating conidiogenous cells and distoseptate conidia (Verkley & Priest 2000, Crous & Braun 2003). In a study of leaf inhabiting fungi from Australia, Swart & Walker (1988) noted that two species of *Hendersonia*, *Hendersonia eucalypticola* A.R. Davis and *Hendersonia eucalyptorum* Hansf. were very similar to species of *Phaeoseptoria* Speg. in their conidiogenesis. However, *P. eucalypti* differed in having larger pycnidia and longer conidia. Swart & Walker (1988) further compared the two species of *Hendersonia* to the type species of *Phaeoseptoria*, and found that it produced smooth-walled euseptate conidia that were different to the rough-walled distoseptate conidia of the *Hendersonia* spp. A new genus, *Sonderhenia* was, therefore, established to accommodate the two species of *Hendersonia*. The *Hendersonia* spp. were transferred to *Sonderhenia* as *S. eucalyptorum* (Hansf.) H.J. Swart & J. Walker and *S. eucalypticola* (A.R. Davis) H.J. Swart & J. Walker. *Sonderhenia* spp. are distinguished from *Phaeophleospora* by having distoseptate conidia (Crous *et al*. 2001a). Both *S. eucalypticola* and *S. eucalyptorum* have been isolated from *Eucalyptus* plantations in

Tasmania and Victoria, Australia, where they appear to be of minor importance (Park & Keane 1984, Carnegie *et al*. 1998, Milgate *et al*.2001).

5.12 *Stenella*

Stenella is based on the type species *Stenella araguata* Syd., which is characterised by the formation of superficial, verruculose, external secondary mycelium, solitary conidiophores that arise laterally or terminally from superficial hyphae, conspicuous conidiogenous loci and conidia that have slightly thickened and darkened conidial hila (Crous *et al*. 2000, Crous & Braun 2003). The production of verrucose superficial hyphae separates *Stenella* from *Mycovellosiella* and *Phaeoramularia* Munt.-Cvetk. (Crous *et al*. 2000, Crous *et al*. 2001a). Four *Stenella* spp. are known to occur on *Eucalyptus* leaves, namely *S. parkii* Crous & Alfenas (*M. parkii*), *Stenella* sp. (*M. scytalidii* Crous & M.J. Wingf.), *Stenella pseudoparkii* Crous & M.J. Wingf. (*Mycosphaerella* sp.) and *Stenella xenoparkii* Crous & M.J. Wingf. (*Mycosphaerella* sp.) (Crous 1998, Crous *et al*. 2006).

5.13 *Trimmatostroma*

Sutton & Ganapathi (1978) described *Trimmatostroma excentricum* B. Sutton & Ganap. from leaves of *E. delegatensis* in New Zealand, and noted that the conidia of *T. excentricum* were regularly asymmetric, which differed to other species of *Trimmatostroma* Corda in which the conidia are irregular and variable in shape and septation. They also found that conidial development of *T. excentricum* was unique within *Trimmatostroma*. Dick (1982) identified *Trimmatostroma bifarium* Gadgil & Dick and *T. excentricum* from *Eucalyptus* leaves in New Zealand where they were responsible for the production of leaf spots on various *Eucalyptus* spp. Dick (1982) stated that the disease was most notable in the lower crown and was of no economic significance. Park & Keane (1982a) identified *T. excentricum* from mature foliage of *E. globulus* in Victoria, Australia where it produced four-celled conidia in chains from conidiophores that are aggregated into a sporodochium. Recently, *Mycosphaerella pseudosuberosa* Crous & M.J. Wingf. was described from *Eucalyptus* leaves in Uruguay and was found to produce a *Trimmatostroma* anamorph in culture (Crous *et al*. 2006).

Trimmatostroma has been further established as an anamorph of *Mycosphaerella* through DNA-based phylogenetic studies. Taylor *et al*. (2003) used DNA sequence data from the ITS gene region to show that *Mycosphaerella microspora* (Joanne E. Taylor & Crous)

Joanne E. Taylor & Crous (anamorph: *Trimmatostroma microspora* Joanne E. Taylor & Crous), a leaf pathogen of *Protea* spp., groups within *Mycosphaerella*. Furthermore, Crous *et al*. (2006) used ITS sequence data to shown that *M. pseudosuberosa* (anamorph: *Trimmatostroma* sp.) also grouped within *Mycosphaerella*. This is the first *Trimmatostroma* sp. to be linked as an anamorph to those *Mycosphaerella* spp. occurring on *Eucalyptus*.

5.14 *Uwebraunia*

The hyphomycete genus *Uwebraunia* Crous & M.J. Wingf. is characterised by having smooth, olivaceous, obclavate, 1-septate conidia with unthickened hila, produced on pale medium brown conidiogenous cells with several percurrent proliferations (Crous & Wingfield 1996, Crous 1998). This genus was established for three species of *Mycosphaerella* occurring on *Eucalyptus*, *Mycosphaerella juvenis* Crous & M.J. Wingf. (*Uwebraunia juvenis* Crous & M.J. Wingf.), *Mycosphaerella ellipsoidea* Crous & M.J. Wingf. (*Uwebraunia ellipsoidea* Crous & M.J. Wingf.) and *M. lateralis* (*Uwebraunia lateralis* = *Dissoconium dekkeri*) (Crous & Wingfield 1996). As stated above, the current status of *Uwebraunia* is uncertain.

6.0 IDENTIFICATION TECHNIQUES

Identification of *Mycosphaerella* spp. is difficult, as species frequently occur without their presumed anamorphs. Therefore, morphological characteristics of the teleomorph were initially used for the identification of *Mycosphaerella* spp. However, the teleomorph morphology is relatively conserved, and only a few characters can be used for species identification (Crous *et al*. 2000). Thus, subsequent studies on *Mycosphaerella* spp. have concentrated on morphological characters of the many anamorph genera known to be associated with this genus (Crous 1998, Crous *et al*. 2004a, Crous *et al*. 2006). Furthermore, the advent of DNA sequence-based technologies has provided several techniques that can be applied to fungal species identification. This is particularly true for *Mycosphaerella* spp., where current identifications rely on both morphological characteristics and DNA-based techniques.

Ascospore germination patterns can be helpful for the initial identification of *Mycosphaerella* spp. Following discharge and germination, ascospores of *Mycosphaerella* spp. exhibit various morphologies. Park & Keane (1982a) first used ascospore germination patterns while investigating the taxonomy of *M. nubilosa*, *M. cryptica* and *M. parva* R.F. Park

& Keane occurring on *E. globulus* subsp. *globulus* in Victoria, Australia. Crous (1998) subsequently described 14 different ascospore germination patterns (Type A–N) for those species of *Mycosphaerella* occurring on *Eucalyptus* spp. Ascospores can germinate and form germ tubes that grow parallel to the long axis of the spore (Type B, C, F), perpendicular to the long axis of the spore (Type, A, L, M, N) and ascospores may change in colour and become verruculose (Type E, G, H, L, N) (Crous 1998). These characters are useful for placing unidentified *Mycosphaerella* spp. into more specific groups, thus narrowing down the possible identity of the *Mycosphaerella* isolate.

The use of Randomly Amplified Polymorphic DNA (RAPDs) has been effective in distinguishing between morphologically closely related *Mycosphaerella* spp. Carnegie *et al.* (2001) employed RAPDs to effectively distinguish between *M. cryptica*, *M. gregaria* Carnegie & Keane, *M. nubilosa* and *M. marksii* Carnegie & Keane. This technique was also applied to separate four banana leaf pathogens, *M. fijiensis* M. Morelet, *M. musicola* R. Leach ex J.L. Mulder, *M. musae* (Speg.) Syd. & P. Syd. and *M. minima* Stahel (Johanson *et al.* 1994). DNA-based methods such as RAPDs that may distinguish between species based on differences in DNA sequence are particularly advantageous. Reliability and standardisation of RAPDs across laboratories is, however, problematic and a disadvantage of this technique.

Species-specific DNA primers have proven effective for distinguishing between morphologically similar *Mycosphaerella* species. Johanson & Jeger (1993), developed species-specific primers for the banana pathogens, *M. fijiensis* and *M. musicola*. The development of these primers was based on differences in the DNA sequence of these two pathogens within the ITS regions of the rDNA operon. These primers showed high specificity for their particular *Mycosphaerella* sp. and could amplify the target *Mycosphaerella* DNA from leaf material either at an early or late necrotic stage of infection. Species-specific primers have also been developed for species of *Mycosphaerella* occurring on *Eucalyptus*. Kularatne *et al*. (2004) developed a PCR-RFLP technique for the effective identification of *M. nubilosa* and *M. cryptica*. This technique also allowed for these species to be detected directly from infected *Eucalyptus* leaf material. Furthermore, Maxwell *et al* (2005) developed species-specific primers for *M. cryptica*, *M. lateralis*, *M. marksii*, *M. nubilosa* and *M. parva*. All of these species are known to occur in Australia and several other countries where they cause MLD (Crous 1998). The developed primers were also able to amplify DNA from the selected *Mycosphaerella* spp. from infected *Eucalyptus* leaves. Such a technique is particularly applicable to quarantine facilities, seed banks and nurseries where the presence of specific *Mycosphaerella* spp. can be detected *in planta*.

Genomic, or PCR based Restriction Fragment Length Polymorphisms (RFLP`s) have been applied to the identification *Mycosphaerella* spp. Carlier *et al*. (1994), developed genomic RFLP probes to study the genetic relatedness of *M. fijiensis* isolates and found that these probes showed a high level of intraspecific polymorphism between isolates of *M. fijiensis*. Hunter (2002) employed the restriction enzyme *Hae*III to distinguish between species of *Mycosphaerella* occurring on *Eucalyptus* leaves and found that this enzyme was effective in grouping species into smaller groups based on their RFLP banding profile.

Sequencing of various DNA gene regions has become the commonly chosen technique used for the identification of *Mycosphaerella* spp. occurring on *Eucalyptus* trees. DNA sequence data from the ITS region has become the traditional gene region used for *Mycosphaerella* spp. identification (Crous *et al*. 2000, 2001a, b, Hunter *et al*. 2004a, b). However, the ITS gene region does not always provide sufficient resolution to distinguish between *Mycosphaerella* spp. and their anamorphs (Verkley & Starink-Willemse 2004). Therefore, several other nuclear gene regions such as the Large Subunit (LSU), Actin (ACT), Translation Elongation Factor 1-alpha (EF-1α) and Beta tubulin (Bt) gene regions have also recently been used to examine species boundaries and cryptic taxa within *Mycosphaerella* (Crous *et al*. 2006, Hunter *et al*. 2006). Undoubtedly, the generation of more DNA sequence datasets from various nuclear and mitochondrial gene regions and the combination of these datasets will contribute to the understanding of species concepts within *Mycosphaerella* in the future and give an indication of teleomorph and anamorph morphologies that are phylogenetically informative.

The application of several DNA-based molecular techniques to the identification of *Mycosphaerella* spp. has become markedly easier. Furthermore, identifications based on DNA data are more conclusive and trustworthy than initial identifications that were solely based on morphological characteristics. It is important though to link results from DNA-based identification methods to sexual or asexual *Mycosphaerella* morphologies to obtain features of these states that are phylogenetically reliable and informative.

7.0 PHYLOGENY

With the advent of molecular biology and DNA-based studies of fungal genomes, it has become possible to study evolutionary relationships among many fungi. In *Mycosphaerella* the ITS regions have been commonly used to answer questions regarding speciation and phylogeny. However, recently, DNA sequence data from other gene regions have been

incorporated into existing DNA sequence datasets to increase resolution among *Mycosphaerella* spp. and elucidate phylogenetic species concepts (Ávila *et al*. 2005, Hunter *et al*. 2006).

Surveys to identify *Mycosphaerella* spp. infecting *Eucalyptus* spp. have become common with the increased importance of plantation forestry in the Southern Hemisphere, especially in Australia and South Africa. This has led to the identification of many previously undescribed *Mycosphaerella* spp. From surveys in South Africa during the 1990`s, Crous & Wingfield (1996) identified several new *Mycosphaerella* spp. From this study it was hypothesized that *Mycosphaerella* was a heterogeneous group of fungi that were polyphyletic with a diverse group of monophyletic anamorph genera (Crous & Wingfield 1996, Crous 1998). Crous *et al*. (2000) noted that it was unclear whether *Mycosphaerella* is a monophyletic group or if the morphology was derived. They further stated that if the morphology was derived then *Mycosphaerella* would be paraphyletic or polyphyletic. Monophyletic groups within *Mycosphaerella* could then be characterised according to their anamorphs. This hypothesis was contradicted by Goodwin *et al*. (2001) and Crous *et al*. (2001b) who found *Mycosphaerella* to be largely monophyletic with polyphyletic anamorph genera. It was also noted that *Mycosphaerella* was of ancient origin and that the morphology of the teleomorph has been retained through natural selection, but that the anamorph characteristics seem to be highly mutable and thus anamorphs should not be used to delimit phylogenetic relationships.

Crous *et al*. (2000) sequenced the ITS region of approximately 46 *Mycosphaerella* spp. and found that three major clades could be resolved. These included a major monophyletic *Mycosphaerella* clade, a second clade corresponding to *Dissoconium* anamorphs and a third representing isolates of *Ramulispora* Miura. Crous *et al*. (1999, 2001a) were also able to prove that several isolates of *Mycosphaerella* spp., corresponding to various anamorph genera, grouped at various places within a larger *Mycosphaerella* clade suggesting that several anamorph genera within *Mycosphaerella* have evolved more than once within the genus. This was supported by Verkley *et al*. (2004b) and Verkley & Starink-Willemse (2004) who showed that *Septoria* was polyphyletic within *Mycosphaerella* and that it has evolved more that once within *Mycosphaerella*. Maxwell (2003) also showed that *Mycosphaerella* is composed of polyphyletic anamorph genera, with different lineages of anamorph genera grouping on separate branches within the *Mycosphaerella* morphology.

8.0 SPECIES COMPLEXES

DNA sequencing has increased the genetic resolution that is now available to study species boundaries and species concepts. This is particularly true for species of *Mycosphaerella*. Early studies into *Mycosphaerella* spp. on *Eucalyptus* relied predominantly on morphological descriptions (Crous 1998). However, by employing DNA sequence results from various gene regions it has become evident that several *Mycosphaerella* spp. represent a complex of morphologically similar yet phylogenetically distinct taxa (Crous *et al*. 2004a, 2006, Hunter *et al*. 2006). Several species complexes have now been identified from those *Mycosphaerella* spp. occurring on *Eucalyptus* (Crous *et al*. 2004a, 2006).

Mycosphaerella heimii, *M. crystallina* Crous & M.J. Wingf., *M. irregulariramosa* and *M. heimioides* are all species that are known from *Eucalyptus* (Crous 1998). All of these species have *Pseudocercospora* anamorphs and produce pale brown, smooth to finely verruculose, obclavate to subcylindrical conidia, with internal or external mycelia, small, dense fascicles on brown stromata. They all cluster together in a well supported phylogenetic clade when sequenced and as such are considered to all represent varieties of *M. heimii* and are referred to as the *M. heimii* complex (Crous *et al*. 2000, Crous *et al*. 2001a). This view was supported by Maxwell (2003) who found that these species grouped together in a separate clade based on ITS sequence data.

Pseudocercospora eucalyptorum has a wide geographic and eucalypt host range. From a phylogenetic analysis of *Mycosphaerella* spp. occurring on *Eucalyptus*, Crous *et al*. (2004a) showed that isolates of *P. eucalyptorum* collected from several locations did not form a monophyletic clade, but, instead were dispersed within a larger *Pseudocercospora* clade. Thus, isolates of *P. eucalyptorum* actually represent a species complex.

Many isolates of *Mycosphaerella* from several different hosts may be morphologically indistinguishable but phylogenetically may represent distinct entities and eventual phylogenetic species (e.g. *Cercospora apii* complex) (Crous & Braun 2003, Groenewald *et al*. 2005, 2006). By sequencing other loci of the *Mycosphaerella* genomes we may be able to achieve increased resolution between *Mycosphaerella* spp., thereby identifying new cryptic morphological species that were once thought of as well defined morphological species (Crous *et al*. 2004a). Maxwell (2003) found that there was a high level of intra-specific variation between certain species of *Mycosphaerella* based on an ITS phylogeny and suggested that this variation may be due to the fact that some species may represent species complexes.

By employing ITS DNA sequence data from more that 295 *Mycosphaerella* isolates from *Eucalyptus*, Crous *et al*. (2006) identified many new *Mycosphaerella* species complexes. These species complexes included the *Colletogloeopsis zuluensis*, *M. molleriana*, *M. cryptica*, *M. suttonii*, *M. suberosa*, *M. marksii*, *M. heimii*, *M. flexuosa*, *M. parva* and *M. endophytica* species complexes. Employing more *Mycosphaerella* isolates in phylogenetic studies, therefore, allows for the clearer elucidation of species complexes (Hunter *et al*. 2006).

9.0 POPULATION BIOLOGY OF *MYCOSPHAERELLA* SPECIES

Many *Mycosphaerella* spp. are important pathogens of economically important crops. In the past, most studies of *Mycosphaerella* spp. have focussed on their taxonomy, phylogeny, epidemiology and host associations. Recently, however, the study of population dynamics of fungal pathogens, and particularly *Mycosphaerella* spp., has helped to understand the population structure of many important *Mycosphaerella* spp. Limited population biology research has, however, been conducted on those *Mycosphaerella* spp. occurring on *Eucalyptus* leaves. In contrast, extensive research into the population biology of several other *Mycosphaerella* spp. such as *Mycosphaerella graminicola* (Fuckel) J. Schröt (Linde *et al*. 2002, Zhan *et al*. 2003), *M. fijiensis* (Carlier *et al*. 1996, Hayden *et al*. 2003a) and *M. musicola* (Hayden *et al*. 2003b) have been published in recent years. Results from these studies have led to and increased understanding of aspects of population dynamics within *Mycosphaerella*, such as population structure, distribution of genetic diversity, gene flow, centres of origin and mating strategies. Results of population biology studies from other *Mycosphaerella* pathosystems provides knowledge that can be used for future population biology studies of *Mycosphaerella* spp. occurring on *Eucalyptus* spp.

9.1 CENTRE OF ORIGIN

It is important to determine the centre of origin and diversity of a particular fungal pathogen. This is because these centres of origin and diversity would be a primary location in which to search for resistant or tolerant plant host genotypes. It is generally accepted that centres of origin for species would be those geographic areas that have the greatest gene diversity (McDonald 1997).

Putative centres of origin have been determined for certain *Mycosphaerella* spp. such as *M. fijiensis*, *M. graminicola* and *M. nubilosa* that are well-characterised pathogens of

banana, wheat and *Eucalyptus* respectively. Carlier *et al*. (1996) determined that populations of *M. fijiensis* from south-east Asia had greater allelic and gene diversity than *M. fijiensis* populations from Africa, the Pacific Islands and Latin America. Here, more than 88 % of the alleles detected in the African, Pacific Islands and Latin American populations were also found in the south-east Asian *M. fijiensis* population, indicating that south-east Asia most likely represents the centre of origin for *M. fijiensis*.

Zhan *et al*. (2003) investigated the global structure of the wheat pathogen *M. graminicola* by employing data generated from genomic RFLPs and found that the *M. graminicola* populations from the Middle East (Israel and Syria) exhibited higher gene diversity values than *M. graminicola* populations from America, Australia, Europe or North America. It was, therefore, suggested that *M. graminicola* originates from the Middle East where wheat was also first domesticated (Zhan *et al*. 2003).

9.2 DISTRIBUTION OF GENETIC DIVERSITY

A question that is often asked in population biology studies is how the genetic diversity of the pathogen population is distributed and on what scale is this diversity perpetuated (McDonald 1997). Answers to this question have important implications on control strategies and host resistance breeding. Distribution of genetic diversity has been effectively determined for a number of *Mycosphaerella* pathosystems.

It has been shown that the majority of genetic diversity (> 90 %) for populations of *M. fijiensis* is distributed within banana plantations (Rivas *et al*. 2004). A similar pattern of genetic distribution has also been found for *M. graminicola* where more than 79–93 % of the genetic diversity of a *M. graminicola* population is observed to occur within plots of a wheat field (Boeger *et al*. 1993, Zhan *et al*. 2003). The wheat glume blotch pathogen, *Phaeosphaeria nodorum* (E. Müll.) Hedjar. (anamorph: *Stagonospora nodorum* (Berk.) E. Castell. & Germano), also exhibits most of its gene diversity within wheat fields, and it has been found that 96 % of the gene diversity of *P. nodorum* populations from Texas, Oregon and Switzerland is found on a local level within a wheat field (Keller *et al.* 1997).

The scale of genetic diversity can be very small, such as within single lesions. Several different genotypes of *M. graminicola* have been found occupying a single lesion on a wheat leaf (Schneider *et al*. 2001, Linde *et al*. 2002, Zhan *et al*. 2003). From these studies it appears that the genetic distribution of certain *Mycosphaerella* spp. occurs at a limited spatial scale with several unique genotypes occupying a single plot or plant within a plantation or field.

9.3 MATING STRATEGIES

Fungi are typically obligately outcrossing (heterothallic) or interbreeding (homothallic). Fungal reproductive modes play an important role in the biology and population dynamics of fungal pathogens. This is also true for *Mycosphaerella* spp. where the majority of these pathogens tend to be heterothallic. Through heterothallism and sexual reproduction novel alleles are introduced into a population that allows a fungal pathogen to overcome host resistance (Milgroom 1996, Zhan *et al*. 2001, Zhan & McDonald 2004). In contrast, homothallism generally results in progeny that are identical to the parent. Through homothallism, specific fungal clones are perpetuated through time and space. It is important, therefore, to determine the reproductive strategy of fungal pathogens as it influences the population structure and ability of these pathogens to overcome resistant host genotypes. Reproductive modes of several *Mycosphaerella* spp. have been determined by investigating the number and frequency of genotypes, through determining the indices of multilocus disequilibria or by the amplification and sequencing of mating type genes.

Many *Mycosphaerella* spp. exhibit a heterothallic mating strategy. Much research has been conducted on the wheat blotch pathogen *M. graminicola*. Zhan *et al*. (2003), investigated the global diversity of *M. graminicola* and found there to be a high level of genotypic diversity in *M. graminicola* populations from America, Europe, Australia, the Middle East and North Africa. It was also found that there was random association of alleles among 14 RFLP loci, suggesting that sexual reproduction occurs within *M. graminicola*. Sexual reproduction in *M. graminicola* was further substantiated by (Zhan *et al*. 2002) who found both mating type genes (*MAT* 1-1 and *MAT* 1-2) present in equal frequency in populations of *M. graminicola* collected from 12 different countries.

Heterothallism is known to occur in the banana pathogen *M. fijiensis*. Carlier *et al.* (1996) showed that there was a high level of gametic disequilibrium between RFLP loci of populations of *M. fijiensis* from south-east Asia and Africa suggesting that these were randomly mating heterothallic populations. Hayden *et al.* (2003a) studied *M. fijiensis* populations from Papua New Guinea and several islands of the Torres Strait and found through the Fischer exact test and the index of association tests that there was no significant gametic disequilibrium among these *M. fijiensis* populations, also indicating that it is heterothallic.

Although the majority of *Mycosphaerella* spp. are known to be heterothallic, there are examples of *Mycosphaerella* spp. that exhibit a homothallic mating strategy. Milgate *et al.* (2005) showed that there was significant linkage disequilibrium in a population of the *Eucalyptus* leaf pathogen, *M. cryptica*, from Tasmania. This indicates that there is a lack of recombination and suggests that this is not a strictly heterothallic fungus.

9.4 GENE FLOW

Gene flow is an important aspect when considering the population dynamics of pathogens. Gene flow can be defined as the change in a population due to the movement of gametes, individuals or groups of individuals from one place to another (Slatkin 1987). Gene flow can serve to homogenise distant pathogen populations and prevent such populations from evolving into different species (Slatkin 1987, Zhan & McDonald 2004). The determination of gene flow within *Mycosphaerella* pathosystems is important and gives an indication of the similarity that exists between distant *Mycosphaerella* populations.

Gene flow has been determined for several populations of *Mycosphaerella* spp. The most important method of gene flow between populations of *Mycosphaerella* spp. is through airborne ascospores, and it has been shown that gene flow is one of the evolutionary forces acting to maintain within-population variation (Boeger *et al*. 1993, Zhan & McDonald 2004). Furthermore, alternative hosts may also facilitate gene flow of *Mycosphaerella* spp. between their primary hosts (Boeger *et al*. 1993). Keller *et al*. (1997) showed that populations of *Phaeosphaeria nodorum*, separated by 2000–7000 km, were not significantly differentiated, and showed that this was due to extensive gene flow. Gene flow has also served to homogenise populations of the maize pathogen *Cercospora zeae-maydis* Tehon & E.Y. Daniels from Uganda, Kenya and Rwanda (Okori *et al*. 2003).

10.0 SPREAD AND CONTROL OF *MYCOSPHAERELLA* SPECIES

The movement and spread of fungal pathogens is of primary importance when considering management strategies for their control. This is particularly true for the many species of *Mycosphaerella* that are known to be important pathogens of economically important crop plants. *Mycosphaerella* spp. can be spread to new areas in several ways and thus begin new infections in native and exotic environments.

Mycosphaerella spp. are known to infect many woody or herbaceous hosts. Many *Mycosphaerella* spp. are primarily pathogens of a single plant host. However, *Mycosphaerella* spp. may also be infecting alternative hosts, apart from their preferred host. This has been hypothesized for *M. graminicola*, causing leaf spot on wheat (Boeger *et al*. 1993). It is known that *M. graminicola* can also infect annual blue grass and if this alternative host also occurs between wheat fields it may act as a source for continued ascospore inoculum (Boeger *et al*. 1993). It has also been hypothesised that species of *Mycosphaerella* occurring on *Eucalyptus* may infect alternative hosts in attempts to move to their primary *Eucalyptus* hosts. This scenario has been termed the "pogo stick hypothesis" and is evident through the identification of fungal pathogens on non-native hosts in low levels (Crous & Groenewald 2005). Recently, isolates of *M. citri*, primarily a pathogen of citrus, have been found to infect and cause lesions on *Acacia mangium* from Thailand (Crous *et al*. 2004b).

Infected seed may serve as a vehicle for the movement of *Mycosphaerella* spp. into non-native environments (Maxwell *et al*. 2003). Seed that are transferred between countries or breeding programs should thus be tested for pathogen propagules (Boeger *et al*. 1993). The movement of seed has also been suggested as a possible method of movement of *Colletogloeopsis zuluensis* around the world to countries where *Eucalyptus* spp. are commercially grown (Cortinas *et al*. 2006b). Infected seed may also be tested for the presence of *Mycosphaerella* spp. by using species-specific primers that have been developed for several *Mycosphaerella* spp. that infect *Eucalyptus* leaves (Kularatne *et al*. 2004, Maxwell *et al*. 2005).

Control of MLD is difficult and largely depends on a combination of several factors. Hybrid tree species are known to be more tolerant to various *Mycosphaerella* spp. when compared to certain pure tree species. The use of hybrid poplar tree species has been suggested for the control and management of *M. populorum* (anamorph: *Septoria musiva* Peck), the causal agent of leaf spot and cankers of various *Populus* spp. (Feau *et al*. 2005). However, certain *Eucalyptus* hybrids have been found to be more susceptible to MLD than parental *Eucalyptus* species. Dungey *et al*. (1997) and Carnegie & Ades (2002) found that F1 *E. globulus* × *E. nitens* hybrids were more susceptible to *M. cryptica* and *M. nubilosa* than any of the parent *Eucalyptus* species. Carnegie & Ades (2002) further suggested that *Eucalyptus* hybrids not be used in environments where MLD is severe. Thus, hybrid tree species should be propagated in areas where MLD is known to be of lesser importance and such hybrid *Eucalyptus* spp. combined with a diversity of *Eucalyptus* spp. would provide the

most opportunity to combat MLD on *Eucalyptus* (Purnell & Lundquist 1986, Wingfield *et al.* 2001).

It is also known that pure host species do exist that show elevated levels of tolerance to *Mycosphaerella* infection (Patton & Spear 1983). For example, Carnegie *et al.* (1998) found significant variation in MLD disease incidence between 13 *Eucalyptus* species and that *Eucalyptus cypellocarpa* L. Johnson, *E. nitens* and *E. globulus* were most susceptible to MLD while *Eucalyptus elata* Dehnh. and *Eucalyptus oreades* R.T. Bak. were more resistant to MLD.

The use of various *Eucalyptus* species provenances may also provide a means to combat the development of MLD. Initial studies of *E. nitens* provenances in South Africa for commercial forestry resulted in the finding that New South Wales provenances of *E. nitens* were generally more resistant to MLD than Victorian *E. nitens* provenances (Purnell & Lundquist 1986). This has been further substantiated by Hood *et al.* (2002) who, during an *E. nitens* provenance trial in New Zealand, found that leaf retention of New South Wales provenances of *E. nitens* was higher than that of Victorian provenances when exposed to MLD.

Variation in susceptibility to MLD has also been determined at the *Eucalyptus* sub-species level. Carnegie & Ades (2005) found significant differences in MLD disease severity of mature foliage between the four sub-species of *E. globulus* namely *E. globulus* ssp. *bicostata* (Maiden *et al.*) Kirkpatr., *E. globulus* ssp. globulus Labill., *E. globulus* ssp. *maidenii* (F. Muell.) Kirkpatr. and *E. globulus* ssp. *pseudoglobulus* (Naudin ex Maiden) Kirkpatr. Here, it was found that *E. globulus* ssp. *bicostata* was more susceptible to *M. cryptica* than any of the other *E. globulus* sub-species while *E. globulus* ssp. *maidenii* exhibited the highest level of tolerance to *M. cryptica*.

Considering that Australia represents the origin of *Eucalyptus* spp. and most likely the centre of diversity for *Mycosphaerella* spp. that occur on these trees, it seems reasonable to expect that co-evolution has occurred between *Mycosphaerella* spp. and their *Eucalyptus* hosts in this country. Therefore, resistant *Eucalyptus* species and genotypes should be sourced from Australia, alternatively, natural *Eucalyptus* land races may also serve as source to *Mycosphaerella* resistance.

Available moisture levels play an important part in the development of *Mycosphaerella* ascomata and ascospores. Knowing the optimal amount of leaf moisture necessary for the development and maturation of ascomata and ascospores of *Mycosphaerella* spp., the frequency and length of watering in nursery systems could be adjusted to decrease

the levels of inoculum (Mondal & Timmer 2002). Furthermore, any dead or decomposing leaf material present in a nursery or plantation acts as an inoculum source, as *Mycosphaerella* pseudothecia are still capable of development in such material (Park & Keane 1987). Therefore, it would be beneficial to remove any such material from a nursery system. Overhead irrigation mechanisms should also be avoided in nurseries as water will accumulate on leaf surfaces and stimulate the production of ascospores. Alternatively drip irrigation may be used for watering nursery stock, thereby avoiding the high humidity levels and the periods that water may accumulate on leaf surfaces.

Fungicide applications may provide a means to control the development of *Mycosphaerella* spp. In a nursery environment this method may be feasible due to the smaller size of the *Eucalyptus* seedlings and the growth tunnels in which they are housed. Dick (1982) suggested that the use of fungicides should be considered in nurseries for the control of *M. cryptica*. Furthermore, it has been suggested that the cost of fungicide applications may be reduced by spraying during the vegetative period of the host (Park 1988b). Carnegie & Ades (2003) showed that the spraying of both a protectant and systemic fungicide significantly reduced the development of MLD on both juvenile and adult foliage of *E. globulus* during a fungicide spray trial in Victoria, Australia. Disease forecasting systems may also be used to determine the most appropriate time for fungicide application (Jacome *et. al*. 1991). However, once deployed into the field on a plantation level, the use of fungicide applications would be economically unviable for forestry companies.

The movement of infected plant material between countries and continents appears to be on the increase (Wingfield *et al*. 2001). Therefore, many fungal pathogens will most likely be introduced into new environments (Wingfield 1999). Quarantine measures should consequently be strictly implemented and updated to reduce the risk of fungal pathogens being introduced into non-native environments. This is particularly true for *Mycosphaerella* spp. occurring on *Eucalyptus*. There are approximately 100 *Mycosphaerella* spp. known from *Eucalyptus* occurring in many countries (Crous 1998, Crous *et al*. 2004a, Crous *et al*. 2006) and it is important that these *Mycosphaerella* spp. be incorporated into quarantine regulations and actionable lists.

11.0 CONCLUSION

From published literature, it is clear that extensive research has been conducted on species of *Mycosphaerella*. This knowledge has increased our understanding into various aspects of their taxonomy, epidemiology, host associations, host susceptibility and phylogeny.

The taxonomy of *Mycosphaerella* species is particularly complex. It has become apparent that the teleomorph stage is morphologically conserved and does not offer sufficient morphological characteristics to discriminate between *Mycosphaerella* species. This has resulted in the fact that morphological characteristics of the anamorph state has become important in *Mycosphaerella* species identification. Many different genera representing the coelomycetes and hyphomycetes are now recognised as anamorph genera of *Mycosphaerella*. It is clear though that the generic circumscription of several anamorph genera is debatable, and should be re-evaluated in terms of their morphological similarities to other anamorph genera, that are newly linked to *Mycosphaerella*. This may lead to the synonymy of several anamorph genera and to an overall decrease in the number of anamorph genera associated with *Mycosphaerella*.

Many morphological and DNA-based methods have been used to identify and characterise species of *Mycosphaerella*. The use of DNA sequencing though has now become the definitive technique that is used in species identification. This has allowed for the realisation that one morphological species does not always represent a single monophyletic taxon. Instead, from such studies, it has become apparent that many species complexes exist within *Mycosphaerella*. The use of DNA sequence data from several nuclear gene regions has thus allowed for increased resolution both at the species level and deeper nodes within *Mycosphaerella* as a whole. Undoubtedly, further studies employing DNA sequence results from a broader range of gene regions will increase out discriminatory power within *Mycosphaerella* and allow the effective identification of species but also link DNA sequences to morphologies that are phylogenetically informative.

Population biology studies on species of *Mycosphaerella* have been limited to a few well-known plant pathogens. These studies have increased our knowledge into the structure of *Mycosphaerella* populations, their diversity and movement. However, existing population biology research on *Mycosphaerella* has focussed on species occurring on various other hosts other than *Eucalyptus*. Therefore, further population biology research should be conducted into *Mycosphaerella* species occurring on this host. This research should incorporate results of population biology studies of other *Mycosphaerella* pathosystems and combine that

information generated from those *Mycosphaerella* species occurring on *Eucalyptus*. Such information will be important from an academic standpoint but also from a forestry and quarantine perspective. Population biology knowledge acquired of *Mycosphaerella* species occurring on *Eucalyptus* will aid in the establishment effective *Eucalyptus* breeding programmes and will be added to quarantine action lists to prohibit the introduction of novel more virulent *Mycosphaerella* species and genotypes.

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