

Mycosphaerella species associated with leaf disease of *Eucalyptus globulus* in Ethiopia

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Summary

Eucalyptus spp. are among the most widely planted exotic trees in Ethiopia. Several damaging leaf pathogens are known from *Eucalyptus* spp. worldwide. Of these, *Mycosphaerella* spp. are among the most important, causing the disease known as *Mycosphaerella* leaf disease (MLD). Characteristic symptoms of MLD include leaf spot, premature defoliation, shoot and twig dieback. Recent disease surveys conducted in Ethiopian *Eucalyptus* plantations have revealed disease symptoms similar to those caused by *Mycosphaerella* spp. These symptoms were restricted to *E. globulus* trees growing in several localities in south, south western and western Ethiopia. The aim of this study was to identify the fungi associated with this disease. This was achieved by examining ascospore germination patterns, anamorph associations and sequence data from the Internal Transcribed Spacer (ITS) region of the rRNA operon, for representative isolates. Several different ascospore germination patterns were observed, suggesting that more than one species of *Mycosphaerella* is responsible for MLD on *E. globulus* in Ethiopia. Analysis of sequence data showed that three *Mycosphaerella* spp., *M. marksii*, *M. nubilosa* and *M. parva* were present. This is the first report of these three species from Ethiopia and represents a valuable basis on which to build further studies in the region.

1 Introduction

Plantations of exotic tree species are widely utilized in the tropics and subtropics for the production of solid timber products and pulp. *Pinus*, *Eucalyptus*, *Cupressus* and Australian *Acacia* spp. are among the most extensively planted exotic species in these situations. Plantations of *Eucalyptus* spp. cover approximately 10 million ha of land worldwide (ELDRIDGE et al. 1997). In Ethiopia, planting of exotic species commenced with the introduction of *Eucalyptus globulus* Labill. approximately 110 years ago (PERSSON 1995). Thereafter, several *Eucalyptus* spp. including *E. camaldulensis* Dehnh., *E. saligna* Sm., *E. grandis* Hill ex Maid and *E. citriodora* Hook were introduced. It has been estimated that plantations of *Eucalyptus* spp. constitute about one-third of the total plantation area of approximately 200 000 ha in Ethiopia (ANONYMOUS 1994). The wood from *Eucalyptus* plantations is commonly used for construction purposes, fuel, poles and posts and is an important resource for subsistence farmers.

Mycosphaerella spp. are important leaf pathogens of *Eucalyptus* spp. and they are distributed worldwide (CORLETT 1991; CROUS 1998). They include both saprophytes and aggressive pathogens (VON ARX 1983). More than 60 *Mycosphaerella* spp. have been described associated with diseases of *Eucalyptus* spp. (CROUS 1998; CARNEGIE 2000; MILGATE et al. 2001; MAXWELL et al. 2003; CROUS et al. 2004; HUNTER et al. 2004a). *Mycosphaerella* leaf disease (MLD), of *Eucalyptus* spp. is characterized by leaf spot,

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defoliation, stunting, stem canker, twig and shoot dieback (BERESFORD 1978; DICK and GADGIL 1983; LUNDQUIST and PURNELL 1987; CROUS 1998).

Mycosphaerella leaf disease reduces the photosynthetic capacity of trees, reducing tree growth and in severe cases causes shoot dieback, resulting in multistemmed trees (DICK 1982; PARK and KEANE 1982a; CARNEGIE 2000). LUNDQUIST and PURNELL (1987), showed that MLD causes a reduction in growth of *E. nitens* (Deane et Maid.) Maid. trees in South Africa when more than 25% of the juvenile crown is defoliated. Similarly, a positive correlation between severities of MLD defoliation with growth of *E. globulus* was observed in Australia (CARNEGIE et al. 1994). It has also been shown that the provenances of some *Eucalyptus* spp. such as *E. globulus*, *E. nitens* and *E. regnans* F. Muell. vary in resistance to *Mycosphaerella* infection (DICK and GADGIL 1983; PURNELL and LUNDQUIST 1986; CARNEGIE et al. 1994). In South Africa, for example, it is recommended that New South Wales provenances of *E. nitens* are planted, as they are more tolerant to infection than Victorian provenances (PURNELL and LUNDQUIST 1986; WINGFIELD and ROUX 2000).

Several different *Mycosphaerella* spp. can infect individual *Eucalyptus* trees, and often more than one *Mycosphaerella* sp. can be found on a single leaf and even fruiting within the same lesion (CROUS and WINGFIELD 1996). MILGATE et al. (2001), for example, found that *M. grandis* Carnegie & Keane was found associated with older lesions of *M. tasmaniensis* Crous & M.J. Wingf., *M. nubilosa* (Cooke) Hansf. and *M. cryptica* (Cooke) Hansf. *Mycosphaerella parva* R. F. Park & Keane, a saprophytic species, is commonly associated with older lesions of *M. nubilosa* and *M. cryptica* (PARK and KEANE 1982b; CROUS et al. 1993; CARNEGIE and KEANE 1994). In this manner, multiple infections of trees can take place, compounding the impact of MLD on susceptible *Eucalyptus* trees. Such multiple infections often result in defoliation (PARK and KEANE 1982b) and they also complicate identification of the causal agents.

The occurrence of *Mycosphaerella* spp. on *Eucalyptus* leaves can vary with the foliage phase of the tree. *Mycosphaerella cryptica* is able to infect both juvenile and adult foliage (PARK and KEANE 1982b), while *M. nubilosa* is commonly associated only with juvenile foliage (PARK and KEANE 1982a). Both species cause severe defoliation of juvenile foliage of *E. globulus*. *Mycosphaerella marksii* is commonly associated with older juvenile leaves (CARNEGIE and KEANE 1994). Succession of infections by different *Mycosphaerella* spp. thus results in susceptible trees being affected at all stages of their rotation. In cases where only juvenile leaves are attacked, for example, *M. nubilosa* on *E. nitens* in South Africa, trees can outgrow the problem as they change to their adult leaf stage, normally during their second year of growth (LUNDQUIST and PURNELL 1987).

In Ethiopia, symptoms of MLD have been reported causing severe damage on juvenile *E. globulus* leaves in several plantations in most areas where this tree species is planted (ALEMU et al. 2003). The *Mycosphaerella* spp. involved in causing the disease have, however, not been identified. Therefore, this study was conducted to identify the fungi associated with MLD on *E. globulus* in Ethiopia. To accomplish this, a suite of identification techniques, including examination of ascospore germination patterns, cultural characteristics as well as sequencing of the Internal Transcribed Spacer (ITS) regions of the ribosomal RNA operon, were used.

2 Materials and methods

2.1 Sample collection and isolations

In a previous survey conducted in *Eucalyptus* plantations in Ethiopia, symptoms similar to those of MLD were observed in most *E. globulus* plantations investigated (ALEMU et al. 2003). The samples used in the present study were thus obtained from *E. globulus*

Table 1. *Mycosphaerella* isolates used in this study

Species	Isolate number	Host	Origin	Collector	Accession number
<i>Mycosphaerella africana</i>	CMW4945/STE-U-794	<i>Eucalyptus viminalis</i>	South Africa	P. W. Crous	AF309602
<i>M. flexuosa</i>	CMW5224/STE-U-1109	<i>E. globulus</i>	Colombia	M.J. Wingfield	AF309603
<i>M. beinii</i>	CMW4942/STE-U-760	<i>Eucalyptus</i> sp.	Madagascar	P.W. Crous	AF309606
<i>M. beinii</i>	CMW5705	<i>Eucalyptus</i> sp.	Brazil	P.W. Crous	AF452508
<i>M. intermedia</i>	CMW7163	<i>E. saligna</i>	New Zealand	K. Dobbie	AY725546
<i>M. intermedia</i>	CMW7164	<i>E. saligna</i>	New Zealand	K. Dobbie	AY725547
<i>M. madeirae</i>	CBS112895/CPC3745	<i>E. saligna</i>	Madeira	S. Denman	AY725553
<i>M. marksii</i>	CMW10190 ¹	<i>E. globulus</i>	Ethiopia	Alemu Gezahgne, J. Roux	AY244404
<i>M. marksii</i>	CMW9090	<i>E. grandis</i>	South Africa	M.J. Wingfield	AF468870
<i>M. marksii</i>	CMW9091	<i>E. grandis</i>	South Africa	M.J. Wingfield	AF468871
<i>M. molleriana</i>	CMW4940/STE-U-1214	<i>E. globulus</i>	Portugal	S. McCrae	AF309620
<i>M. molleriana</i>	CMW2734/STE-U-784	<i>E. globulus</i>	USA	M.J. Wingfield	AF309619
<i>M. nubilosa</i>	CMW11148 ¹	<i>E. globulus</i>	Ethiopia	Alemu Gezahgne, J. Roux	AY244409
<i>M. nubilosa</i>	CMW11149 ¹	<i>E. globulus</i>	Ethiopia	Alemu Gezahgne, J. Roux	AY244411
<i>M. nubilosa</i>	CMW11150 ¹	<i>E. globulus</i>	Ethiopia	Alemu Gezahgne, J. Roux	AY244410
<i>M. nubilosa</i>	CBS116005	<i>E. globulus</i>	Australia	A.J. Carnegie	AF309618
<i>M. obnova</i>	CBS112896	<i>E. globulus</i>	South Africa	M.J. Wingfield	AF309604
<i>M. obnova</i>	CBS112973	<i>E. grandis</i>	South Africa	M.J. Wingfield	AF309605
<i>M. parkii</i>	CMW3358/CBS387.92	<i>E. grandis</i>	Australia	A.J. Carnegie	AF309590
<i>M. parva</i>	CBS116289	<i>Eucalyptus</i> sp.	South Africa	P.W. Crous	AY725576
<i>M. parva</i>	CBS110503	<i>E. globulus</i>	Australia	A. Maxwell	AY626980
<i>M. parva</i>	CMW10186 ¹	<i>E. globulus</i>	Ethiopia	Alemu Gezahgne, J. Roux	AY244405
<i>M. parva</i>	CMW10187 ¹	<i>E. globulus</i>	Ethiopia	Alemu Gezahgne, J. Roux	AY244406
<i>M. parva (grandis)</i>	CMW8554	<i>E. globulus</i>	Chile	M.J. Wingfield	DQ267584
<i>M. parva (grandis)</i>	CMW8557	<i>E. globulus</i>	Chile	A. Rortella	DQ267583

¹ Isolates collected from *E. globulus* in Ethiopia and sequenced in this study. All isolates are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. CBS refers to cultures from the collection of the Centraalbureau voor Schimmeldcultures, Utrecht, the Netherlands, STE refers to those from Stellenbosch University and CPC to those from the Culture collection of P. W. Crous housed at CBS.

plantations in south, south western and western Ethiopia (Table 1). At each locality where trees showed leaf spot symptoms, five to 10 symptomatic leaves per tree were collected per tree sampled.

The method described by CROUS (1998), was used to isolate and identify the *Mycosphaerella* spp. Two to four leaves were selected from each sample and four leaf discs containing lesions were excised from each leaf. These discs were then immersed in water for 2 h to moisten the pseudothecia and facilitate ascospore release. The leaf discs were then attached to the insides of Petri dish lids with the pseudothecia facing downwards over 2% malt extract agar (MEA; Biolab, Midrand, South Africa). The Petri dishes were kept in the dark at room temperature for 24 h for the active release and germination of the ascospores. After 24 h, plates were examined for germinating ascospores using a light microscope (Zeiss Axioskope, Carl Zeiss, Jena, Germany). Single germinating ascospores were picked up and transferred to 2% MEA plates and incubated at 25°C in the dark. Cultures resulting from germinated ascospores were incubated at 25°C under continuous light. Isolates obtained in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

2.2 Morphological characterization

Cultural characteristics, ascospore germination patterns and anamorph associations were used to differentiate the *Mycosphaerella* spp. associated with MLD in Ethiopia. Colony colour was determined using mycological colour charts (RAYNER 1970). Germinating ascospores for each sample were mounted in lactophenol on microscope slides and the germination patterns noted. Ascospore germination patterns were studied using a light microscope (Zeiss Axioskope) and compared with those described for *Mycosphaerella* spp. on *Eucalyptus* (CROUS 1998). To identify the anamorph states of the *Mycosphaerella* spp., isolates were grown on 1.5% water agar (Biolab) containing sterilized carnation leaves at 25°C under near ultraviolet light (NUV; 250 nm).

2.3 DNA extraction and PCR amplification

Isolates for DNA extraction (Table 1) were selected based on differences detected in culture morphology and ascospore germination patterns. Mycelium used for DNA extraction was scraped directly from the surface of cultures on agar plates. Mycelia were placed in Eppendorf tubes and freeze-dried under vacuum. DNA was isolated according to the method described by HUNTER et al. (2004a). Isolated DNA was visualized under ultraviolet light after electrophoresis on a 1% agarose gel containing ethidium bromide.

The ITS regions and 5.8S gene of the ribosomal RNA operon were amplified using Primers ITS 1 (5'-TCC GTA GGT GAA CCT GCG G-3'; WHITE et al. 1990) and LR 1 (5'-GGT TGG TTT CTT TTC CT-3'; VILGALYS and HESTER 1990). The PCR reaction mixture and reaction conditions were the same as those described by HUNTER et al. (2004a), except that the annealing temperature was set at 55°C and not 53°C. PCR amplicons were visualized on a 1.5% agarose gel, stained with ethidium bromide and viewed under UV illumination. Sizes of the PCR fragments were estimated using a 100 bp molecular weight marker (XIV; Roche Diagnostics, Johannesburg, South Africa). Prior to sequencing the PCR products were cleaned using the High Pure PCR product purification kit (Roche Diagnostics).

2.4 DNA sequencing and phylogenetic analysis

The purified PCR products were used as templates for DNA sequencing using an ABI Prism, Big Dye Terminator Cycle sequencing reaction kit (Perkin-Elmer Applied

Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Primers ITS 1 and LR 1 were used to sequence both strands of the amplicons. Sequencing reactions were analysed using an ABI PRISM™ 3100 automated DNA sequencer (Perkin-Elmer, Norwalk, CT, USA).

DNA sequences of the Ethiopian isolates used in this study were compared with sequences deposited in GenBank [National Centre for Biotechnology Information (NCBI), US National Institute of Health Bethesda, <http://www.ncbi.nlm.nih.gov/BLAST>] for preliminary identification. MAFFT (multiple alignment program for amino acid or nucleotide sequences) ver.5.667 (KATO et al. 2005) was used to align sequences. Sequences were aligned against those of *Mycosphaerella* spp. from an extensive in-house database emerging from previous studies (CROUS et al. 2001, 2004; HUNTER et al. 2004a,b) and obtained from GenBank (Table 1). Phylogenetic analysis of the aligned sequences was conducted using Phylogenetic Analysis Using Parsimony (PAUP) version 4.0b10 (SWOFFORD 2002). All uninformative and ambiguous data were excluded from the analysis. The sequences were analysed using parsimony, with the most parsimonious trees generated by heuristic searches, simple addition and Tree Bisection Reconstruction (TBR) branch swapping, with MULPAR effective. Bootstrap values for the branching points were calculated using 1000 replicates (FELSENSTEIN 1993). For the phylogenetic analysis, *Botryosphaeria ribis* Grossenb. & Duggar (CMW7773) was used as the outgroup taxon.

3 Results

3.1 Sample collection and isolation

Symptoms of MLD were found on juvenile foliage of *E. globulus* at several localities, including Wondo Genet, Hossana, Endibir, Bedele, Menagesha, Holeta and Addis Alem (Fig. 1). Disease symptoms, including shoot dieback and leaf blotch were common. In some cases, almost 100% of the juvenile leaves and leaf surfaces on a tree were affected. Lesions varied in size from small to large spots spreading over the whole leaf surface. Some lesions coalesced to form larger lesions. The lesions were light brown in colour and had raised brown margins. On some leaves, lesions were confined to the margins of the leaves. Other samples had leaf spots that extended through the leaf laminae, with lesions visible on both leaf surfaces with a light brown colour and a faint red margin.

Ascospores germinated within 24 h on 2% MEA. *Mycosphaerella* spp. were successfully isolated from samples collected from 16 trees. Ascospores from a number of samples failed to germinate, while some isolates died shortly after germination. Representative isolates were, however, obtained from all areas sampled.

3.2 Morphological characterization

When the growth of the *Mycosphaerella* spp. on MEA was considered, three culture morphologies were found. Group I isolates, collected from *E. globulus* leaves from Addis Alem, Endibir and Hossana had colonies of which the colour of the upper sides were olivaceous black 27''''m. Group II isolates, obtained from Hossana, Endibir, Holeta and Bedele showed a dark olivaceous grey colour, 23''''i. The third group included only one isolate (CMW10190) obtained near Hossana. This isolate had a pale olivaceous grey colour (23''''f).

Examination of the ascospore germination patterns of *Mycosphaerella* isolates obtained from Ethiopia showed three different germination patterns. These germination patterns could be directly correlated with the morphological groups defined based on culture morphology. Isolates belonging to Group I had an ascospore germination pattern closely

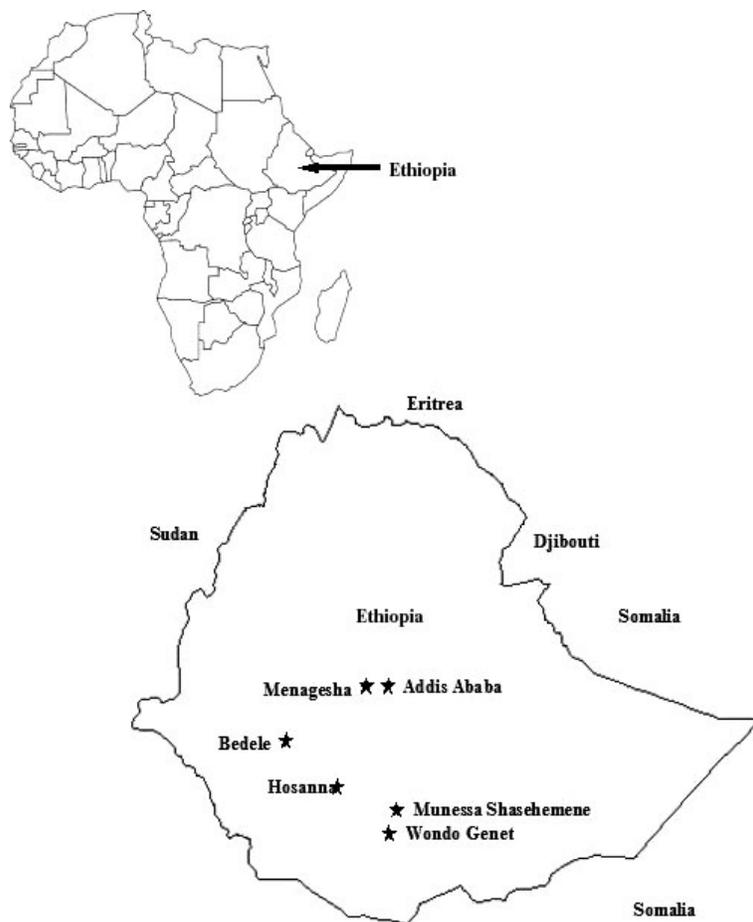


Fig. 1. Map of Ethiopia showing the main towns near collection points. Map from <http://www.About.com> (<http://geography.about.com/library/blank/blxethiopia.htm>)

resembling a Type F pattern as defined by CROUS (1998). This pattern is characteristic of *M. nubilosa* (CROUS et al. 2004). The isolates in Group II had Type C germination patterns. This type of germination is characteristic of *M. heimii*, *M. gregaria* Carnegie & Keane, *M. molleriana* and *M. walkerii* R. F. Park & Keane (CROUS 1998). The isolate obtained from Hossana had a Type B germination pattern which is associated with *M. gracilis* Crous & Alfenas and *M. marksii* (CROUS 1998). No anamorph structures were found for any of the Ethiopian *Mycosphaerella* isolates.

3.3 DNA sequencing and phylogenetic analysis

Amplification of the ITS region of the rRNA operon produced a fragment of approximately 600 bp for all *Mycosphaerella* isolates obtained from Ethiopia. A BLAST search using sequences of Ethiopian *Mycosphaerella* isolates showed that these isolates were closely related to three different *Mycosphaerella* spp. When the Ethiopian sequence data were incorporated into a larger database of sequences from previous studies (HUNTER

et al. 2004a,b), including those in GenBank, and analysed, one tree was generated through heuristic searches (Fig. 2). The number of characters in the analysed data set was 190, after the exclusion of constant and uninformative characters. The phylogenetic tree had consistency index (CI) and retention index (RI) values of 0.6294 and 0.8587 respectively. One of the Ethiopian *Mycosphaerella* isolates (CMW10190; Group III), grouped with *M. marksii* with 100% bootstrap support. Isolates from morphological Group I, grouped with *M. parva* (100% bootstrap support) and those from Group II resided in the *M. nubilosa* clade (100% bootstrap support).

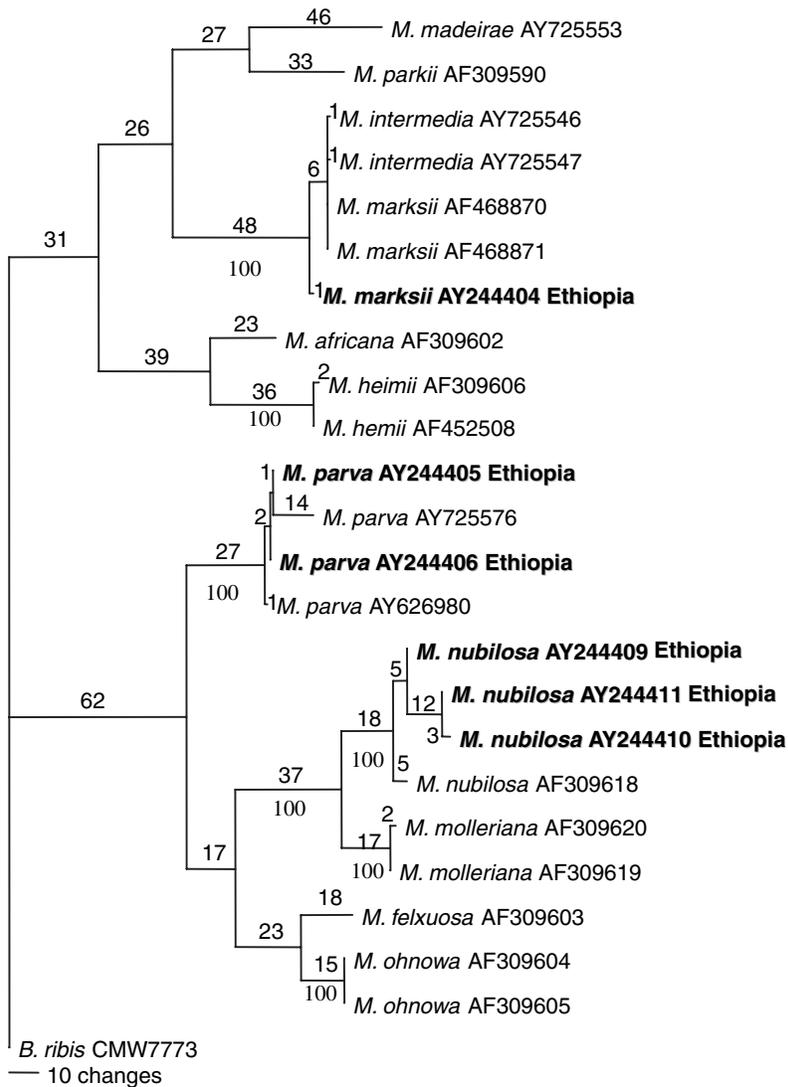


Fig. 2. A most parsimonious tree of the Internal Transcribed Spacer (ITS) sequence data of *Mycosphaerella* spp. Consistency index (CI) = 0.6294 and retention index (RI) = 0.8587. Bootstrap values are shown below each branch, while branch lengths appear above the branches

4 Discussion

Mycosphaerella leaf disease was the most common foliage disease observed on *E. globulus* in Ethiopia during surveys in 2000 and 2001 (ALEMU et al. 2003). Results of the present study provide the first identification of this group of fungi on *Eucalyptus* in Ethiopia. Three *Mycosphaerella* spp., namely, *M. marksii*, *M. nubilosa* and *M. parva* were thus identified and this study represents the first report of these species on *Eucalyptus* spp. from Ethiopia.

Ascospore germination patterns present a useful method to differentiate between *Mycosphaerella* spp. (PARK and KEANE 1982a; CROUS 1998). CROUS (1998) described 14 types of ascospore germination patterns for *Mycosphaerella* spp. from *Eucalyptus* spp. Examination of the ascospore germination patterns from Ethiopia revealed several different germination types. Living isolates could be grouped into three germination types, suggesting that three different species of *Mycosphaerella* were linked to MLD in Ethiopia. The occurrence of three different species was supported by DNA sequence data confirming the value that germination patterns have when identifying *Mycosphaerella* spp. Additional surveys might, however, be able to produce living isolates belonging to some of the other germination patterns observed.

A single isolate of *M. marksii* was found from a leaf sample collected from *E. globulus* near Hossana. Previous studies have shown that *M. marksii* occurs on several *Eucalyptus* spp., including *E. globulus*, *E. grandis*, *E. nitens* and *E. saligna* (CARNEGIE and KEANE 1994). It has also been shown that *M. marksii* is phylogenetically similar to *M. intermedia* M. A. Dick & Dobbie, with these two species grouping together in the same clade in a study published by CROUS et al. (2004). This is similar to the situation found in the present study, where we could not distinguish between these two species based on ITS sequence data alone. *Mycosphaerella marksii* was first described in Australia and it is now known to occur in South Africa, Indonesia, Portugal and Uruguay (CARNEGIE and KEANE 1994; CROUS and WINGFIELD 1996; CROUS 1998). This fungus is common in Australia and South Africa, but has not been reported to cause significant damage (CARNEGIE 2000; HUNTER et al. 2004a). Because the fungus was collected only from a single leaf, it is probably not an important component of the MLD problem in Ethiopia.

Mycosphaerella parva was found on samples collected from Addis Alem, Endibir and Hossana. This fungus was first described from Australia on *E. globulus* (PARK and KEANE 1982b), causing necrotic lesions at the margins of leaves. This type of symptom was common in Ethiopia, suggesting that *M. parva* is one of the more important components of MLD in the country. Ethiopia is only the third country from which it has been reported, and based on its relative abundance among isolates collected in this study, might play a major role in MLD outbreaks in this country.

Mycosphaerella nubilosa was found in several areas including Endibir, Holeta, Hossana and Bedele. This species mostly affects juvenile leaves of *E. globulus* and is one of the most common and destructive pathogens of *E. globulus* in Australia and New Zealand (PARK and KEANE 1982a; CARNEGIE et al. 1994) and *E. nitens* in South Africa (PURNELL and LUNDQUIST 1986; HUNTER et al. 2004a,b). *Mycosphaerella nubilosa* was associated with severe damage to *E. globulus* in South Africa in the early 1900s and resulted in the abandonment of this species (PURNELL and LUNDQUIST 1986). It has also been reported from a number of other African countries, including Kenya, Tanzania and Zambia (CROUS et al. 2004). Thus, the presence of *M. nubilosa* in Ethiopia explains the serious defoliation of *E. globulus* observed in this country. This fungus should be placed on the list of more important constraints to *E. globulus* propagation in the future.

This study has shown that MLD is common, wherever *E. globulus* is grown in Ethiopia. Previous studies have shown that infection by *Mycosphaerella* spp. not only causes premature defoliation and retarded growth, but can also lead to the abandonment of

planting certain *Eucalyptus* spp. (LUNDQUIST and PURNELL 1987). As *E. globulus* is a widely planted species in Ethiopia, the discovery of *M. nubilosa* is of concern, especially given the fact that plantations of *E. globulus* are likely to be expanded in the future. Wide variation in susceptibility to MLD within families and provenances of *E. globulus* has been observed (CARNEGIE et al. 1994; DUNGEY et al. 1997; MILGATE et al. 2005). Selection of species and provenances with tolerance to MLD might, therefore, help to minimize loss of yield caused by *Mycosphaerella* species in Ethiopia in the future.

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Résumé

Espèces de Mycosphaerella associées à une maladie foliaire d'Eucalyptus globulus en Ethiopie

Les *Eucalyptus* comptent parmi les essences d'arbres exotiques les plus plantées en Ethiopie. Plusieurs pathogènes foliaires sont connus dans le monde pour occasionner des dégâts sur *Eucalyptus*. Parmi ceux-ci, les espèces de *Mycosphaerella* sont parmi les plus importantes, causant la maladie connue comme Maladie Foliaire à *Mycosphaerella* (MFM, MLD en anglais). Les symptômes caractéristiques de la MFM comprennent des taches foliaires, une défoliation précoce et des dépérissements de pousses et de rameaux. Des campagnes de surveillance menées récemment dans les plantations éthiopiennes d'*Eucalyptus* ont révélé la présence de tels symptômes. Ces symptômes sont uniquement observés sur *E. globulus* dans plusieurs localités du sud, sud-ouest et ouest de l'Ethiopie. L'objectif de cette étude était d'identifier les champignons associés à cette maladie. Pour cela, des isolats représentatifs ont été étudiés pour les modalités de germination des ascospores, les anamorphes associés ainsi que les données de séquence de la région ITS de l'opéron ADNr. Différentes modalités de germination des ascospores ont été observées, suggérant que plusieurs espèces de *Mycosphaerella* seraient associées à la MFM sur *E. globulus* en Ethiopie. L'analyse des données de séquence a montré la présence de 3 espèces : *M. markesii*, *M. nubilosa* et *M. parva*. Ceci constitue la première mention de ces 3 espèces en Ethiopie et une première étape pour envisager d'autres études dans cette région.

Zusammenfassung

Mit Blattkrankheiten an Eucalyptus globulus assoziierte Mycosphaerella-Arten in Äthiopien

Eucalyptus-Arten sind die am häufigsten angepflanzten exotischen Bäume in Äthiopien. An *Eucalyptus* kommen verschiedene Blattkrankheiten vor, wobei die *Mycosphaerella*-Arten als Verursacher der *Mycosphaerella*-Blattkrankheit (MLD) am bedeutendsten sind. Charakteristische Symptome der MLD sind Blattnekrosen und vorzeitiger Blattfall sowie Trieb- und Zweigsterben. Bei der Inventur von Krankheiten in äthiopischen *Eucalyptus*-plantagen wurden Symptome entdeckt, die denen von *Mycosphaerella* spp. ähnlich waren. Diese traten nur an *E. globulus* lokal in S-, SW- und W-Äthiopien auf. Ziel dieser Untersuchung war es, die damit assoziierten Pilze zu identifizieren. Hierzu wurde an repräsentativen Isolaten das Keimverhalten der Ascosporen, das Vorkommen von Anamorphen und die ITS-Sequenz des rRNA-Operons untersucht. Es wurden verschiedene Keimungstypen der Ascosporen beobachtet, was darauf schliessen liess, dass mehr als eine *Mycosphaerella*-Art für die Krankheit an *E. globulus* in Äthiopien verantwortlich ist. Anhand der Sequenzen wurden *M. markesii*, *M. nubilosa* und *M. parva* identifiziert. Dies ist der Erstdachweis für diese drei Arten in Äthiopien und eine Grundlage für weitere Studien.

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