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A diverse assemblage of Botryosphaeriaceae infect *Eucalyptus* in native and non-native environments

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The Botryosphaeriaceae cause endophytic infections of leaves and bark of various trees, including *Eucalyptus*, and they apparently persist in this state for extended periods of time. Under conditions of stress, these fungi cause many different disease symptoms on *Eucalyptus*, of which stem and branch cankers and die-back are the most prominent. Given their cryptic, endophytic nature, the Botryosphaeriaceae are easily overlooked when moving seeds and plants around the world. It is, therefore, not surprising to see a growing number of examples of introductions of Botryosphaeriaceae into new environments. In the past, three species were commonly reported from *Eucalyptus*, namely *Botryosphaeria dothidea, Neofusicoccum ribis* (reported as *B. ribis*) and *Lasiodiplodia theobromae*. It is now known that *B. dothidea* and *N. ribis* are generally rare on *Eucalyptus*, and that *Aplosporella yalgorensis*, *B. mamane, N. parvum*, *N. eucalyptorum*, *N. eucalypticola*, *N. australe*, *N. macroclavatum*, *N. andinum*, *N. mangiferum*, *Dichomera eucalypti*, *Dichomera versiformis*, *Fusicoccum ramosum*, *Pseudofusicoccum stromaticum*, *P. adansoniae, P. ardesiarum, P. kimberleyense*, *Lasiodiplodia crassispora, L. gonubiensis, L. pseudotheobromae* and *L. rubropurpurea* also infect this host. Interestingly, different species dominate on *Eucalyptus* in different regions of the world, irrespective of whether other species occur in that environment or not. As examples, in parts of eastern Australia, *N. eucalyptorum* and *N. eucalypticola* dominate, although *N. australe* is common on *Acacia* spp. in this area, while in Western Australia *N. australe* dominates. In South Africa and Chile *N. parvum*, *N. eucalyptorum* and *N. eucalypticola* are common, despite the presence of *N. ribis* and *N. australe* on related hosts such as *Syzygium*. In Venezuela, there are five other species not common on *Eucalyptus* elsewhere, but *L. theobromae* dominates. In Colombia, *B. dothidea* and *N. ribis,* and in Uganda and Ethiopia, *L. theobromae* and *N. parvum,* are most common. These fascinating patterns of distribution are explored, while their pathogenicity and potential influence on *Eucalyptus* plantations and surrounding native plant communities are considered.

Keywords: *Botryosphaeria*, Botryosphaeriaceae, canker, die-back, endophyte, *Eucalyptus*, latent pathogen

Introduction

The fungal family Botryosphaeriaceae includes thousands of described species from around the world that occur on various, primarily woody hosts (von Arx 1987, Index Fungorum Partnership 2004, Crous et al. 2006). Many species of the Botryosphaeriaceae are known as pathogens, most commonly causing die-back and canker diseases on twigs, branches and trunks of trees, and more rarely diseases such as seed-capsule abortion, witches-broom, leaf diseases, seedling diseases and root cankers (Sinclair and Lyon 2005, Slippers and Wingfield 2007). Species of Botryosphaeriaceae are, however, treated as opportunistic pathogens, because the diseases they cause are almost always associated with stress or wounding to their host plants. Despite their pathogenic abilities, Botryosphaeriaceae are also well known as endophytes of above-ground parts of woody plants, apparently existing for long periods of time in the absence of symptoms (Slippers and Wingfield 2007).

Eucalyptus species, both in their native and introduced ranges, are known to be commonly infected by various species of the Botryosphaeriaceae (Smith et al. 1994, Slippers et al. 2004a, Burgess et al. 2005, Mohali et al. 2007, and others cited in the remainder of this paper). While these fungi are often not as aggressive as some primary pathogens, the die-back and canker diseases caused by Botryosphaeriaceae on *Eucalyptus* are amongst the most common and, under some conditions, the most serious diseases affecting these trees. For this reason, and also because of their wide distribution and ability to infect healthy trees, these fungi have been amongst the pathogens having the greatest negative impact on *Eucalyptus*. This is especially true in non-native environments where *Eucalyptus* have been grown in plantations or woodlots under marginal or less than ideal conditions.

The taxonomy of the Botryosphaeriaceae is complex and impossible to treat based solely on morphology, due to the paucity of defining characters that can clearly delimit the multitude of species (Denman et al. 2000, Slippers et al. 2004b, Crous et al. 2006). Similarly, host association, which has been used to define species in some cases, can be drastically misleading because some species appear to be host specific, while others have broad host ranges. For these reasons, the taxonomic history of the group is confusing. Such confusion has seriously hampered the correct identification of the Botryosphaeriaceae on *Eucalyptus*, and consequently also attempts to make sense of their biology, distribution and means to manage diseases that they cause.

In recent years, molecular tools have contributed substantially to resolve the taxonomic difficulties that have characterised the Botryosphaeriaceae (Crous et al. 2006). Information obtained from sequence data, PCR-RFLPs, species-specific primers, ISSRs, RAPDs and SSRs have all been used successfully to distinguish species in this group (Slippers and Wingfield 2007). These data have frequently been combined with morphological characters and in particular conidial morphology, to characterise and describe species (Smith et al. 2001, de Wet et al. 2003, Pavlic et al. 2004, Slippers et al. 2004a, Burgess et al. 2005, and others cited in the remainder of this paper). By means of these combined morphological and molecular phylogenetic studies, consistent groups have become evident within the Botryosphaeriaceae, typified by specific anamorph types. Crous et al. (2006) extended these observations to describe several genera amongst species that were typically referred to as *Botryosphaeria* species or asexual states (anamorphs) of the genus.

Molecular genetic tools and recent developments in the taxonomy of the Botryosphaeriaceae have been applied to studies of the causal agents of Botryosphaeria diseases on *Eucalyptus*. The results have revealed a diverse assemblage of Botryosphaeriaceae infecting this host. This has significant implications regarding our understanding of the biology, ecology and control of this important group of fungi on *Eucalyptus*. We review the most important of these implications in this paper.

Identification and recent taxonomic changes

The lack of distinguishing morphological and ecological characters to delineate species of the Botryosphaeriaceae has necessitated the distinguishing power of molecular tools to accurately and objectively separate and identify species. For this reason, any identification of species in this group must begin with analysis of sequence data, particularly for ITS rDNA sequence data and comparisons with well-characterised species. It is, however, recognised that molecular data should be interpreted with caution and that they should incorporate all knowledge of the organism. This is true, even for taxonomic specialists working with species from new hosts and areas, but even more so for plant pathologists and others performing routine identification of pathogens.

Subsequent to the first application of ITS rDNA sequence data to distinguish species and genera in the Botryosphaeriaceae (Jacobs and Rehner 1998), more than 1 000 sequences have been produced for this locus of members of this group (GenBank, http://www.ncbi.nlm.nih.gov; and see representative sample in Figure 1). All of the species known to occur on *Eucalyptus* are represented by sequences in GenBank, some by a number of isolates. This provides an appropriate starting point for both specialist and non-specialist alike. Caution should, however, be applied when identifying species solely based on GenBank BLAST analysis. This is because the latest taxonomy for the Botryosphaeriaceae is not reflected in the binomials assigned to sequences in the database; neither have isolates from which sequences are derived always been correctly identified. Identification of the closest-related sequences through a BLAST search should ideally be followed by phylogenetic analysis using sequences from various authenticated samples (from GenBank sequences linked to taxonomic papers and/or ex-type isolates) and including the sequence found by BLAST as the closest relative. A number of datasets of taxonomic importance can also be found in the alignment database TreeBASE (http://www.treebase.org).

Numerous studies have shown that there are cryptic species within taxa identified by morphology, sometimes even when combined with ITS sequence data. Recently diverged species are expected to show little sequence divergence in any one particular locus. Deciding where the species delimitation is, based on sequence divergence alone, can be very subjective (Taylor et al. 2000). A more objective measure, which has been applied widely in the group, is to consider concordance between sequence datasets for multiple unlinked loci (e.g. Taylor et al. 2000, de Wet et al. 2003, Slippers et al. 2004a, 2004b, 2004c, Burgess et al. 2005, Mohali et al. 2006). In this way species such as *Diplodia pinea* and *D. scrobiculata* (de Wet et al. 2003), *Neofusicoccum eucalyptorum* and *N. eucalypticola* (Slippers et al. 2004a), *N. parvum* (syn. *Botryosphaeria parva*) and *N. ribis* (syn. *B. ribis*) (Slippers et al. 2004b), *N. luteum* and *N. australe* (Slippers et al. 2004c), have been successfully separated and subsequently characterised based on morphology, geographical distribution and ecology. The distinction of these cryptic species has made a significant contribution to the understanding of the evolution and ecology of these important pathogens. In the above-mentioned studies partial sequence data for the Elongation Factor 1-α (commonly) and β-tubulin (less commonly) gene loci have most frequently been used, together with ITS rDNA sequence data.

Multilocus markers can be used for population genetic analyses, but they have also been successfully used to distinguish species in the Botryosphaeriaceae. RAPD data have, for example, been used to confirm the separation of

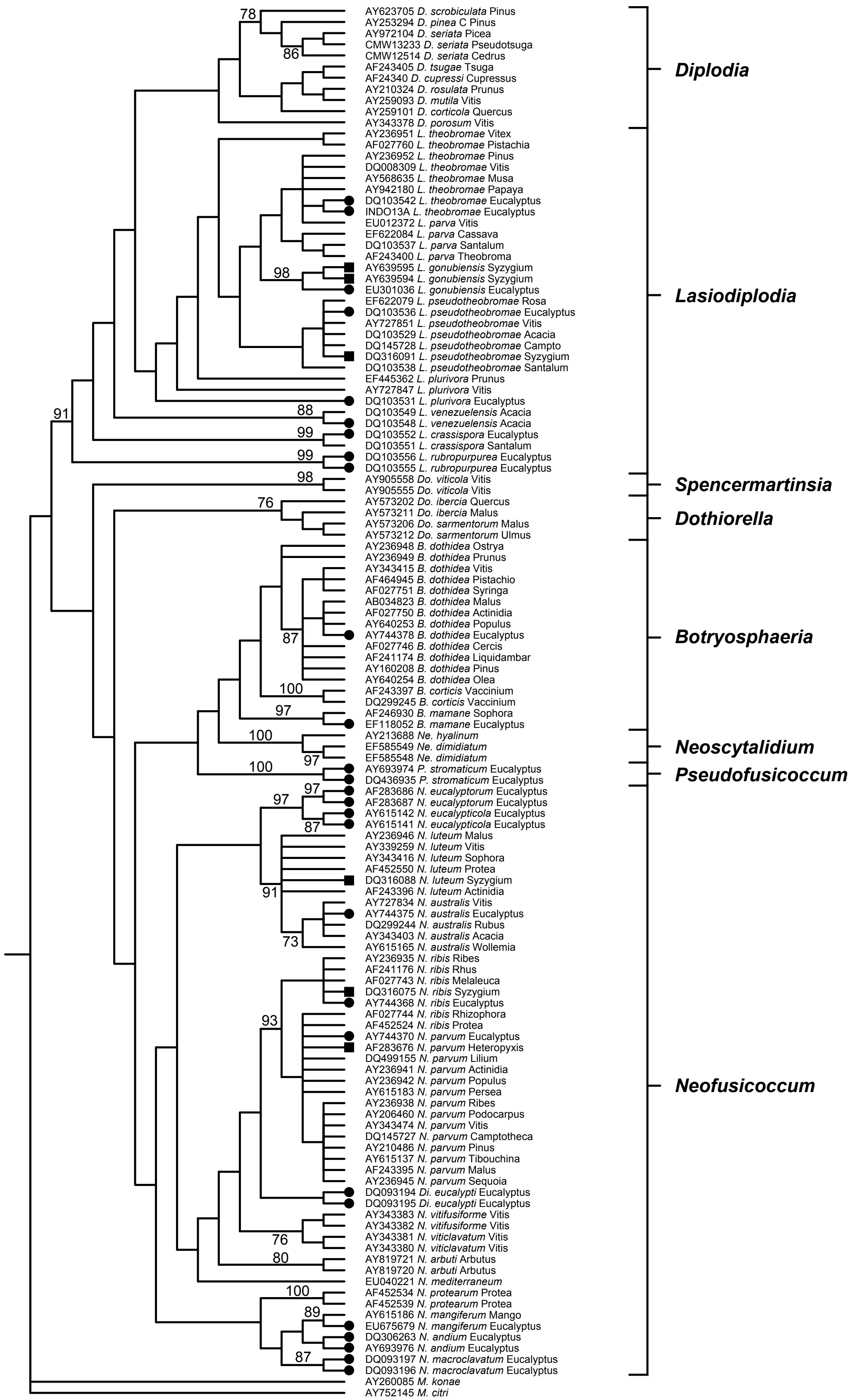

Figure 1: A simple phylogeny of the Botryosphaeriaceae based on ITS rDNA sequence data available in GenBank. Isolates from *Eucalyptus* are labeled with a solid circle and isolates from other Myrtaceae with a solid square. Bootstrap values >70% are indicated beside the branches

the different 'morphotypes' of *D. pinea* (Smith and Stanosz 1995). These forms were subsequently recognised as distinct species (de Wet et al. 2003). Smith and Stanosz (2001) have also used ISSRs to distinguish various *Neofusicoccum* and *Fusicoccum* species. Likewise, SSR markers can be very powerful in delineating cryptic species of the Botryosphaeriaceae (de Wet et al. 2003).

Once species have been identified from a particular host or area, fingerprinting techniques can be used to screen large numbers of isolates rapidly and reliably. For example, PCR-RFLP has been widely used for this purpose, also on isolates from *Eucalyptus*. Slippers et al. (2004a) used the restriction enzymes *Cfo*I, *Ksp*I and *Sty*I with ITS rDNA amplicons to distinguish five species occurring on *Eucalyptus* in South Africa and elsewhere. Mohali et al. (2006) used the same technique with *Cfo*I on ITS rDNA amplicons, as well as an unknown locus, to distinguish six species of Botryosphaeriaceae from *Eucalyptus* in Venezuela. Alves et al. (2005) were able to distinguish 10 Botryosphaeriaceae species using a larger PCR amplicon of the rDNA operon and treating this with six restriction enzymes.

An approach that is currently under-explored, but rising in popularity, is the application of species-specific primers for identification. Such primers have been developed for *D. pinea* and are proving very sensitive in identification of this pathogen *in vivo* (Luchi et al. 2005, Smith and Stanosz 2006). Development of species-specific primers for Botryosphaeriaceae that occur on *Eucalyptus* in South Africa is part of an ongoing project (authors' unpublished data). These primers allow fast and effective *in vivo* identification of Botryosphaeriaceae, and they are bound to be very valuable in studying ecological aspects of these fungi. They could also be useful to screen apparently healthy plant material that is destined for export.

Morphology remains the basis of taxonomic descriptions of fungal taxa, including those in the Botryosphaeriaceae. It is also a route to compare new records with species that were described long ago and for which no living specimens are available. The most useful morphological characters for species identification are the conidial size, shape, septation, wall characters and colour. The anamorphs of the Botryosphaeriaceae are fortunately also commonly encountered on infected tissue in nature. When the anamorph has not been observed *in vivo,* it can be readily induced in culture, most effectively on water agar supplemented with a substrate such as a host twig or pine needles. Culture morphology has also proven to be a fairly robust characteristic to help identify certain species.

The recent description of a number of new genera in the Botryosphaeriaceae (Crous et al. 2006) impacts heavily on the taxonomy of these fungi on *Eucalyptus*. For many years, two main groups were recognised within 'Botryosphaeria', namely species with *Fusicoccum*-like, hyaline and mostly narrow (fusiform) conidia, and those with *Diplodia*-like, often darker and broader (elipsoidal) conidia. Crous et al. (2006) used a larger sample and sequence dataset than most previous studies and distinguished 10 lineages within the Botryosphaeriaceae. Many of these were recognised as genera, corresponding largely to anamorph conidial characters. Species of four of these genera occur on *Eucalyptus*, namely *Botryosphaeria* (anamorph *Fusicoccum*), *Neofusicoccum* and *Pseudofusicoccum* (formally in *Fusicoccum*), and *Lasiodiplodia* (Table 1, Figure 2). Of these, *Neofusicoccum* is the most common and diverse genus on *Eucalyptus* in most areas of the host distribution, while *Lasiodiplodia* spp. tend to dominate in tropical environments.

Botryosphaeriaceae recorded from *Eucalyptus*

Prior to 1995, 27 taxa in the genera *Botryosphaeria*, *Diplodia*, *Dothiorella* and *Lasiodiplodia* had been described from *Eucalyptus* tissue and these have a cosmopolitan distribution (Sankaran et al. 1995). Of these taxa, *B. ribis* (Davison and Tay 1983, Webb 1983, Shearer et al. 1987, Crous et al. 1989, Old et al. 1990) and *B. dothidea* (Barnard et al. 1987, Fisher et al. 1993, Smith et al. 1994) have most commonly been reported as the causal agents of disease, especially of cankers and die-back, in various temperate areas of the world where *Eucalyptus* spp. are grown. From tropical environments, *Lasiodiplodia theobromae* (syn. *B. rhodina* and *Botryodiplodia theobromae*) has commonly been reported (Sharma et al. 1984, Sankaran et al. 1995, Roux et al. 2000, 2001). Given the taxonomic confusion for the group as a whole, the taxonomic validity of some of these names is in question. In some cases, they are known to have been confused with other fungi. Their taxonomic placement is, however, not the focus of this review.

During the course of the last five years, 23 species of Botryosphaeriaceae have been confirmed or described as new from *Eucalyptus,* using contemporary identification tools and taxonomic conventions (Table 1). Some classifications are only clear after reanalysis of the original data. For example, Mohali et al. (2005) reported *L. theobromae* from *Eucalyptus* in many tropical countries. However, reevaluation of data following the recent descriptions of *L. crassispora* and *L. rubropurpurea* (Burgess et al. 2006a), *L. pseudotheobromae* and *L. parva* (Alves et al.*,* 2008) have determined that *L. theobromae*, *L. pseudotheobromae, L. crassipora* and *L. rubropurpurea* have all been isolated from eucalypts in the tropics.

It is clear from currently unpublished work that there are more undescribed species occurring on eucalypts (*Eucalyptus* and *Corymbia*) from previously unexplored areas, as well as from newly established *Eucalyptus* plantations around the world. The most common of recently reported Botryosphaeriaceae are *N. parvum* in temperate areas of Africa, Australia and South America, *N. australe* in Western Australia, *N. eucalyptorum* and *N. eucalypticola* in Chile and eastern Australia, South African temperate areas and *Lasiodiplodia* spp*.* in tropical areas. The two most common and diverse Botryosphaeriaceae genera on *Eucalyptus* are *Neofusicoccum* and *Lasiodiplodia*, while others such as *Diplodia* and *Dothiorella* are notably absent, even though they are present on other hosts in the areas where *Eucalyptus* are grown.

Some of the Botryosphaeriaceae occurring on *Eucalyptus* have been reported only rarely and from confined geographic areas. For example, *B. mamane, N. andinum*, *P. stromaticum* and *L. crassispora* have thus far been reported only from *Eucalyptus* in Venezuela (Burgess et al.

Table 1: Botryosphaeriaceae reported from *Eucalyptus* spp. in recent years of which the identity has been confirmed using molecular data. Note that this does not consider the total geographic range of these fungi, reported from other hosts

Species	Country	Reference
Aplosporella yalgorensis	Western Australia	Taylor et al. (2009)
Botryosphaeria dothidea (anamorph *Fusicoccum aesculi*)	Colombia, South Africa, Uruguay, Australia	Mohali et al. (2007), Pérez et al. (2008), Maleme (2009), Rodas et al. (2009), Taylor et al. (2009), T Burgess (unpublished data)
B. mamane	Venezuela	Mohali et al. (2007)
Fusicoccum ramosum	Western Australia	Pavlic et al. (2008)
Neofusicoccum parvum	Australia, Chile, China, Ethiopia, Indonesia, South Africa, Uganda, Uruguay, Venezuela	Ahumada (2003), Gezahgne et al. (2004), Slippers et al. (2004a), Barber et al. (2005), Mohali et al. (2007), Pérez et al. (2008), Maleme (2009), T Burgess (unpublished data)
N. ribis[1]	Eastern Australia, China, Colombia	Barber et al. (2005), Mohali et al. (2007), Rodas et al. (2009), T Burgess (unpublished data)
N. australe	Western Australia, South Africa	Burgess et al. (2005, 2006b), Taylor et al. (2009)
N. macroclavatum	Western Australia	Burgess et al. (2005)
N. mangiferum	China	T Burgess (unpublished data)
N. eucalyptorum	Eastern Australia, Chile, South Africa, Uruguay	Ahumada (2003), Slippers et al. (2004a), Pérez et al. (2008)
N. eucalypticola	Eastern Australia, Chile, South Africa	Ahumada (2003), Slippers et al. (2004a), Pérez et al. (2008)
N. andinum	Venezuela	Mohali et al. (2006)
Neofusicoccum sp. (syn. *Dichomera eucalypti*)	Australia, South Africa	Barber et al. (2005), Maleme (2009), Taylor et al. (2009)
Neofusicoccum sp. (syn. *Dichomera versiformis*)	Eastern Australia	Barber et al. (2005)
Pseudofusicoccum stromaticum	Venezuela	Mohali et al. (2006)
P. adansoniae	Western Australia	Pavlic et al. (2008)
P. ardesiarum	Western Australia	Pavlic et al. (2008)
P. kimberleyense	Western Australia	Pavlic et al. (2008)
Lasiodiplodia theobromae	Wastern Australia, Congo, Uganda, Venezuela	Roux et al. (2000, 2001), Nakabonge (2002), Burgess et al. (2006a), Mohali et al. (2007), T Burgess (unpublished data)
L. crassispora	Venezuela	Burgess et al. (2006a)
L. gonubiensis	Eastern Australia	T Burgess (unpublished data)
L. pseudotheobromae	Eastern Australia, Venezuela	Mohali et al. (2005) (following reanalysis after Alves et al. 2008)
L. rubropurpurea	Eastern Australia	Burgess et al. (2006a)

[1] The taxonomy of this species is uncertain. While these identifications confirmed that these isolates are related to *B. ribis* type isolates, there was also some phylogenetic distance between them

2006a, Mohali et al. 2006), while *Aplosporella yalgorensis*, *N. macroclavatum*, *Dichomera versiformis* and *L. rubropurpurea, Fusicoccum ramosum*, *P. adansoniae, P. ardesiarum* and *P. kimberleyense* have only been isolated from this host in localised areas of Australia (Burgess et al. 2005, 2006a, Pavlic et al. 2008, Taylor et al. 2009). These species most likely originated from native tree hosts in their areas of occurrence, as has been shown for various other species. The areas of Venezuela and Australia treated in the above-mentioned studies were sampled fairly intensively. This revealed the rarer species and it is likely that they, or other species, might be present elsewhere, but just have not yet been sampled.

Interestingly, some species of Botryosphaeriaceae seem to infect *Eucalyptus* only in some parts of the distribution of these trees. For example, *N. australe* is known to occur commonly and on various hosts throughout Australia and South Africa (Slippers et al. 2004b, 2004c, Pavlic et al. 2007), but it infects *Eucalyptus* only in western Australia, and it is the dominant species (>90% of over 300 isolates) of the Botryosphaeriaceae on this host in that area (Burgess et al. 2005, 2006b). *Neofusicoccum mangiferum* has been isolated from cankered *Eucalyptus* in China, but never in Australia or South Africa even though it is present on mangoes in these countries. *Neofusicoccum parvum* dominates in eastern Australia, but has not been found reported from Western Australia (TB, unpublished data). Neither *N. australe* nor *N. parvum* have been recorded in the island state of Tasmania, where *N. eucalyptorum* and *N. eucalypticola* are found (Burgess et al. 2006b).

A phylogeny of Botryosphaeriaceae for which ITS sequence data is available in GenBank (Figure 1), shows that the Botryosphaeriaceae occurring on *Eucalyptus* are not all closely related, not even those occurring on native *Eucalyptus* in Australia. Thus, there appears to be little host-associated coevolution or cospeciation (see also de Wet et al. 2008). The only two sister species that might have speciated on *Eucalyptus* are *N. eucalyptorum* and *N. eucalypticola*. These species are known only from *Eucalyptus* and they are common on this host in areas where these trees are native, such as eastern Australia and Tasmania (Slippers et al. 2004a, Burgess et al. 2006b). *Neofusicoccum macroclavatum* (western Australia) and *N. andinum* (Andes in Venezuela) are also sister taxa, but from two geographically separated areas. This lack of host-associated coevolution is not entirely surprising, given that many Botryosphaeriaceae

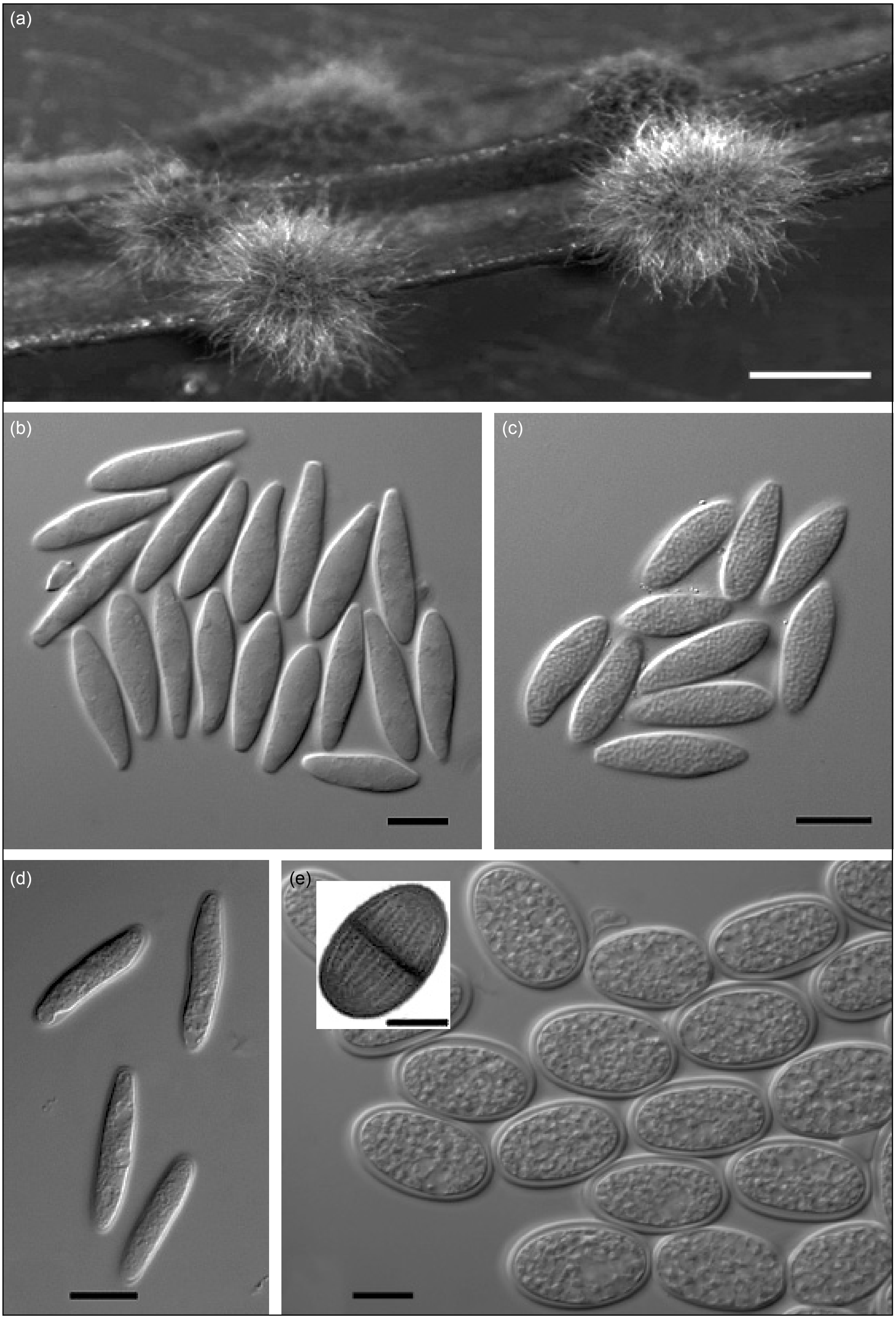

Figure 2: *In vitro* sporulation of representatives of anamorph Botryosphaeriaceae genera associated with *Eucalyptus.* (a) Typical pycnidia-covered mycelium forming on pine needles on water agar medium. This technique has been applied on most Botryosphaeriaceae from *Eucalyptus* to stimulate *in vitro* sporulation. Scale bar = 100 µm. (b) *Fusicoccum* anamorph of *Botryosphaeria*. (c) Conidia of a *Neofusicoccum* sp. (d) *Pseudofusicoccum* sp. conidia with persistent mucus layer. (e) Immature ellipsoid conidia of a *Lasiodiplodia* sp., which become dark, septate and longitudinally striate with age (insert). Scale bars = 10 µm

have broad host ranges. However, it does reflect the importance of considering host jumps and new host associations in Botryosphaeriaceae evolution and control. This would be from related or even unrelated hosts onto *Eucalyptus* in native and non-native areas (also see Slippers et al. 2005, Slippers and Wingfield 2007).

The threat of Botryosphaeriaceae to *Eucalyptus* and related plants following anthropogenic movement

The Botryosphaeriaceae are not usually thought of as organisms of serious quarantine importance, although there are species that occur on quarantine lists in some countries. There are nevertheless several reasons to consider them important as invasive or potentially invasive organisms (Wingfield et al. 2001, Desprez-Loustau et al. 2007, Slippers and Wingfield 2007). Firstly, they are very common in most environments and virtually all seedling and more mature germplasm moved across the world is bound to contain some infections of Botryosphaeriaceae. Some Botryosphaeriaceae can also be seed-borne (Cilliers et al. 1993, Gure et al. 2005). Furthermore, their cryptic, endophytic habit for a large part of their life cycle makes them very difficult to detect.

Anthropogenic-driven climate change is now accepted as a reality that will have far-reaching influences on plant communities and their interactions with pests and pathogens worldwide (Coakley et al. 1999, Desprez-Loustau et al. 2006). Not all these influences will be negative, but factors such as increasing temperatures, droughts, floods, range expansions for pests and pathogens, pressure on mutualistic partners and other factors are likely to increase stress on many plant communities. Under these conditions the Botryosphaeriaceae, as stress-associated opportunistic pathogens with wide occurrence, are likely to cause serious disease problems (Desprez-Loustau et al. 2006, Slippers and Wingfield 2007).

There are numerous examples of Botryosphaeriaceae being moved across the world, sometimes apparently in high frequency. For example, *D. pinea* is more diverse in South Africa than in some areas of its native range in Europe (Burgess et al. 2004). *Pinus* spp. are introduced in the Southern Hemisphere and, therefore, the host-specific *D. pinea* has moved with its host. This intriguing, yet unfortunate, situation could have occurred only following recurrent introductions from various native areas in the Northern Hemisphere (Wingfield et al. 2001). In fact, the study of Burgess et al. (2004) showed a direct correlation between the numbers and routes of seed introduction with the diversity of this pathogen in the countries considered. There is clearly a great need to characterise additional populations of Botryosphaeriaceae with the same vigour as has been applied for *D. pinea*, to better understand their pathways and the extent of introductions and invasions. These fungi would also serve as excellent bioindicators to map the extent of fungal introductions from different areas of the world.

Once a species of Botryosphaeriaceae has been introduced into a new area, it is quite possible that it would spread to and infect other hosts, both related or unrelated to the host with which it entered the new environment. In a study of Botryosphaeriaceae occurring on native Myrtaceae in South Africa and the related, introduced *Eucalyptus*, two species (*N. parvum* and *L. theobromae*) were found to coinfect both hosts, and they are thus likely to move between them (Pavlic et al. 2007). Consequently, there is a threat of native Botryosphaeriaceae in this area causing disease on the introduced hosts and, likewise, for introduced Botryosphaeriaceae from *Eucalyptus* to cause disease on native plants. A study presented at the IUFRO WP 2.08.03 congress in Durban, October 2007, investigated the similarities in Botryosphaeriaceae infecting native Myrtaceae and introduced *Eucalyptus* in Uruguay, and it also shows that such host jumps are occurring (Pérez et al. 2008). These occurrences might thus not be uncommon. Where novel host–pathogen encounters occur following host jumps of pathogens after introduction, the naïve recognition and defense systems of the new hosts could have very serious consequences for disease and epidemic development (Slippers et al. 2005).

Burgess et al. (2006b) showed high levels of gene flow of *N. australe* between native *Eucalyptus* forests and *E. globulus* plantations (non-native in Western Australia), which highlights the threat to these plant communities if virulent pathogens were to be introduced together with high levels of germplasm movement that is occurring in some areas (see also Burgess and Wingfield 2002a, 2002b). This is especially important within the context of increasing populations of local pathogens on non-native *Eucalyptus* in other parts of the world. Such populations would be present in higher than normal densities, causing increased propagule pressure and thus their risk of introduction.

Control

The Botryosphaeriaceae are predominantly stress-related, opportunistic pathogens (Slippers and Wingfield 2007). Thus, their management on *Eucalyptus* predominantly relies on stress management. This is especially important where large areas are planted to specific species or clones (Wingfield et al. 1991), which represent genetically uniform potential hosts. If such plantings are in areas that are 'off site' for the particular *Eucalyptus* species or clone, the plants are likely to be severely affected by Botryosphaeriaceae. Even on appropriate sites that are ineffectively managed or are attacked by insects, resulting in stress, the trees are likely to be damaged due to infections by the Botryosphaeriaceae (Carnegie 2007). Poor silviculture in terms of planting density or over-mature stands is also likely to promote infection of the Botryosphaeriaceae and diseases associated with these fungi. While the Botryosphaeriaceae do not require wounds for infection, cankers caused by these fungi often develop from wounds. These wounds would also be a risk for infection by other pathogens and should be avoided or minimised.

A number of pathogenicity trials have shown variable degrees of resistance or tolerance of *Eucalyptus* clones to infection by species of Botryosphaeriaceae (Mohali et al. 2009, Rodas et al. 2009). Such trials are quite easily implemented via routine stem inoculations and subsequently measuring the resulting lesions that develop after a few weeks. There is thus an opportunity to screen breeding material or selected clones for resistance to infection by Botryosphaeriaceae before genetic stock is commercialised and planted over large areas. Any pathogenicity trials, however, must be preceded by careful identification of the pathogens to characterise the species infecting the given host in a particular area. Population genetic studies on the pathogen will also reveal the extent of population diversity, and existence of sexual recombination, knowledge of which should be incorporated into screening strategies for resistance. A diverse population with sexual recombination is likely to vary in its virulence, and could also more easily overcome resistance in the host over time.

Because species of the Botryosphaeriaceae are very difficult, if not impossible, to control once established in an area, their continual movement into new environments should be considered more seriously (Burgess and Wingfield 2002a, Slippers and Wingfield 2007). Quarantine of these fungi remains a first priority for their management. Effective quarantine strategies most likely lie in general regulations of treatment of seed and plant material before it enters new environments.

Future perspectives

The Botryosphaeriaceae have emerged as important pathogens of *Eucalyptus* in area where these trees are native, as well as where they have been introduced into new environments. Recent research has shown that a diverse assemblage of species of the Botryosphaeriaceae is associated with these trees, and that they are different in different areas. It seems clear that new species will continue to be discovered and described from areas previously not sampled, or areas where more thorough sampling is done. Many of these species are found in very low numbers and thus appear to contribute less to current disease epidemics. It is nevertheless important to monitor both the rare and the more common species for increases in their incidence. This will especially be true under conditions of changing climate and increasing conditions of stress.

A number of recent studies have begun the process of evaluating the species composition of the Botryosphaeriaceae. In one case, the gene flow between those species occurring on native Myrtaceae and introduced *Eucalyptus*, or between native forests and non-native *Eucalyptus* plantations, has also been considered (see Burgess et al. 2006, Pavlic et al. 2007). Clearly, more work is needed to characterise the overlap between species and gene flow between native and introduced *Eucalyptus* and other hosts. This is particularly true in the case of the species occurring on the Myrtaceae. Given the importance of host jumps and the origin of emerging pathogens (Slippers et al. 2005), it is imperative to better understand this process.

Because of their endophytic nature and occurrence in asymptomatic tissue, the Botryosphaeriaceae are well suited to being accidentally moved internationally together with germplasm. A number of species, such as *N. parvum*, *N. eucalyptorum*, *N. eucalypticola* and *L. theobromae*, occur on *Eucalyptus* in various countries and on different continents. Clearly these fungi, especially the apparently specialised *Eucalyptus*-infecting species, have been moved between these countries. It is unfortunate that this has most likely been by humans who have been responsible for moving infected *Eucalyptus* germplasm. The patterns and extent of such movements are, however, not known for most of the species and these need to be studied more specifically. Such information will help to identify ways to control the introduction of Botryosphaeriaceae and potentially other latent pathogens.

The basis of resistance of plants to Botryosphaeriaceae is currently unknown. The variation in susceptibility of trees observed in clonal trials to some species of the Botryosphaeriaceae indicates a possible genetic basis for resistance. The opportunity thus exists to search for molecular markers for resistance that could be used in breeding programs. Such long-term research should be actively pursued.

References

Ahumada R. 2003. Fungal diseases affecting *Eucalyptus* plantations in Chile. MSc thesis, University of Pretoria, South Africa.

Alves A, Phillips AJL, Henriques I, Correia A. 2005. Evaluation of amplified ribosomal DNA restriction analysis as a method for the identification of *Botryosphaeria* species. *FEMS Microbiology Letters* 245: 221–229.

Alves A, Crous PW, Correia A, Phillips AJL. 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Diversity* 28: 1–13.

Barber PA, Burgess TI, Hardy GEStJ, Slippers B, Keane PJ, Wingfield MJ. 2005. *Botryosphaeria* species from *Eucalyptus* in Australia are pleoanamorphic, producing *Dichomera* synanamorphs in culture. *Mycological Research* 109: 1347–1363.

Barnard EL, Geary T, English JT, Gilly SP. 1987. Basal cankers and coppice failure of *Eucalyptus grandis* in Florida. *Plant Disease* 71: 358–361.

Burgess TI, Barber PA, Hardy GEStJ. 2005. *Botryosphaeria* spp. associated with eucalypts in Western Australia including description of *Fusicoccum macroclavatum* sp. nov. *Australasian Plant Pathology* 34: 557–567.

Burgess TI, Barber PA, Mohali S, Pegg G, de Beer ZW, Wingfield MJ. 2006a. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. *Mycologia* 98: 423–435.

Burgess TI, Sakalidis M, Hardy GEStJ. 2006b. Gene flow of the canker pathogen *Botryosphaeria australis* between *Eucalyptus globulus* plantations and native eucalypt forests in Western Australia. *Austral Ecology* 31: 559–566.

Burgess TI, Wingfield MJ, Wingfield BD. 2004. Global distribution of *Diplodia pinea* genotypes revealed using simple sequence repeat (SSR) markers. *Australasian Plant Pathology* 33: 513–519.

Burgess T, Wingfield MJ. 2002a. Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the Southern Hemisphere. *International Forestry Review* 4: 56–64.

Burgess T, Wingfield MJ. 2002b. Impact of fungal pathogens in natural forest ecosystems: a focus on *Eucalyptus*. In: Sivasithamparam K, Dixon KW (eds), *Microorganisms in plant conservation and biodiversity*. Dordrecht: Kluwer Academic Press. pp 285–306.

Carnegie AJ. 2007. Forest health condition in New South Wales, Australia, 1996–2005. II. Fungal damage recorded in eucalypt plantations during forest health surveys and their management. *Australasian Plant Pathology* 36: 225–239.

Cilliers AJ, Swart WJ, Wingfield MJ. 1993. A review of *Lasiodiplodia* *theobromae* with particular reference to its occurrence on coniferous seeds. *South African Forestry Journal* 166: 47–52.

Coakley SM, Scherm H, Chakraborty S. 1999. Climate change and plant disease management. *Annual Review of Phytopathology* 37: 399–426.

Crous PW, Knox-Davies PS, Wingfield MJ. 1989. Newly-recorded foliage fungi of *Eucalyptus* spp. in South Africa. *Phytophylactica* 21: 85–88.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Philips AJL, Alves A, Burgess T, Barber P, Groenewald JZ. 2006. Phylogenetic lineages in the Botryosphaeriaceae. *Studies in Mycology* 55: 235–253.

Davison EM, Tay CS. 1983. Twig, branch and upper trunk cankers of *Eucalyptus marginata*. *Plant Disease* 67: 1285–1287.

Denman S, Crous PW, Taylor JE, Kang JC, Pascoe I, Wingfield MJ. 2000. An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology* 45: 129–140.

Desprez-Loustau M-L, Marcais B, Nageleisen LM, Piou D, Vannini A. 2006. Interactive effects of drought and pathogens in forest trees. *Annals of Forest Science* 63: 597–612.

Desprez-Loustau M-L, Robin C, Buee M, Courtecuisse R, Garbaye J, Suffert F, Sache I, Rizzo DM. 2007. The fungal dimension of biological invasions. *Trends in Ecology and Evolution* 22: 472–480.

de Wet J, Burgess T, Slippers B, Preisig O, Wingfield BD, Wingfield MJ. 2003. Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycological Research* 107: 557–566.

de Wet J, Slippers B, Preisig O, Wingfield BD, Wingfield MJ. 2008. Phylogenetic analysis reveals patterns of host association in the Botryosphaeriaceae. *Molecular Phylogenetics and Evolution* 46: 116–126.

Fisher PJ, Petrini O, Sutton BC. 1993. A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. *Sydowia* 45: 1–14.

Gezahgne A, Roux J, Slippers B, Wingfield MJ. 2004. Identification of the causal agent of Botryosphaeria stem canker in Ethiopian *Eucalyptus* plantations. *South African Journal of Botany* 70: 241–248.

Gure A, Slippers B, Stenlid J. 2005. Seed-borne *Botryosphaeria* spp. from native *Prunus* and *Podocarpus* trees in Ethiopia, with a description of the anamorph *Diplodia rosulata* sp. nov. *Mycological Research* 109: 1005–1014.

Index Fungorum Partnership. 2004. Index Fungorum. Available at http://www.indexfungorum.org [accessed on 1 June 2009].

Jacobs KA, Rehner SA. 1998. Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* 90: 601–610.

Luchi N, Capretti P, Surico G, Orlando C, Pazzagli M, Pinzani P. 2005. A real-time PCR assay for the detection of *Sphaeropsis sapinea* from inoculated *Pinus nigra* shoots. *Journal of Phytopathology* 153: 37–42.

Maleme H. 2009. Characterization of latent Botryosphaeriaceae on diverse *Eucalyptus*. MSc thesis, University of Pretoria, South Africa.

Mohali S, Burgess T, Wingfield MJ. 2005. Diversity and host association of the tropical tree endophyte *Lasiodiplodia theobromae* revealed using simple sequence repeat markers. *Forest Pathology* 35: 385–396.

Mohali S, Slippers B, Wingfield MJ. 2006. Two new *Fusicoccum* species from *Acacia* and *Eucalyptus* in Venezuela, based on morphology and DNA sequence data. *Mycological Research* 110: 405–413.

Mohali S, Slippers B, Wingfield MJ. 2007. Identification of Botryosphaeriaceae from *Eucalyptus*, *Acacia* and *Pinus* in Venezuela. *Fungal Diversity* 25: 103–125.

Mohali SR, Slippers B, Wingfield MJ. 2009. Pathogenicity of seven species of the Botryosphaeriaceae on *Eucalyptus* clones in Venezuela. *Australasian Plant Pathology* 38: 135–140.

Nakabonge G. 2002. Diseases associated with plantation forestry in Uganda. MSc thesis, University of Pretoria, South Africa.

Pavlic D, Slippers B, Coutinho TA, Venter M, Wingfield MJ. 2004. *Lasiodiplodia gonubiensis* sp. nov, a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Studies in Mycology* 50: 313–322.

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ. 2007. Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. *Plant Pathology* 56: 624–636.

Pavlic D, Barber PA, Hardy GEStJ, Slippers B, Wingfield MJ, Burgess TI. 2008. Seven new species of the Botryosphaeriaceae discovered on baobabs and other native trees in Western Australia. *Mycologia* 100: 851–866.

Pérez CA, Altier N, Simeto S, Wingfield MJ, Slippers B, Blanchette RA. 2008. Botryosphaeriaceae from *Eucalyptus* and native Myrtaceae in Uruguay. *Agrociencia* 12: 19–30.

Old KM, Gibbs R, Craig I, Myers BJ, Yaun ZQ. 1990. Effect of drought and defoliation on the susceptibility of *Eucalyptus* to cankers caused by *Endothia gyrosa* and *Botryosphaeria ribis*. *Australian Journal of Botany* 38: 571–581.

Rodas C, Slippers B, Gryzenhout M, Wingfield MJ. 2009. Botryosphaeriaceae associated with *Eucalyptus* canker diseases in Colombia. *Forest Pathology* 39: 110–123.

Roux J, Coutinho TA, Wingfield MJ, Bouillet JP. 2000. Diseases of plantation *Eucalyptus* in the Republic of Congo. *South African Journal of Science* 96: 454–456.

Roux J, Coutinho TA, Mujuni Byabashaija D, Wingfield MJ. 2001. Diseases of plantation *Eucalyptus* in Uganda. *South African Journal of Science* 97: 16–18.

Sankaran KV, Sutton BC, Minter DW. 1995. A checklist of fungi recorded on *Eucalyptus*. *Mycological Papers* 170: 1–376.

Sharma JK, Mohanan C, Maria Florence EJ. 1984. A new stem canker disease of *Eucalyptus* caused by *Botryodiplodia theobromae* in India. *Transactions of the British Mycological Society* 83: 162–163.

Shearer BL, Tippett JT, Bartle JR. 1987. *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiata* in species selection trials. *Plant Disease* 71: 140–145.

Sinclair WA, Lyon HH. 2005. *Diseases of trees and shrubs*. Ithaca: Cornell University Press.

Slippers B, Crous PW, Denman S, Coutinho TA, Wingfield BD, Wingfield MJ. 2004b. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* 96: 83–101.

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Carnegie A, Wingfield MJ. 2004a. Speciation and distribution of *Botryosphaeria* spp. on *Eucalyptus* in Australia and South Africa. *Studies in Mycology* 50: 343–358.

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ. 2004c. Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. *Mycologia* 96: 1028–1039.

Slippers B, Stenlid J, Wingfield MJ. 2005. Emerging pathogens: fungal host jumps following anthropogenic introduction. *Trends in Ecology and Evolution* 20: 420–421.

Slippers B, Wingfield MJ. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* 21: 90–106.

Smith DR, Stanosz GR. 1995. Confirmation of two distinct populations of *Sphaeropsis sapinea* in the north central United States using RAPDs. *Phytopathology* 85: 699–704.

Smith DR, Stanosz GR. 2001. Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum* *aesculi*) from some other fungi with *Fusicoccum* anamorphs. *Mycologia* 93: 505–515.

Smith DR, Stanosz GR. 2006. A species-specific PCR assay for the detection of *Diplodia pinea* and *D. scrobiculata* in dead red and Jack pines with collar rot symptoms. *Plant Disease* 90: 307–313.

Smith H, Crous PW, Wingfield MJ, Coutinho TA, Wingfield BD. 2001. *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* 93: 277–284.

Smith H, Kemp GHJ, Wingfield MJ. 1994. Canker and die-back of *Eucalyptus* in South Africa caused by *Botryosphaeria dothidea*. *Plant Pathology* 43: 1031–1034.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC. 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* 31: 21–32.

Taylor K, Barber PA, Hardy GEStJ, Burgess TI. 2009. Botryosphaeriaceae from tuart (*Eucalyptus gomphocephala*) woodland, including descriptions of four new species. *Mycological Research* 113: 337–353.

von Arx JA. 1987. *Plant pathogenic fungi*. Beheifte zur *Nova Hedwigia* 87. Berlin: J Cramer.

Webb RS. 1983. Seed capsule abortion and twig dieback of *Eucalyptus camaldulensis* in South Florida induced by *Botryosphaeria ribis*. *Plant Disease* 67: 108–109.

Wingfield MJ, Swart WJ, Kemp GHJ. 1991. Pathology considerations in clonal propagation of *Eucalyptus* with special reference to the South African situation. In: Schönau APG (ed.), *Intensive forestry – the role of eucalypts*. *Proceedings of the IUFRO Symposium P2.02-01, productivity of eucalypts, 2–6 September 1991, Durban*. Pretoria: Southern African Institute of Forestry. pp 811–830.

Wingfield MJ, Slippers B, Roux J, Wingfield BD. 2001. Worldwide movement of exotic forest fungi, especially in the tropics and the Southern Hemisphere. *BioScience* 51: 134–140.