Identification of fungal pathogens occurring in eucalypt and pine plantations in Zambia by comparing DNA sequences

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Summary

Commercial forestry plantations in Zambia were initiated during the 1960s. Since then, very little attention has been given to diseases that impact negatively on the production of these plantations. Recent field surveys have highlighted the occurrence and impact of several diseases. This study was undertaken to determine, to species level, the identity of fungal pathogens associated with diseases of eucalypt and pine plantations in the country. Fungal morphology and DNA sequence data of the internal transcribed spacer (ITS) and β-tubulin gene regions were used to characterize isolates. Eleven fungal species were identified of which *Teratospheria zuluensis*, causing Coniothyrium canker, and *Lasiodiplodia theobromae*, causing stem canker and die-back on *Eucalyptus* spp., were the most serious and prevalent. A serious post-emergence damping-off disease of *Pinus oocarpa* and *Pinus kesiya* seedlings in nurseries yielded *Calonectria pauciramosum*. This study, which provides the first detailed species level identification of plantation tree pathogens in Zambia, provides a foundation for future work to develop management strategies aimed at reducing the impact of plantation diseases in the country.

Introduction

During the 1960s, the Forestry Department in Zambia embarked on the planting of non-native tree species to provide timber for the mines and construction industries and to supply fuel wood and poles locally (Sekeli, 1998). Various extractive minning companies in the Copperbelt region of Zambia rely on eucalypt poles for scaffolding and as mine props (Zimba, 2005). By 1967, 57 000 ha of plantations were established for industrial use in the Copperbelt Province alone. Another 10 000 ha were established in other provinces to meet the demand for local communities (Sekeli, 1998). Since then, forestry plantations have been expanding in the country, at the rate of 5000 ha annum^{-1} (O. Shakachite, personal communication).

Non-native tree species that have been planted on a large scale in Zambia include *Eucalyptus cloeziana* F. Muell, *Eucalyptus grandis* W. Hill, *Pinus oocarpa* Schiede and *Pinus kesiya* Royle ex Gordon. Other less commonly planted species include *Eucalyptus camaldulensis* Dehnh, *P. merkusii* De Vries, *Pinus michoacana* Martinez, *E. tereticornis* Sm and a hybrid between *E. camaldulensis* and *E. tereticornis* (Sekeli, 1998). The total area occupied by *Pinus* spp. is estimated to be one and half times more than that of *Eucalyptus* spp. The choice of *E. cloeziana*, *E. grandis*, *P. oocarpa* and *P. kesiya* as the major species for planting was based on superior growth in high rainfall areas and their ability to compete with, and suppress, noxious weeds. Currently, forestry plantations form the basis of raw material for several wood industries, providing construction timber, wood-based panels and various types of pulp products that were previously imported, exerting pressure on Zambia's foreign exchange reserves (Njovu, 2004).

Plantations of *Eucalyptus* and *Pinus* spp. are threatened by an array of problems among which fire and diseases are the most damaging. Recent surveys conducted in forestry plantations in the Copperbelt region of Zambia revealed that diseases caused by fungi constitute a major threat to plantation productivity in the country (Roux *et al.*, 2005; Muimba-Kankolongo *et al.*, 2009). These included the stem canker diseases: Chrysoporthe, Coniothyrium (Roux *et al.*, 2005) and Botryosphaeria canker and leaf blight/spot caused by *Calonectria* (*Cylindrocladium*) spp. and *Kirramyces* spp. (Muimba-Kankolongo *et al.*, 2009).

Previous studies of plantation tree diseases in Zambia mostly only identified pathogens associated with disease symptoms to the genus level (Chungu *et al.*, 2010b). More comprehensive and accurate identification of pathogens is required to develop sound plantation management and quarantine procedures. The aims of our study were to

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extend the areas surveyed in recent studies (Roux *et al.*, 2005; Muimba-Kankolongo *et al.*, 2009) and to identify the fungal pathogens collected up to species level based on molecular and morphology data.

Study area and methods

Study sites and sampling

Eucalypt and pine trees in commercial stands, woodlots and nurseries were surveyed in the Central, Copperbelt, Luapula, Northern and Northwestern Provinces (Figure 1) of Zambia between 2007 and 2008 for the presence of diseases. Random sampling was used in the collection of samples in plantations, woodlots and nurseries. In some cases, local staff reported problems of diseased trees in the survey area. A total of 42 sampling sites were surveyed of which 13 were in the nurseries and 29 in plantations and woodlots. The average rainfall in the areas surveyed ranged between 1200 and 1300 mm per annum and mean annual temperatures were between 24°C and 28°C (Table 1). Leaves, pieces of bark, twigs, segments of stems and roots showing disease symptoms were collected and separately placed in brown paper bags, which were sealed in larger plastic bags to retain moisture, until isolations were done. Samples that could not be processed immediately were kept in cool dry conditions or in a refrigerator at ~4°C.

Isolation of fungi

Isolation techniques were selected based on visual evaluation of morphological characteristics, e.g. pycinidia, of fungi of the most likely cause of the disease observed. Where the pathogen involved was unclear, general isolation media and techniques were used and pure cultures of all isolates are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa.

For isolation of fungal species within the Teratosphaeriaceae, a technique described by Crous (1998) was followed and isolations incubated for 24 h at room temperature on 2 per cent MEAS (Malt Extract Agar containing streptomycin; 20 g Biolab malt extract, 20 g Biolab agar, 1 l deionized water). After incubation, single germinating ascospores were observed on the agar surface, individually transferred onto 2 per cent MEAS to obtain pure cultures and incubated for 30 days at 25°C under cool white light. For fungi in the Calonectriaceae, selected lesions from leaves with leaf blight symptoms were placed in moist chambers and incubated for 10 days at 25°C to promote sporulation. Conidial masses were transferred from conidiophores to 2 per cent MEAS and incubated for 8 days at 25°C under continuous near-ultraviolet light.

Pieces of bark from *Eucalyptus* trees, showing cankers and die-back symptoms typical of the Botryosphaeriaceae, Cryphonectriaceae and Teratosphaeriaceae, were, after surface sterilization with 70 per cent ethanol, placed overnight in moist chambers at 25°C to induce sporulation. Additionally, using a sterile scalpel, the surface of the bark was removed and pycinidia in the bark were cut to expose fungal fruiting bodies and spores. Spore masses were transferred onto 2 per cent MEAS and incubated under cool white light at 25°C. Isolations were also made from leading edges of canker lesions on trees, by plating small pieces (~1 × 5 mm) onto 2 per cent MEAS and incubating them at 25°C. For isolation of fungi in the Cryphonectriaceae, we followed the procedure described by Gryzenhout *et al.* (2009). Isolations from pine cones were done using the technique described by Swart *et al.* (2000).

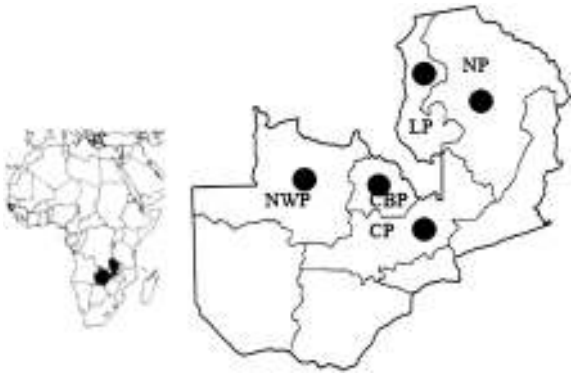

Figure 1. Map of Zambia showing provinces where surveys in *Eucalyptus* and *Pinus* plantations were carried out: Luapula Province (LP), Northern Province (NP), Copperbelt Province (CBP), Central Province (CP) and Northwestern Province (NWP).

To obtain the causal agent of a disease of *Pinus* seedlings collected from a Zambia Forests and Forestry Industrial Corporation pine nursery in Kitwe, four plant pieces, ~2 × 5 mm in size, were aseptically excised from seedling roots and root collars showing symptoms and placed on 2 per cent MEAS. These were incubated for 5 days at 25°C. Pure cultures were made by transferring single hyphal tips from each fungal isolate to fresh 2 per cent MEAS and incubating these for 10 days under continuous near-ultraviolet light at 25°C.

Identification of fungal pathogens

Representative isolates of putative pathogens were selected from each host, geographic area and morphological group (Table 1) and used for DNA extraction. DNA was extracted and purified using the Cetyl Trimethyl Ammonium Bromide method as described in Chungu *et al.* (2010a). The DNA concentrations were determined using a Nanodrop ND-1000 Spectrophotometer v. 3.6 (Thermo Fisher Scientific, Wilmington, NC, USA).

DNA extracts from each isolate were used separately as templates for amplification with the polymerase chain reaction (PCR). The internal transcribed spacer (ITS) regions were amplified for all isolates using primers ITS1 and ITS4 (White *et al.*, 1990). For some fungi, where ITS sequence data are insufficient for identification to species level,

Table 1: Climatic conditions and locations surveyed to collect samples used for identification of pathogens occurring in non-native forestry plantations in Zambia

Sampling area	Average temperature (°C)	Average rainfall (mm/year)	Location	
			GPS	Altitude
Copperbelt Province				
Chati	25	1200	12° 51′ S, 27° 49′ E	1308
Kitwe	26	1200	12° 50′ S, 27° 31′ E	1303
Ndola	26	1200	12° 32′ S, 28° 50′ E	1310
Central Province				
Serenje	24	1100	13° 15′ S, 30° 17′ E	1515
Luapula Province				
Samfya	25	1300	11° 21′ S, 29° 28′ E	1207
Kapweshi	25	1250	S11° 13′ E28° 31′	1216
Northern Province				
Kasama	28	1300	10° 12′ S, 31° 26′ E	1421

GPS = global positioning system.
Source: Meteorological Department of Zambia, 2000.

the β-tubulin (BT) 2 gene region was amplified additionally, using primers Bt2a and Bt2b (Glass and Donaldson, 1995). For *Diplodia* isolates, the BT gene region was amplified using the protocol by De Wet *et al.* (2002) to identify isolates to morphotype. PCR products were visualized and cleaned using procedures described in Chungu *et al.* (2010a).

The purified PCR products were used as template DNA for cycle sequencing reactions using the ABI Prism Big Dye Terminator Cycle sequencing reaction kit v. 3.1 (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. The same primers as used for the PCRs were also used for sequencing reactions. Sequence reactions were run using polyacrylamide gel electrophoresis using an ABI PRISM™ 377 Autosequencer (Applied Biosystems). Sequence electropherograms were analysed using Sequence Navigator version 1.0.1 (Applied Biosystems) and both the forward and reverse sequences for each isolate were obtained. Final sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov) (Table 2). Additional sequences for comparison in the analyses were obtained from Genbank and all sequence alignments and phylogenetic trees in this study have been deposited in TreeBASE (http://www.treebase.org).

Results

Study sites and sampling

In this study, symptoms of several diseases were observed on both *Eucalyptus* and *Pinus* spp. in Zambia. On *Eucalyptus* spp., stem cankers typical of those caused by *Teratosphaeria* spp. (Figure 2A) were observed in the Kitwe and Chati areas in the Copperbelt Province, while symptoms similar to infection by the Botryosphaeriaceae (Figure 2B) were found on *E. grandis* in Kapweshi in Samfya (Luapula Province) and Serenje in the Central Province. Chrysoporthe canker was found on *E. grandis* in the Kapweshi and Serenje areas. A leaf blight disease, typical of *Calonectria* sp. (Figure 2C) was found on *E. grandis* in a plantation in Serenje.

In pine plantations, no particular disease was observed, mainly due to fire damage that obscured disease symptoms. Samples were, however, collected from pine cones to obtain an indication of the presence of endophytic *Diplodia* spp. A fungus typical of *Calonectria* sp. was consistently isolated from root collar lesions of *P. kesiya* and *P. oocarpa* seedlings in a nursery in Kitwe (Figure 2D–E). There was higher mortality of *P. kesiya* (40 per cent) than *P. oocarpa* (25 per cent) seedlings in this nursery.

Identification of fungal pathogens

DNA sequences of the ITS gene regions of isolates recovered from symptoms typical of Coniothyrium stem canker on *E. cloeziana* in Chati and *E. grandis* in Kitwe matched with 100 per cent similarity with reference isolates of *Teratosphaeria zuluensis* (M.J. Wingf., Crous and T.A. Cout.) Andjic and M.J. Wingf. (TreeBase: S10391). Three pathogens isolated from *E. grandis* and *E. cloeziana* trees with stem canker and tip die-back symptoms in Samfya and Kapweshi were identified based on ITS as *Lasiodiplodia theobromae*, *Neofusicoccum parvum*, and *Neofusicoccum eucalyptorum* Crous, H. Smith and M.J. Wingf. (TreeBase: S10391) with 99 per cent similarity. Isolates from cones of *P. oocarpa* in Kasama, in the Northern Province of Zambia, matched with 100 per cent similarity with *Diplodia pinea* (Desm.) J. Kickx morphotype A from South Africa based on the BT gene region (TreeBase: S10391). Isolates collected from Chrysoporthe stem canker symptoms on *E. grandis* in Kapweshi and Serenje matched with 100 per cent similarity with *Chrysoporthe austroafricana* Gryzenhout and M.J. Wingf. based on sequence analyses of the ITS and BT gene regions (TreeBase: S10391).

ITS sequences of leaf pathogens resembling species with 100 per cent similarity in the Mycosphaerellaceae and Teratosphaeriaceae collected in this study matched with those of *Kirramyces epicoccoides* (Cooke and Massee)

Table 2: Zambian isolates used in DNA sequence studies, including information on hosts and origins

Species name	Isolate no.*	Host	Origin	GenBank accession no.
Chrysoporthe austroafricana	CMW30109	*Eucalyptus grandis*	Serenje, Central	FJ805224†, FJ805227‡
Chr. austroafricana	CMW30110	*E. grandis*	Serenje, Central	FJ805225†, FJ805228‡
Chr. austroafricana	CMW30111	*E. grandis*	Kapweshi, Luapula	FJ805226†, FJ805229‡
Cladosporium cladosporioides	CMW30176	*E. grandis*	Ndola, Copperbelt	FJ805222†
Cl. cladosporioides	CMW30177	*Eucalyptus cloeziana*	Samfya, Luapula	FJ805223†
Cl. cladosporioides	CMW30174	*E. grandis*	Kitwe, Copperbelt	FJ805221†
Calonectria pauciramosum	CMW30120	*Pinus oocarapa*	Kitwe, Copperbelt	FJ795546‡
Ca. pauciramosum	CMW30121	*Pinus kesiya*	Kitwe, Copperbelt	FJ795547‡
Ca. pauciramosum	CMW30122	*P. kesiya*	Kitwe, Copperbelt	FJ795548‡
Calonectria spathulatum sensu lato	CMW30135	*E. grandis*	Serenje, Central	FJ795544‡
Ca. spathulatum s.l	CMW30136	*E. grandis*	Serenje, Central	FJ795545‡
Ca. spathulatum s. l	CMW30134	*E. grandis*	Serenje, Central	FJ795543‡
Diplodia pinea Morphotype A	CMW30128	*P. oocarpa*	Kasama, Northern	FJ858719‡
D. pinea Morphotype A	CMW30127	*P. oocarpa*	Kasama, Northern	FJ858718‡
D. pinea Morphotype A	CMW30174	*P. oocarpa*	Kasama, Northern	FJ858720‡
Kirramyces epicoccoides	CMW30613	*E. grandis*	Kitwe, Copperbelt	FJ858712†
K. epicoccoides	CMW30614	*E. grandis*	Kitwe, Copperbelt	FJ858713†
K. epicoccoides	CMW30615	*E. cloeziana*	Serenje, Central	FJ858714†
Lasiodiplodia theobromae	CMW30093	*E. grandis*	Samfya, Luapula	FJ826612†
L. theobromae	CMW30094	*E. grandis*	Samfya, Luapula	FJ826613†
L. theobromae	CMW30092	*E. grandis*	Samfya, Luapula	FJ826611†
Neofusicoccum eucalyptorum	CMW30156	*E. grandis*	Serenje, Central	FJ826606†
N. eucalyptorum	CMW30157	*E. grandis*	Serenje, Central	FJ826607†
N. eucalyptorum	CMW30155	*E. grandis*	Serenje, Central	FJ826605†
N. parvum	CMW30142	*E. grandis*	Kapweshi, Luapula	FJ826608†
N. parvum	CMW30143	*E. grandis*	Kapweshi, Luapula	FJ826609†
N. parvum	CMW30144	*E. cloeziana*	Kapweshi, Luapula	FJ826610†
Teratosphaeria zuluensis	CMW28714	*E. cloeziana*	Kitwe, Copperbelt	FJ617253†
T. zuluensis	CMW28725	*E. cloeziana*	Kitwe, Copperbelt	FJ617254†
T. zuluensis	CMW28709	*E. grandis*	Kitwe, Copperbelt	FJ617252†
Teratosphaeria nubilosa	CMW30190	*E. grandis*	Serenje, Central	FJ805218†
T. nubilosa	CMW30191	*E. grandis*	Serenje, Central	FJ805219†
T. nubilosa	CMW30192	*E. grandis*	Serenje, Central	FJ805220†
T. parva	CMW28772	*E. grandis*	Serenje, Central	FJ858715†
T. parva	CMW28773	*E. grandis*	Serenje, Central	FJ858716†
T. parva	CMW28774	*E. grandis*	Serenje, Central	FJ858717†
T. pseudafricana	CMW30607	*E. grandis*	Kitwe, Copperbelt	FJ826602†
T. pseudafricana	CMW30608	*E. grandis*	Kitwe, Copperbelt	FJ826603†
T. pseudafricana	CMW30609	*E. cloeziana*	Kitwe, Copperbelt	FJ826604†

* CMW refers to culture collection of Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

† Accession numbers refer to sequence data of the ITS gene region.

‡ Accession numbers refer to sequence data of the BT 2 gene region.

J. Walker, B. Sutton and Pascoe, *Teratosphaeria pseudafricana* (Crous and T.A. Cout.) Crous and U. Braum, *Teratosphaeria nubilosa* (Cooke) Crous and U. Braum, *Teratosphaeria parva* (R.F. Park and Keane) Crous and U. Braum and *Cladosporium cladosporioides* Penz, respectively (TreeBase: S10391). *Kirramyces epicoccoides* was collected from *E. grandis* in Kitwe and Serenje, *T. pseudafricana* from *E. grandis* in Kitwe, *T. nubilosa* and *T. parva* from *E. grandis* in Serenje while *Cl. cladosporioides* was collected from *E. cloeziana* in Chati and also from *E. grandis* in Kitwe, Ndola and Samfya.

Two species of *Calonectria* were collected in this study (TreeBase: S10391). Both were identified based on morphological characteristics and molecular techniques. Samples collected from leaf spot symptoms in an *E. grandis* plantation in Serenje matched with 100 per cent similarity with *Calonectria spathulatum sensu lato* El–Gholl, Kimbr., E.L. Barnard, Alfieri and Schoult. The causal agent of the disease of *P. kesiya* and *P. oocarpa* seedlings in a nursery in Kitwe was identified as *Calonectria pauciramosum* C.L. Schoch and Crous.

Discussion

This study provides additional knowledge on the identity, at species level, of fungal pathogens of *Eucalyptus* and *Pinus* spp. in nurseries, plantations and woodlots in Zambia.

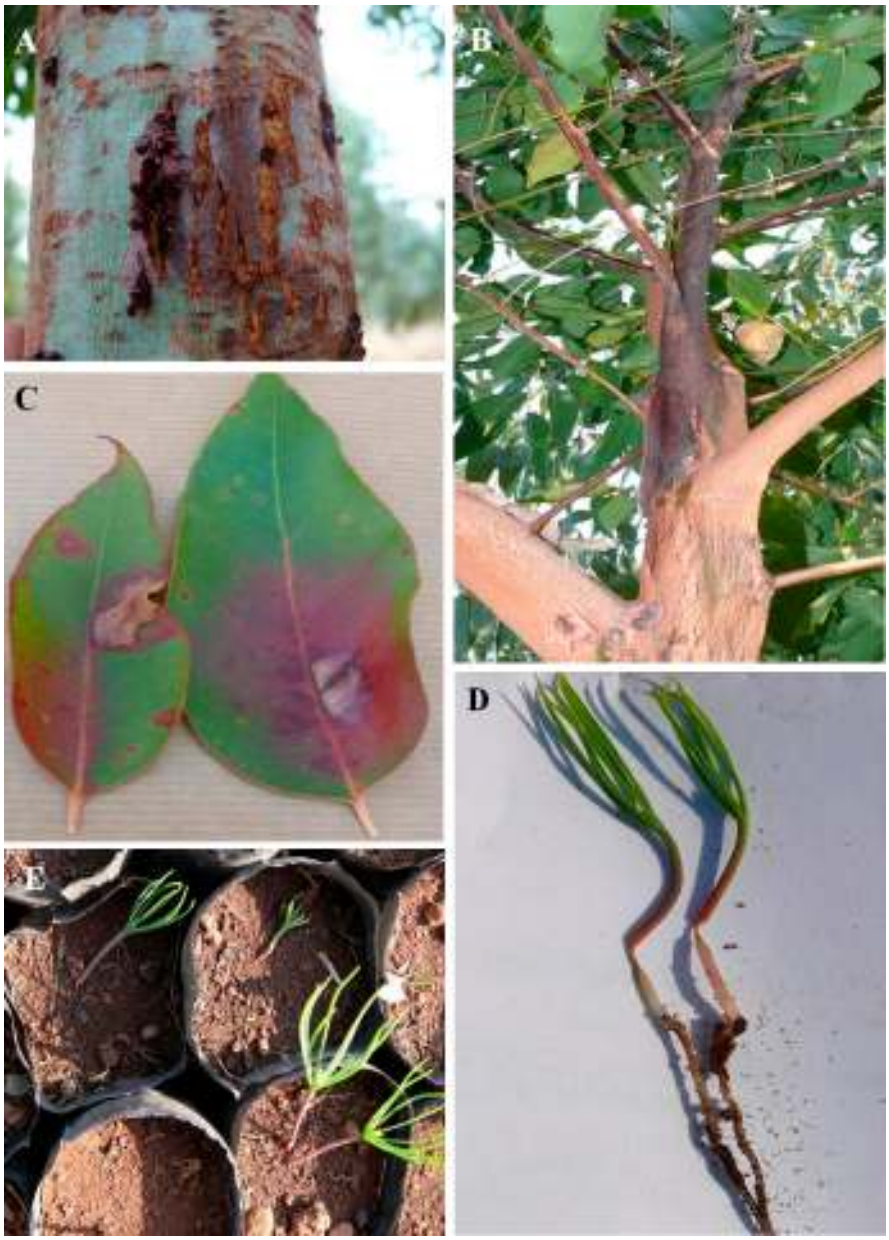

Figure 2. Disease symptoms of fungal pathogens infecting *Eucalyptus* spp. and *Pinus* spp. in Zambia: cankers caused by *Teratosphaeria zuluensis* on *E. cloeziana* (A), tip die-back and canker caused by *Lasiodiplodia theobromae* on *E. grandis* (B), leaf blight caused by *Calonectria spathulatum sensu lato* on *E. grandis* (C) and collar and rot collar caused by *Ca. pauciramosum* on *P. oocarpa* and *P. kesiya* seedlings (D–E).

It also represents the most extensive investigation of plantation tree diseases in the country, greatly expanding knowledge emerging from previous investigations (Shakacite, 1991; Roux *et al.*, 2005; Muimba-Kankolongo *et al.*, 2009). The majority of pathogens identified in this study represent first records for Zambia. Hence, they contribute to a better understanding of the impact, distribution and origin of these pathogens on the African continent and globally.

During our investigation, we identified a stem canker disease, typical of Coniothyrium stem canker, as the most severe disease occurring in eucalypt plantations in Zambia. The causal agent was identified as *T. zuluensis*, a pathogen that has been recorded from Malawi (Cortinas *et al.*, 2006) and South Africa (Wingfield *et al.*, 1997). This pathogen caused disease on *E. cloeziana* and *E. grandis* in the Copperbelt Province only. In a previous study (Muimba-Kankolongo *et al.*, 2009), Coniothyrium canker was considered as one

of the most damaging diseases to *Eucalyptus* plantations in Zambia. Infection results in wood damage, making trees unsuitable for sawn timber or for use in construction. This disease was only observed in the Copperbelt Province and was not reported by Roux *et al.* (2005), suggesting that *T. zuluensis* might have been introduced into the country only recently. However, there is no information regarding the possible origin of this pathogen and its spread, although it is suggested to spread via seed (Cortinas *et al.*, 2006).

Previously, Chrysoporthe canker disease of Eucalypts was only known from the Copperbelt Province (Nakabonge *et al.*, 2006), but its geographic distribution has now been extended to include the Luapula and Central Provinces of Zambia. It was found in five plantations, causing typical basal cankers on trees. Its occurrence in the Luapula and Central Provinces is not surprising as the causal agent, *Chr. austroafricana*, is widespread in southern and eastern Africa (Wingfield *et al.*, 1989; Nakabonge *et al.*, 2006) and is suggested to be native to southern Africa (Heath *et al.*, 2006; Gryzenhout *et al.*, 2009), where it has undergone a host shift from native *Myrtaceae* to non-native *Eucalyptus* spp. (Heath *et al.*, 2006). This pathogen could have a potentially serious impact on plantation forestry in Zambia, as was experienced in South Africa, where it has caused significant losses to eucalypt plantations (Wingfield, 2003; Gryzenhout *et al.*, 2009). Disease caused by *Chr. austroafricana* in South Africa and its close relative *Chr. cubensis* in South America and Asia necessitated extensive breeding programmes to ensure the continued sustainability of plantation forestry in affected countries (Wingfield, 2003; Van Heerden *et al.*, 2005).

Botryosphaeria canker of *Eucalyptus* spp. has been associated with a wide range of fungi in the Botryosphaeriaceae (Slippers and Wingfield, 2007). Three species, namely *L. theobromae*, *N. eucalyptorum* and *N. parvum*, were recovered from symptomatic trees in this study. *Lasiodiplodia theobromae* is a widespread unspecialized canker pathogen with a wide host range worldwide including in Africa, although its centre of origin is unknown (Punithalingam, 1980; Slippers *et al.*, 2004a). *N. eucalyptorum* was first described on *Eucalyptus* spp. from South Africa (Smith *et al.*, 2001) and *N. parvum* from New Zealand (Pennycook and Samuels, 1985). Slippers *et al.* (2004a) reported that *N. eucalyptorum* may be native to Australia and *N. parvum* to New Zealand. Since its first report, *N. parvum* has subsequently been recorded as an important pathogen of *Eucalyptus* spp. in numerous parts of the world, including in Africa (Slippers *et al.*, 2004b; Alemu *et al.*, 2004) and on native *Myrtaceae* in South Africa (Slippers *et al.*, 2004b), though its centre of origin remains uncertain. Fungi in the Botryosphaeriaceae are known as opportunistic, often stress-associated pathogens, causing disease and death of trees suffering, for example, from drought or other stress (Smith *et al.*, 2001; Slippers and Wingfield, 2007). Care should thus be taken in Zambia to ensure optimal site species matching and sound silviculture to reduce the chances of disease caused by species in the Botryosphaeriaceae.

The most serious leaf disease observed during our survey of non-native plantations was associated with *Ca. spathulatum sensu lato* on 4-year-old *E. grandis* trees. This pathogen was first reported in Brazil on *E. viminalis* Labill, where it caused damage to trees in plantations (El-Gholl *et al.*, 1986) and thereafter has been observed in nurseries and plantations worldwide (Crous *et al.*, 1991; Park *et al.*, 2000; Lombard *et al.*, 2010). Its distribution and impact in Zambia requires further study, as it is unlikely that it is restricted only to the single plantation in which we found it. It could have been the cause of the Cylindrocladium disease reported from the Kitwe area by Muimba-Kankolongo *et al.* (2009), but this is not possible to confirm since no living cultures remain from that study. *Calonectria* spp. can result in significant damage to Eucalypt plantations, as has been experienced in India (Sharma and Mohanan, 1991), China (Zhou *et al.*, 2008), Columbia (Rodas *et al.*, 2005) and many other countries (Booth *et al.*, 2000). In Africa, for example, *Ca. theae* has been reported causing total defoliation of Eucalypt trees in the Republic of Congo (Roux *et al.*, 2000).

During our investigation, Mycosphaerella leaf blotch (MLB) was found on a number of *Eucalyptus* trees in different parts of Zambia. *T. nubilosa* was isolated from diseased leaves of juvenile *E. grandis* in Serenje and *T. pseudafricana* on *E. grandis* in Kitwe. MLB observed in this study was not severe and is not considered of economic importance. *T. nubilosa* and *T. pseudafricana* were previously reported from *E. globulus* in Zambia and the latter is known to occur only in this country (Gibson, 1975; Crous *et al.*, 2006). *T. nubilosa* is one of the most important leaf pathogens of *Eucalyptus* spp., particularly on cold-tolerant species, such as *E. globulus* and *E. nitens*, in many parts of the world (Park *et al.*, 2000; Hunter *et al.*, 2004, 2007), including South Africa (Hunter *et al.*, 2004) and Ethiopia (Alemu *et al.*, 2006) in Africa. However, it is unlikely to cause severe damage to *Eucalyptus* spp. in Zambia because species susceptible to the disease are not planted in the country. Other leaf diseases of minor importance, although commonly observed on stressed trees in Kitwe and Serenje, were associated with *T. epicoccoides* and *Cl. cladosporioides*.

No major pine diseases were observed in plantations during this study. The most common cause of tree mortality observed was fire. Fire damage made it impossible to obtain an accurate indication of possible fungal diseases of *Pinus* spp. in Zambia during the time of survey. To obtain some indication of what could be expected, we identified the species of *Diplodia* occurring on *Pinus* in Zambia. *Diplodia pinea* is the best known pathogen of *Pinus* spp. in Southern Africa (De Wet *et al.*, 2002; Burgess *et al.*, 2004) and can be expected to also result in disease in Zambia. This opportunistic pathogen commonly occurs on pine cones (De Wet *et al.*, 2002). We consistently isolated *D. pinea* morphotype A from cones of *P. oocarpa*, particularly in Kasama. This is consistent with previous reports that only *D. pinea* mophortype A is associated with *Pinus* spp. in Africa (De Wet *et al.*, 2002).

Serious disease was only observed in one of the nurseries visited in this study. The cause of the disease was identified as *Ca. pauciramosum*. It was associated with a serious

disease (rotting of the root collars) of seedlings in a nursery of *P. kesiya* and *P. oocarpa* in Kitwe. This is a first report of *Ca. pauciramosum* in Zambia, as it has previously only been reported from Australia, Brazil, Mexico and South Africa (Schoch *et al.*, 1999; Lombard *et al.*, 2010). This is a serious pathogen of *Eucalyptus* and *Pinus* in countries, where it has been observed causing leaf spot, root rot and stem cankers on cuttings and seedlings. *Calonectria pauciramosum* is hypothesized to be native to South or Central America (Schoch *et al.*, 2001) but has recently been introduced to Europe (Polizzi and Crous, 1999) and California (Koike *et al.*, 1999). Diseases caused by *Calonectria* spp. are managed through effective silviculture since this creates conditions that are unfavourable for disease development (Schoch *et al.*, 1999).

This study has provided additional information on the identity of a number of important pathogens associated with diseases of forestry plantations, particularly *Eucalyptus* spp. in Zambia. Many of these, such as *T. zuluensis*, *Ca. pauciramosum*, *N. parvum* and *D. pinea*, represent first reports for the country. These are well-known pathogens elsewhere in the world. However, pathogenicity tests on *Eucalyptus* and *Pinus* spp. grown in Zambia will be important to determine their relative importance on planting stock and for selection of disease-tolerant genotypes. Pathogens included those affecting healthy unstressed trees, such as stem canker caused by *T. zuluensis* and *Chr. austroafricana*, and those that are stress-related pathogens, such as *N. parvum*, *L. theobromae* and *D. pinea*. If left unchecked, the impact of these diseases will likely increase and could result in substantial economic losses to the Zambian forestry industry in future.

Effective measures to reduce losses due to fungal pathogens exist and can be implemented successfully. Planting of disease-tolerant tree species should be the core objective in plantation establishment in order to minimize risk of disease development. The government and private forestry companies should form partnerships to establish sustained tree breeding and selection programmes that should include disease resistance as a key component. Furthermore, strict quarantine measures should be established to prevent introduction of new and important diseases into Zambia.

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Conflict of Interest Statement

None declared.

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