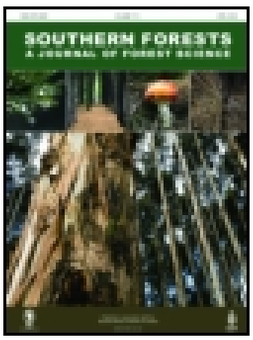

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Diseases on *Eucalyptus* species in Zimbabwean plantations and woodlots

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Zimbabwe embarked on planting *Eucalyptus* species in the early 1900s. Based on a robust breeding programme, it has become a major source of seed for other countries in and outside Africa. Tree health surveys conducted on *Eucalyptus* in some east and southern African countries over the past two decades have revealed several important fungal diseases that were previously not known in the region, but little is known regarding these problems in Zimbabwe. The aim of this study was to identify important *Eucalyptus* diseases across Zimbabwe's agroclimatic regions. Morphological characteristics and DNA sequence data were used to identify pathogens collected to species level. Widespread stem canker diseases, caused by species belonging to the Botryosphaeriaceae and Teratosphaeriaceae, and leaf spot diseases caused by fungi in the Capnodiales, were identified. Armillaria root and stem rot was restricted to a single site in the Eastern Highlands. Fungi that could cause canker or blue stain of timber were isolated from recently harvested stumps and included species of *Ceratocystis* and *Ophiostoma*. This study is the first to identify *Eucalyptus* pathogens to species level in Zimbabwe and we report for the first time the presence of the stem canker pathogen *T. gauchensis* in southern Africa. The results will provide a foundation for the formulation of future disease management strategies in the country.

Keywords: *Armillaria*, Botryosphaeriaceae, Capnodiales, leaf spot, root rot, stem cankers, Teratosphaeriaceae

Introduction

Eucalyptus species were introduced into Zimbabwe in the early1900s to meet the demand for hardwood poles, sawn timber and firewood. Subsequently, several species have been cultivated in plantations, particularly in the Eastern Highlands of the country. By 2001, they occupied approximately 29 314 ha (25%) out of a total of 119 130 ha of plantation area in the country (Mabugu and Chitiga 2002). The major species planted are *Eucalyptus grandis* (Hill) Maiden and *E. cloeziana* F.Muell., but *E. tereticornis* Sm., *E. globulus* Labill., *E. macarthurii* H.Deane & Maiden, *E. microcorys* F.Muell., *E. paniculata* Sm. and *E. robusta* Sm. are also important. Together with pines, *Eucalyptus* species contribute about 3% to GDP (Shumba 2001). Besides being grown in intensively managed plantations, *Eucalyptus* species are also planted extensively in rural areas for the production of poles and fuelwood.

The Forestry Commission, through its Forestry Extension Services Division, implemented the Rural Afforestation Project from 1983 to 1989 in an attempt to solve critical fuelwood shortages in Zimbabwe (Mushaka 1998). The project promoted the establishment of more than 2 000 woodlots by schools, community groups and individuals in rural areas (Bradley and McNamara 1993). Most of these woodlots ranged from 1 to 10 ha, with *E. camaldulensis* Dehn. primarily planted due to its drought tolerance and adaptability to a wide range of soil types (Mushaka 1998; Shumba 2001; Tyynela 2001).

Relatively little is known regarding the microbial pathogens affecting the productivity of *Eucalyptus* species in Zimbabwe. Several disease symptoms have been observed in plantations of these trees, but in most cases the causal agents are unknown. Masuka (1990) reported widespread Botryosphaeria stem canker affecting *E. grandis* and had the view that this was the most important *Eucalyptus* disease in the country. The disease was reported to most commonly affect trees aged two years and older and was characterised by kino exudation, stem swelling and cankers. The causal pathogens were identified as *Botryosphaeria dothidea* (Moug. ex Fr.) Cesati & De Notaris and *B. ribis* Grossenb. & Duggar (Masuka 1990; Keane et al. 2000), now in the genus *Neofusicoccum* (Slippers et al. 2004a). Ganoderma root rot, thought to be caused by *Ganoderma sculpturatum* (Lloyd) Ryvarden, was identified on *E. grandis* at Mtao in Masvingo (Masuka and Nyoka 1995) and an unidentified *Cercospora* sp. was reported to cause a leaf spot disease of *E. grandis* (Masuka 1990).

During the course of the past two decades, various pathogens of *Eucalyptus* species have been identified and studied in countries neighbouring Zimbabwe, and many of these are new to the region. For example, recent studies of

Eucalyptus diseases in Malawi, Mozambique and Zambia identified the presence of several important pathogens, including species of *Armillaria*, Botryosphaeriaceae, *Chrysoporthe*, *Teratosphaeria* and *Mycosphaerella*, causing root rot, stem canker and leaf spot diseases (Roux et al. 2005; Nakabonge et al. 2006; Chungu et al. 2010a, 2010b). These diseases are also known from South Africa, where they result in significant losses to the forestry industry annually (Wingfield et al. 2008, 2010, 2012; Roux et al. 2012). Given the geographic proximity of these countries, it is likely that some of these pathogens occur in plantations and woodlots in Zimbabwe. The aim of this study was thus to carry out a comprehensive study of *Eucalyptus* pathogens in Zimbabwe, with the overall aim of providing a foundation of knowledge to manage these problems in the future.

Materials and methods

Sampling sites, sampling and isolation

Eucalyptus trees in plantations and rural woodlots in Zimbabwe were examined for diseases between February 2011 and June 2012. The areas identified for study were selected so as to include the most commonly planted *Eucalyptus* species in the country, trees of different age classes, and to cover as many ecological zones as possible (Table 1). Forestry companies and district forestry officers were contacted to gain access to recent reports and thus to include current disease problems.

For surveys, transects were made through plantations and woodlots and trees were visually checked for signs of wilting, die-back, kino exudation, cracks and lesions on the stems, leaf spots and blight. Sections of stems, branches and roots, as well as entire leaves, showing signs of pathogen presence were collected in separate paper bags and transported to the laboratory for isolation and identification of the causal agents. Twigs with fruiting structures resembling those of fungi in the Botryosphaeriaceae were also collected at each site visited. Where encountered, bark and wood tissue samples were collected from freshly harvested stumps (two- to four-week-old harvesting wounds) to obtain wound-associated fungi.

Various techniques were used to isolate pathogens from the collected samples. Bark samples with typical Teratosphaeria stem canker symptoms were incubated in moist chambers to induce sporulation of fungi, after which spore drops were transferred to petri dishes containing 2% malt extract agar (MEA; 20 g L^{-1} malt extract, 15 g L^{-1} agar [Biolab, Midrand, South Africa] and 1 L deionised water) and streptomycin sulphate. Fungi were also isolated from stem and branch cankers by surface-sterilising the lesions with 70% EtOH, removing the epidermis and cutting a piece of tissue from the leading edges of infection using a sterile blade and transferring this to 2% MEA for incubation at 25 °C until fungal growth was observed. Wood-decay fungi were isolated directly from mycelial fans formed between the bark and the cambium of affected trees on the same day of collection. Bark was split from the wood to expose fresh mycelial fans, after which small sections of the mycelial mats were excised with a scalpel and plated onto MEA (Coetzee et al. 2000; Roux et al. 2005). Tips of the rhizomorphs produced on the primary isolation plates were transferred to fresh MEA plates and incubated at 25 °C. Leaf spot pathogens residing in the Capnodiales were isolated from spots using the method described by Crous (1998), in which ascospores were discharged onto the surface of MEA and single germinating spores were transferred to sterile MEA plates. Species of Microascales and Ophiostomatales were isolated directly from infected tissue by transferring spore masses from the apices of ascomata onto MEA in petri dishes. All primary isolations were purified by transferring single hyphal tips or spore drops to fresh MEA. Representative isolates of all putative pathogens obtained in this study were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Fungal identification

Isolates of fungi obtained during this study were grouped based on culture morphology on MEA as well as the characteristics of fruiting structures, where these were present. Representative isolates from each morphological group were selected for further characterisation using DNA sequence studies.

DNA extraction and PCR amplification

For fungal species identification, mycelium was scraped from the surfaces of young, actively growing cultures using a sterilised surgical blade, transferred into 2 mL Eppendorf tubes and freeze-dried overnight for preservation. Small amounts of freeze-dried mycelium were ground

Table 1: Details of survey areas in Zimbabwe including host species and climatic conditions

Survey area	Designation	Nearest town	Host species[1]	Province	Agroecological region	Mean annual rainfall (mm)
Chesa	Forest Research Station	Bulawayo	GC, *E. paniculata*, *E. propinqua*	Bulawayo	IV	450–650
Imbeza	Plantation	Mutare	*E. grandis*, *E. paniculata*	Manicaland	I	>1 000
John Meikle	Forest Research Station	Mutare	*E. grandis*, GS, GU	Manicaland	I	>1 000
Madziwa	Woodlots	Bindura	*E. camaldulensis*	Mashonaland Central	II	750–1 000
Moyomakaza	Plantation	Rusape	*E. grandis*	Manicaland	II	750–1 000
Mtao	Plantation	Masvingo	*E. grandis*, *E. paniculata*	Midlands	III	650–800
Rushinga	Woodlots	Mount Darwin	*E. camaldulensis*	Mashonaland Central	IV	450–650
Shangani	Woodlots	Gweru	*E. camaldulensis*	Matabeleland South	III	650–800

[1] GC = *Eucalyptus grandis* × *E. camaldulensis* hybrid, GS = *E. grandis* × *E. saligna* hybrid, GU = *E. grandis* × *E. urophylla* hybrid

to a fine powder in sterile 2 mL Eppendorf tubes using a metal ball that had been washed in 70% alcohol and 1% HCl, and autoclaved before use. DNA was extracted using the protocol described by Möller et al. (1992). Each sample was treated with 2 μL RNase (1 mg mL^{-1}) and left overnight to digest RNA. Final DNA working concentrations were adjusted to c. 75 ng μL^{-1} using a Thermo Scientific NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

As a first level of identification, the internal transcribed spacer (ITS) region of the ribosomal DNA gene, including ITS1 and ITS2 as well as the 5.8S operon, of each sample was amplified using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-GCTGCGTTCTTCATCGATGC-3′) (White et al. 1990). For some genera, primer ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes and Bruns 1993) was used as the forward primer, with ITS4 as the reverse primer. For identification to species level, exons 3 to 6 and the respective introns of the β-tubulin gene (BT2) region were amplified using primers βt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and βt2b (5′-AACCTCAGTGTAGTGACCCTTGGC-3′) (Glass and Donaldson 1995). For *Ceratocystis* species, βt1a (5′-TTCCCCCGTCTCCACTTCTTCATG-3′) and βt1b (5′-GACGAGATCGTTCATGTTGAACTC-3′) (Glass and Donaldson 1995) were used for amplification of the β-tubulin 1 (BT1) gene region. In addition, the intron sequences of the translation elongation factor 1-α (TEF1-α) gene were amplified using primers EF1-728F (5′-CATCGAGAAGTTCGAGAAGG-3′) and EF1-986R (5′-TACTTGAAGGAACCCTTACC) (Carbone and Kohn 1999). The primer set EF1F (5′-TGCGGTGGTATCGACAAGCGT-3′) and EF2R (5′-AGCATGTTGTCGCCGTTGAAG-3′) (Jacobs et al. 2004) was used for *Ceratocystis* isolates. The primer pair P-1 (5′-TTGCAGACGACTTGAATGG-3′) (Hsiau 1996) and 5S-2B (5′-CACCGCATCCCGTCTGATCTGCG-3′) (Coetzee et al. 2000) was used to amplify the IGS-1 region of *Armillaria* isolates.

The PCR mix for each sample was prepared by combining 5 μL 5× MyTaq reaction buffer (Bioline, Memphis, TN, USA), 0.5 μL MyTaq DNA polymerase (Bioline), 0.5 μL forward primer, 0.5 μL reverse primer, 17.5 μL Sabax water and 1 μL DNA template to give a total volume of 25 μL in an Eppendorf tube. For the ITS region, PCR reactions consisted of initial denaturation for 4 min at 96 ºC, followed by cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min repeated 10 times, followed by 25 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min with 5 s extensions after every two cycles. A final elongation step of 7 min at 72 °C was included. For the BT gene regions, the PCR reaction conditions consisted of 3 min at 96 °C as initial denaturation and cycles of 30 s at 95 °C, 45 s at 57 °C, 45 s at 72 °C, repeated 40 times. For the TEF1-α, 3 min at 96 °C, and cycles of 30 s at 95 °C, 45 s at 54 °C, 45 s at 72 °C, were repeated 40 times with 5 s extensions after every two cycles. A final elongation step of 7 min at 72 °C was included. PCR products were assessed for amplification on 2% agarose gels stained with Gel Red (Biotium, Haward, CA, USA) in 1× TAE buffer (0.4 M Tris base, 1% acetic acid, 0.5 M EDTA, pH 8.0) and visualised under ultraviolet illumination. PCR products were purified using 6% Sephadex G-50 columns (Sigma-Aldrich, Steinheim, Germany) following the instructions provided by the manufacturer.

DNA sequencing and sequence analyses

PCR products for each sample were sequenced in both directions using the same primers as used for primary PCR. The sequencing PCR reaction mixture for each sample was prepared by adding 2.5 μL sequencing buffer, 0.5 μL Big Dye, 1 μL forward or reverse primer, 4 μL Sabax water and 4 μL DNA template, giving a total volume of 12 μL. The thermal cycling conditions comprised 25 cycles of 10 s at 96 °C, 5 s at 52 °C and 4 min at 60 °C. Sequencing PCR products were cleaned using 6% Sephadex G-50 columns (Sigma-Aldrich, Steinheim, Germany). Sequences of isolates collected from Zimbabwe were compared with ITS, BT, TEF-1α and IGS sequences obtained from GenBank (National Centre for Biotechnology Information, US National Institute of Health, Bethesda, MD, USA). Parsimony analyses were conducted considering single and combined gene sequences. Most parsimonious (MP) trees were generated using PAUP* 4.0b10 (Swofford 2002).

Results

Surveys conducted in *Eucalyptus* plantations and woodlots revealed the existence of at least five diseases. Based on culture and spore morphology, fungi in the genera *Ceratocystis*, *Pesotum* and *Ophiostoma* were obtained from freshly harvested stumps. From leaf spot symptoms, species in the Capnodiales were obtained. Stem canker symptoms yielded fungi resembling species of Botryosphaeriaceae and Teratosphaeriaceae. DNA sequences of the ITS gene region grouped the fungi collected in this study in seven genera. These comprised *Armillaria*, *Ceratocystis*, *Mycosphaerella*, *Neofusicoccum*, *Ophiostoma*, *Teratosphaeria* and *Valsa*. To identify the obtained fungi to species level, additional gene regions, appropriate for each genus, were sequenced.

Stem canker diseases

Pathogens belonging to the genus *Teratosphaeria* were isolated from stems where they were associated with ‘measle-like’ lesions (Figure 1a and b) on *Eucalyptus* species at Chesa, Mtao as well as Madziwa, Rushinga and Shangani rural areas. At Chesa, the disease affected *E. grandis* × *E. camaldulensis* hybrid trees, *E. paniculata* and *E. propinqua* Deane & Maiden. At Mtao, *E. grandis* was affected. *Eucalyptus camaldulensis* was the most severely affected species in Madziwa, Rushinga and Shangani rural areas. Phylogenetic analyses of single and combined ITS, BT2 and TEF-1α DNA sequences showed fungi isolated from the lesions to represent the pathogen previously described as *T. gauchensis* (M.N.Cortinas, Crous & M.J.Wingf.) M.J.Wingf. & Crous (Table 2).

Symptoms consistent with those of Botryosphaeria stem canker (Figure 1c and d) were observed at all sites sampled. All species surveyed, including *E. camaldulensis*, *E. grandis*, *E. grandis* × *E. camaldulensis* hybrid and *E. paniculata*

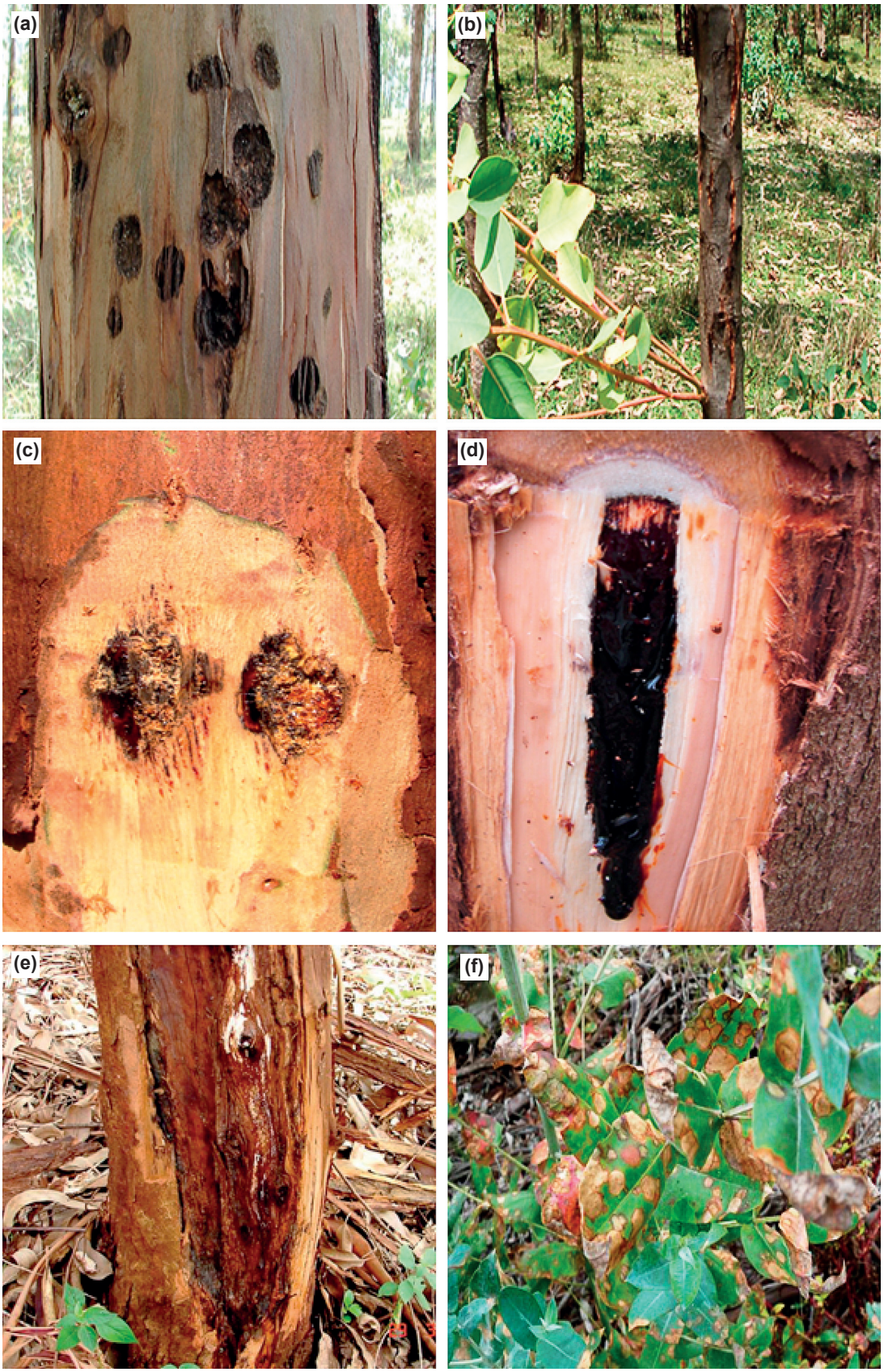

Figure 1: Symptoms of some of the most important diseases of *Eucalyptus* species in Zimbabwe. (a) Lesions and (b) epicormic shoots caused by *T. gauchensis*. (c) Kino pockets and (d) kino typical of Botryosphaeria stem canker. (e) Stem butt deformed by *Armillaria* infection and (f) Mycosphaerella leaf disease

Table 2: Details of the isolates collected in Zimbabwe on various *Eucalyptus* species in 2012 and others used for comparative purposes in this study

Fungal species	Isolate no.	Host[1]	Origin	GenBank accession no.			Reference
				ITS	BT/IGS*	EF-α	
Armillaria fuscipes	CMW2717	*Pinus elliottii*	South Africa	AY882971	AF204821*	–	Coetzee et al. (2000)
A. fuscipes	CMW2740	*Pinus patula*	South Africa	AY882970	AF204822*	–	Coetzee et al. (2005)
A. fuscipes	CMW3164	*Pelargonium asperum*	La Reunion	AY882975	AF204824*	–	Coetzee et al. (2000)
A. fuscipes	CMW4874	*Araucaria cunninghamii*	Zimbabwe	AY882967	AF489481*	–	Mwenje et al. (2003)
A. fuscipes	CMW10165	*Prunus persica*	Zimbabwe	AY882966	AF489482*	–	Coetzee et al. (2005)
A. heimii	CMW3173	*Tectona grandis*	Zambia	AY882981	AF204825*	–	Coetzee et al. (2000)
A. mellea	Iso94-7		Japan	AB510863	AB510819	–	Coetzee et al. (2005)
A. sp.	CMW4455	*Camellia sinensis*	Zimbabwe	AY882985	AF489486*	–	Mwenje et al. (2003)
A. sp.	CMW4456	*Brachystegia utilis*	Zimbabwe	AY882984	AF489485*	–	Mwenje et al. (2003)
A. sp.	CMW10115	*Acacia albida*	Zimbabwe	AY882983	AF489483*	–	Mwenje et al. (2003)
A. sp.	CMW10116	*Newtonia buchananii*	Zimbabwe	AY882982	AF489484*	–	Mwenje et al. (2003)
A. sp.	CMW38628	*Eucalyptus grandis*	Zimbabwe	KF878323	KF941286*	–	Present study
A. sp.	CMW38630	GU	Zimbabwe	KF923251	KF941287*	–	Present study
A. sp.	CMW38631	GU	Zimbabwe	KF878324	KF941288*	–	Present study
Ceratocystis atrox	CMW19383	*Eucalyptus grandis*	Australia	EF070414	EF070430	EF070402	van Wyk et al. (2007)
C.atrox	CMW19385	*Eucalyptus grandis*	Australia	EF070415	EF070431	EF070403	van Wyk et al. (2007)
C. corymbiicola	CMW29120	*Corymbia variegata*	Australia	HM071902	HM071914	HQ236453	Kamgan Nkuekam et al. (2012)
C. corymbiicola	CMW29349	*Eucalyptus pilularis*	Australia	HM071919	HQ236455	HM071905	Kamgan Nkuekam et al. (2012)
C. manginecans	CMW38736	*Eucalyptus grandis*	Zimbabwe	KF878325	KF878334	KF878345	Present study
C. manginecans	CMW38737	*Eucalyptus grandis*	Zimbabwe	KF878326	KF878335	KF878346	Present study
C. manginecans	CMW38739	*Eucalyptus grandis*	Zimbabwe	KF878327	KF878336	KF878347	Present study
C. sp.	CMW38735	*Eucalyptus grandis*	Zimbabwe	KF878328	KF878337	KF878348	Present study
C. sp.	CMW38636	*Eucalyptus grandis*	Zimbabwe	KF878329	KF878338	KF878349	Present study
C. obpyriformis	CMW23807	*Acacia mearnsii*	South Africa	EU245004	EU244976	EU244936	Heath et al. (2009)
C. obpyriformis	CMW23808	*Acacia mearnsii*	South Africa	EU245003	EU244975	EU244935	Heath et al. (2009)
C. pirilliformis	CMW6569	*Eucalyptus nitens*	Australia	AF427104	DQ371652	AY528982	Barnes et al. (2003)
C. pirilliformis	CMW6579	*Eucalyptus nitens*	Australia	AF427105	DQ371653	AY528983	Barnes et al. (2003)
C. polychroma	CMW11424	*Syzygium aromaticum*	Indonesia	AY528970	AY528966	AY528978	van Wyk et al. (2004)
C. polychroma	CMW11436	*Syzygium aromaticum*	Indonesia	AY528971	AY528967	AY528979	van Wyk et al. (2004)
C. polyconidia	CMW23809	*Acacia mearnsii*	South Africa	EU245006	EU244978	EU244938	Heath et al. (2009)
C. polyconidia	CMW23818	*Acacia mearnsii*	South Africa	EU245007	EU244979	EU244939	Heath et al. (2009)
C. tanganyicensis	CMW15991	*Acacia mearnsii*	Tanzania	EU244997	EU244969	EU244929	Heath et al. (2009)
C. tanganyicensis	CMW15999	*Acacia mearnsii*	Tanzania	EU244998	EU244970	EU244939	Heath et al. (2009)
C. tsitsikammensis	CMW14276	*Rapanea melanophloeos*	South Africa	EF408555	EF408569	EF408576	Kamgan Nkuekam et al. (2008)
C. tsitsikammensis	CMW14278	*Rapanea melanophloeos*	South Africa	EF408556	EF408570	EF408577	Kamgan Nkuekam et al. (2008)
C. virescens	CMW11164	*Fagus americana*	USA	DQ520639	EF070441	EF070413	van Wyk et al. (2007)
C. zambeziensis	CMW35958	*Combretum imberbe*	South Africa	KC691458	KC691482	KC691506	Mbenoun et al. (2014)
C. zambeziensis	CMW35959	*Combretum imberbe*	South Africa	KC691459	KC691483	KC691507	Mbenoun et al. (2014)
Mycosphaerella marksii	CMW38632	*Eucalyptus grandis*	Zimbabwe	KF878319	–	KF878343	Present study
M. marksii	CMW38633	*Eucalyptus grandis*	Zimbabwe	KF878320	–	KF878344	Present study
Neofusicoccum eucalyptorum	CMW40036	*Eucalyptus grandis*	Zimbabwe	KF923239	KF923260	KF923272	Present study
N. eucalyptorum	CMW40037	*Eucalyptus grandis*	Zimbabwe	KF923240	KF923261	KF923273	Present study
N. eucalyptorum	CMW40038	*Eucalyptus grandis*	Zimbabwe	KF923241	KF923262	KF923274	Present study
N. parvum	CMW38722	*Eucalyptus grandis*	Zimbabwe	KF923242	KF923263	KF923275	Present study
N. parvum	CMW38723	*Eucalyptus grandis*	Zimbabwe	KF923243	KF923264	KF923276	Present study
N. parvum	CMW38724	*Eucalyptus grandis*	Zimbabwe	KF923244	KF923265	KF923277	Present study
Ophiostoma quercus	CMW40055	*Eucalyptus grandis*	Zimbabwe	KF923245	KF923257	KF923266	Present study
O. quercus	CMW40056	*Eucalyptus grandis*	Zimbabwe	KF923246	KF923258	KF923267	Present study
O. quercus	CMW40057	*Eucalyptus grandis*	Zimbabwe	KF923247	KF923259	KF923268	Present study
Teratosphaeria gauchensis	CMW37812	*Eucalyptus camaldulensis*	Zimbabwe	KF878313	KF878330	KF878339	Present study
T. gauchensis	CMW37818	GC	Zimbabwe	KF878314	KF878331	KF878340	Present study
T. gauchensis	CMW37821	*Eucalyptus propinqua*	Zimbabwe	KF878316	KF878333	KF878342	Present study
T. ohnowa	CMW38639	*Eucalyptus grandis*	Zimbabwe	KF878317	KF923252	KF923278	Present study
T. ohnowa	CMW38640	*Eucalyptus grandis*	Zimbabwe	KF878318	KF923253	KF923279	Present study
Valsa fabianae	CMW40048	*Eucalyptus camaldulensis*	Zimbabwe	KF923248	KF923254	KF923269	Present study
V. fabianae	CMW40051	*Eucalyptus camaldulensis*	Zimbabwe	KF923249	KF923255	KF923270	Present study
V. fabianae	CMW40052	*Eucalyptus camaldulensis*	Zimbabwe	KF923250	KF923256	KF923271	Present study

[1] GC = *Eucalyptus grandis* × *E. camaldulensis* hybrid, GU = *E. grandis* × *E. urophylla* hybrid

had symptoms of this disease. The symptoms observed included cankers on stems and branches, kino pockets in the wood beneath the bark, kino exudation and cracked bark (Figure 1d). The pathogens associated with the disease also formed fruiting bodies on branches from which they were isolated. Fungi isolated from samples with Botryosphaeria stem canker symptoms were identified using ITS, BT2 and TEF-1α sequence data as *Neofusicoccum eucalyptorum* Crous, H.Smith and M.J.Wingf. and *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L.Phillips (Table 2).

Root rot disease

Armillaria root and stem rot (Figure 1e) was observed at John Meikle Forest Research Station in the Eastern Highlands of Zimbabwe. The disease affected *E. grandis* and its hybrids with *E. saligna* Sm. and *E. urophylla* S.T.Blake. Symptoms included cracked and deformed stem bases, the presence of thick mats of mycelium and rhizomorphs beneath the bark of infected trees. Multi-gene (ITS and IGS-1) phylogenies of *Armillaria* isolates collected from *Eucalyptus* in Zimbabwe, and those previously collected from a wide range of native hosts in the country, showed that they formed a clade of closely related species. The isolates collected in this study formed a separate subgroup, among the previously reported Groups II and III (Mwenje and Ride 1996; Mwenje et al. 2003) (Figure 2).

Wound-associated fungi

Fungi resembling species of *Ceratocystis* were found on the stumps of freshly harvested *E. grandis* in the Mtao area of the Masvingo Province. DNA sequence data revealed the presence of two species. The first group of isolates were tentatively identified as *C. manginecans* M.van Wyk, A.Adawi & M.J.Wingf based on phylogenetic analyses of sequences for the single and combined ITS, BT1 and TEF1-α gene regions. The second *Ceratocystis* sp. formed a separate group close to *C. obpyriformis* R.N.Heath & Jol. Roux, *C. pirilliformis* Barnes & M.J.Wingf. and *C. polyconidia* R.N.Heath & Jol.Roux (Figure 3).

Species of *Ophiostoma* were also found on the fresh stem wounds on *E. camaldulensis* and the GC hybrid at Chesa Forest Research Station and from *E. grandis* at Imbeza, Moyomakaza and Mtao. DNA sequences of the ITS, BT2 and TEF1-α gene regions of isolates matched with 100 percent similarity with reference isolates of *Ophiostoma quercus* (Georgévitch) Nannf. Single and multi-gene phylogenetic analyses using DNA sequences of the three gene regions confirmed the identity of the fungus as *O. quercus* (Table 2).

Leaf spot diseases

Symptoms consistent with those of Mycosphaerella leaf spot diseases (MLD; Figure 1f) were observed on *E. camaldulensis*, *E. grandis*, GC, *E. paniculata* and *E. tereticornis* at all sites surveyed. Fruiting bodies of the

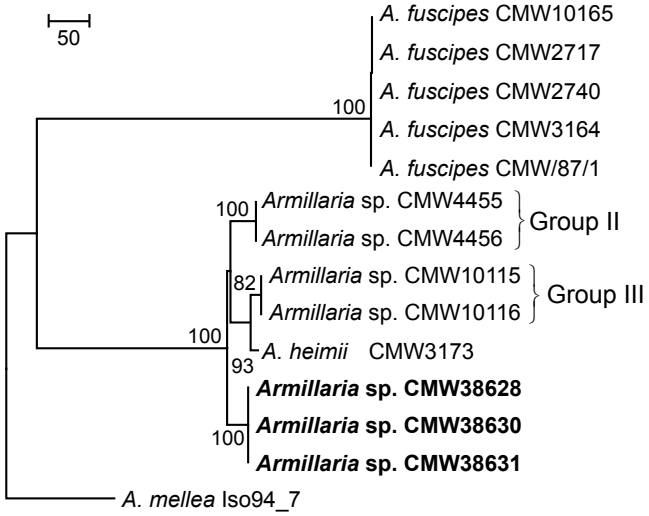

Figure 2: First of 1 000 equally most parsimonious trees obtained from a heuristic search with 14 random taxon additions of combined ITS and IGS sequences alignment using PAUP* 4.0b10. Bootstrap support values after 1 000 replicates are shown at the nodes. *Armillaria mellea* was used as the outgroup. Isolates in bold were obtained in this study

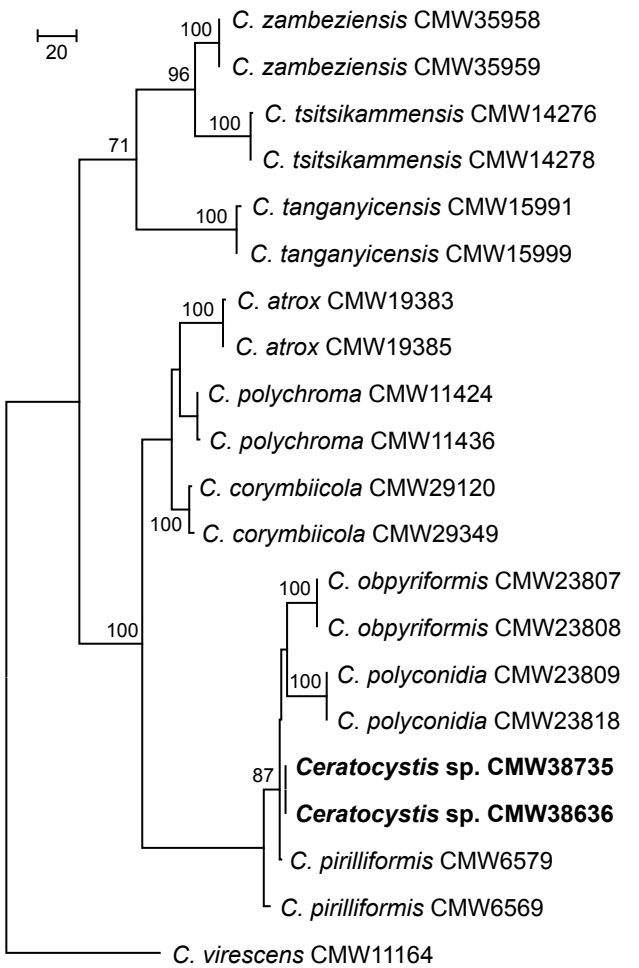

Figure 3: First of 1 000 equally most parsimonious trees obtained from a heuristic search with 21 random taxon additions of combined ITS, BT1 and TEF1-α sequences alignment using PAUP v4.0b10. Bootstrap support values after 1 000 replicates are shown at the nodes. *Ceratocystis virescens* was used as the outgroup. Isolates in bold were obtained in this study

fungal pathogens were visible in the necrotic patches produced by these fungi. Fungi isolated from *Eucalyptus* leaves with symptoms of MLD were identified using ITS, BT2 and TEF-1α gene sequences. Large necrotic leaf spots yielded isolates of *Mycosphaerella marksii* Carnegie & Keane, whereas smaller spots were caused by *Teratosphaeria ohnowa* Crous & M.J.Wingf. (Table 2).

Other fungi

Some endophytic fungi were isolated from abscised *Eucalyptus* branches sampled for the isolation of pathogens in the Botryosphaeriaceae. Single and multi-gene phylogenetic analyses of the ITS, BT2 and TEF1-α DNA sequences identified the fungus to be *Valsa fabianae* G.C.Adams, M.J.Wingf. & Jol.Roux (Table 2). This fungus was isolated from a wide range of hosts including *E. camaldulensis*, *E. grandis* and *E. paniculata* from all the sites sampled.

Discussion

This study represents the most comprehensive evaluation of the fungal pathogens in *Eucalyptus* plantations in Zimbabwe. All of the pathogens found during surveys are reported from Zimbabwe for the first time, although all but one of them are known in neighbouring countries (Roux et al. 2005; Chungu et al. 2010a, 2010b; Roux et al. 2012). The exception was the stem canker pathogen, *T. gauchensis*, which was previously not known from southern Africa. The most commonly encountered disease was Botryosphaeria stem canker, but significant damage was also associated with infections by *T. gauchensis* and root rot caused by an *Armillaria* sp. Leaf spots were common in all areas, but were found to result in obvious damage only on *E. grandis* and *E. camaldulensis* and in relatively limited areas.

The identification of *T. gauchensis* in Zimbabwe was unexpected given that this pathogen was previously known only from Argentina, Uruguay (Cortinas et al. 2006), Hawaii (Cortinas et al. 2004), Ethiopia (Gezahgne et al. 2005) and Uganda (Roux et al. 2005). In contrast, the closely related *T. zuluensis* M.J.Wingf., Crous & T.A.Cout., is well known in southern Africa, including South Africa (Wingfield et al. 1997), Malawi, Mozambique (Roux et al. 2005) and Zambia (Chungu et al. 2010a, 2010b). *Teratosphaeria gauchensis* is widespread in Zimbabwe, associated with severe disease on *E. grandis*, *E. camaldulensis*, *E. paniculata* and *E. propinqua*. Symptoms of Teratosphaeria stem canker were observed in Zimbabwe for the first time in 2010 and it will be important to determine the origin of *T. gauchensis* in this country and to initiate a programme to manage this disease.

Symptoms of Botryosphaeria stem canker were observed mainly on *E. grandis*, *E. camaldulensis* and *E. tereticornis* in all the areas sampled in this study. The associated pathogens were identified as *N. eucalyptorum* and *N. parvum*. Masuka (1990) suggested that Botryosphaeria stem canker was the most widespread and important disease of *E. grandis* in Zimbabwe, although he suggested *B. dothidea* as the causal agent. Due to considerable taxonomic confusion relating to this group of fungi, the name *B. dothidea* was used loosely for species in the Botryosphaeriaceae (Smith et al. 2001; Slippers et al. 2004a, 2004b). More recent taxonomic studies (e.g. Slippers et al. 2004a; Phillips et al. 2013) showed that isolates previously identified as *B. dothidea* in South Africa represented *N. parvum* and it seems probable that *N. eucalyptorum* and *N. parvum* found in this study were the same as the fungus previously reported by Masuka (1990).

The two *Neofusicoccum* species found on *Eucalyptus* species in this study have both previously been found on these trees in Africa. *Neofusicoccum eucalyptorum* was described by Smith et al. (2001) from *Eucalyptus* species in South Africa and has since been reported from several other African countries (Slippers et al. 2004b; Chungu et al. 2010a). *Neofusicoccum parvum* was described from New Zealand (Pennycook and Samuels 1985) and was previously also reported to cause cankers on *Eucalyptus* in Africa (Slippers et al. 2004a, 2004b; Gezahgne et al. 2004a; Pavlic et al. 2007; Chungu et al. 2010a). While *N. eucalyptorum* appears to be specialised to the Myrtaceae, especially *Eucalyptus* (Slippers et al. 2004b; Perez et al. 2009), *N. parvum* has been found on a wide range of hosts in many different parts of the world (e.g. Golzar and Burgess 2011; Heath et al. 2011; Yu et al. 2013). The Botryosphaeriaceae are opportunistic pathogens that exist as endophytes in healthy plant tissue and cause disease when trees are exposed to stresses such as those emerging from frost, hail, drought and physical damage (Smith et al. 2001; Slippers and Wingfield 2007). They have significant potential to cause increasingly common problems to *Eucalyptus* plantings under conditions of changing climate, particularly drought situations, and where appropriate silviculture is not applied timeously.

Armillaria root rot is a commonly encountered disease of trees in Africa, including Zimbabwe (e.g. Mwenje and Ride 1996; Mwenje et al. 1998; Coetzee et al. 2000; Keane et al. 2000; Mwenje et al. 2003; Gezahgne et al. 2004b; Roux et al. 2005; Wingfield et al. 2009). Although this study represents the first report of Armillaria root and stem rot of *E. grandis* and hybrids of this species with *E. saligna* and *E. urophylla* in Zimbabwe, the disease has previously been reported on various tree species (Keane et al. 2000). In Africa, Armillaria root rot has been reported from *Eucalyptus* species in Kenya (Roux et al. 2005), South Africa (Coetzee et al. 2000), Tanzania and Tunisia (Keane et al. 2000). Armillaria root and stem rot is caused by two known species in southern Africa, *A. fuscipes* Petch and *A. heimii* Pegler (Gezahgne et al. 2004b; Pérez-Sierra et al. 2004; Coetzee et al. 2005; Mwenje et al. 2006). A number of additional *Armillaria* species that are recognised in Africa have not yet been given names. These include Zimbabwean isolates that could be separated into three distinct groups (Groups I–III) (Mwenje and Ride 1996; Mwenje et al. 2003). The isolates collected from *Eucalyptus* in this study resided in a group separate from those previously recognised, suggesting the presence of an additional undescribed pathogen in this genus.

Mycosphaerella leaf spot diseases observed in this study have previously been reported from several African countries (Hunter et al. 2004; Roux et al. 2005; Gezahgne et al. 2006; Chungu et al. 2010a, 2010b). These diseases are

caused by many species in the families Mycosphaerellaceae and Teratosphaeriaceae (Capnodiales) (Gezahgne et al. 2003; Roux et al. 2005; Gezahgne et al. 2006; Chungu et al. 2010a, 2010b) and those found in the present study were identified as *M. marksii* and *T. ohnowa. Mycosphaerella marksii* was first described in Australia (Carnegie and Keane 1994) and is not considered to be an aggressive pathogen (Carnegie and Keane 1994; Hunter et al. 2004). In Africa, *M. marksii* has been reported from Ethiopia (Gezahgne et al. 2006) and South Africa (Carnegie and Keane 1994; Crous and Wingfield 1996). *Teratosphaeria ohnowa* was previously known only from South Africa and Australia (Crous et al. 2004; Hunter et al. 2006). Zimbabwe thus represents a new geographic region for these fungi. Neither species is considered to be a serious threat to *Eucalyptus* forestry in Zimbabwe.

Species of *Ceratocystis* and *Ophiostoma* are commonly associated with wounds on trees. These genera include important tree pathogens (e.g. Kile 1993; Roux and Wingfield 2009) with *Ceratocystis* species emerging as important pathogens of *Eucalyptus* and *Acacia* species grown in plantations globally (Roux and Wingfield 1997; Roux et al. 1999, 2004; Roux and Wingfield 2009). In this study, the identified *Ceratocystis* and *Ophiostoma* species were not associated with disease symptoms and occurred only on wounds resulting from harvesting. The *Ceratocystis* species tentatively identified include those previously treated in the *Ceratocystis fimbriata* complex, including many tree pathogens such as those of *Eucalyptus* and this should be tested. *Ophiostoma quercus* found in this study is a cosmopolitan species with a wide host range and probably represents a species complex (Grobbelaar et al. 2008; Kamgan Nkuekam et al. 2012). The fungus has previously been reported from *Eucalyptus* in Australia (Kamgan Nkuekam et al. 2011), South Africa (de Beer et al. 2003; Kamgan Nkuekam et al. 2012), Tanzania (Grobbelaar et al. 2009), Uganda (Kamgan Nkuekam et al. 2008) and Uruguay (Harrington et al. 2001), but is not considered to be a pathogen.

Conclusions

Eucalyptus pathogens reported in this study represent first reports for Zimbabwe. Most of the pathogens identified have been known from neighbouring African countries for a number of years and it is thus not surprising that they were found in Zimbabwe. The identification of *T. gauchensis* in the country represents a significant and unexpected finding. This study is the first report of the pathogen in southern Africa, where previously only *T. zuluensis* was known. The occurrence of *T. gauchensis* in Zimbabwe, surrounded by *T. zuluensis* in other countries, requires further study and raises numerous important questions regarding the introduction and spread of this important *Eucalyptus* stem canker pathogen. This is particularly important since Zimbabwe is still a major supplier of *Eucalyptus* seed to other African countries. Overall, it is clear that *Eucalyptus* forestry in Zimbabwe will be challenged by pathogen problems and there is an urgent need to establish a robust research programme to assist tree farmers in dealing with these problems.

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