Forest Pathology WILEY-BLACKWELL

For. Path. doi: 10.1111/efp.12264

SHORT COMMUNICATION

First report of *Teratosphaeria gauchensis* causing stem canker of *Eucalyptus* in Kenya

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Summary

Teratosphaeria stem canker is an important disease of *Eucalyptus* species in many parts of the world where these trees are intensively propagated in plantations. Symptoms similar to those of Teratosphaeria stem canker were observed on *Eucalyptus grandis* and a *E. grandis* × *E. camaldulensis* hybrid clone in the Central Highlands of Kenya. Symptomatic bark samples were collected from two sites and the associated fungus isolated and identified using DNA sequence analyses of multiple gene regions. The pathogen was identified as *Teratosphaeria gauchensis*. This represents the first report of the disease and the pathogen in Kenya.

1 Introduction

Teratosphaeria stem canker is one of the most important diseases to have emerged on *Eucalyptus* species grown as non-natives in intensively managed plantations outside the native range of these trees. The disease was first observed in South Africa in the late 1980s (Wingfield et al. 1997) and has subsequently been reported from several countries in Africa, the Americas, Asia and Europe. The disease was originally ascribed to a single pathogen, *Coniothyrium* (now *Teratosphaeria*) *zuluensis* M.J. Wingf., Crous & T.A. Cout. DNA sequence analyses later showed that the fungus considered as a single species represented two distinct, but closely related species (Cortinas et al. 2006). The second species was described as *Colletogloeopsis* (now *Teratosphaeria*) *gauchensis* M.N. Cortinas, Crous & M.J. Wingf.

Teratosphaeria zuluensis is known to cause disease in China, Malawi, Mexico, South Africa, Thailand, Uganda, Vietnam and Zambia (Roux et al. 2005; Cortinas et al. 2006; Chungu et al. 2010; Jimu et al. 2014). *Teratosphaeria gauchensis* is known from Argentina, Ethiopia, Hawaii, Portugal, Uganda, Uruguay and Zimbabwe (Roux et al. 2005; Cortinas et al. 2006; Silva et al. 2014; Jimu et al. 2015a).

The symptoms of Teratosphaeria stem canker (Fig. 1) include dark brown circular lesions that sometimes coalesce to give rise to larger lesions, subepidermal dark brown pycnidia on the lesions, kino exudation and epicormic shoots

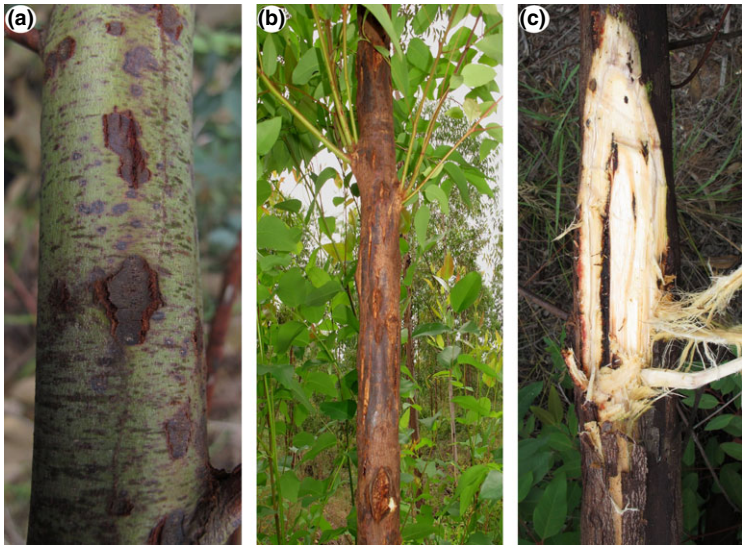

Fig. 1. Typical symptoms of Teratosphaeria stem canker on *Eucalyptus* observed in Kenya. (a) Discrete necrotic lesions on a young stem, (b) Large coalescing lesions giving rise to the production of epicormic shoots, (c) Kino pockets deep in the wood.

Received: 28.10.2015; accepted: 12.1.2016; editor: S. Woodward

(Wingfield et al. 1997; Cortinas et al. 2006). The disease impacts negatively on growth but is especially relevant where sawn timber is produced for construction and where kino pockets weaken the wood and detracts from its aesthetic value.

During a disease survey of *Eucalyptus* species in Kenya in 2013, symptoms similar to those known for Teratosphaeria stem canker were observed in some *Eucalyptus* plantations in the Central Highlands of the country. The aim of this study was to identify the causal agent of the stem canker disease in the region.

2 Materials and methods

2.1 Sampling and isolation

Diseased bark samples were collected from stems and branches of *Eucalyptus grandis* at Nanyuki KTDA (0.05221°N; 37.1719°E) and from a *E. grandis* × *camaldulensis* hybrid at Muranga Gatharaini plantation (0.49943°N; 37.08859°E) in the Central Highlands of Kenya. The bark samples were placed in moist chambers, and freshly exuding spore masses were picked from pycnidia and transferred to Petri dishes containing MEA (20 g/l malt extract, 15 g/l agar Biolab, Midrand, South Africa, and 1 l deionized water). Single hyphal tips from developing colonies were transferred to fresh MEA plates after a week. After about 3 weeks, two plates of each culture were deposited into the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

2.2 DNA extraction, PCR and sequencing

For DNA extraction, mycelium was scraped from actively growing cultures using a sterilized surgical blade, transferred into 2-ml Eppendorf tubes, freeze-dried and ground to a powder using a Retsch Mixer MM 301. DNA was extracted, and the

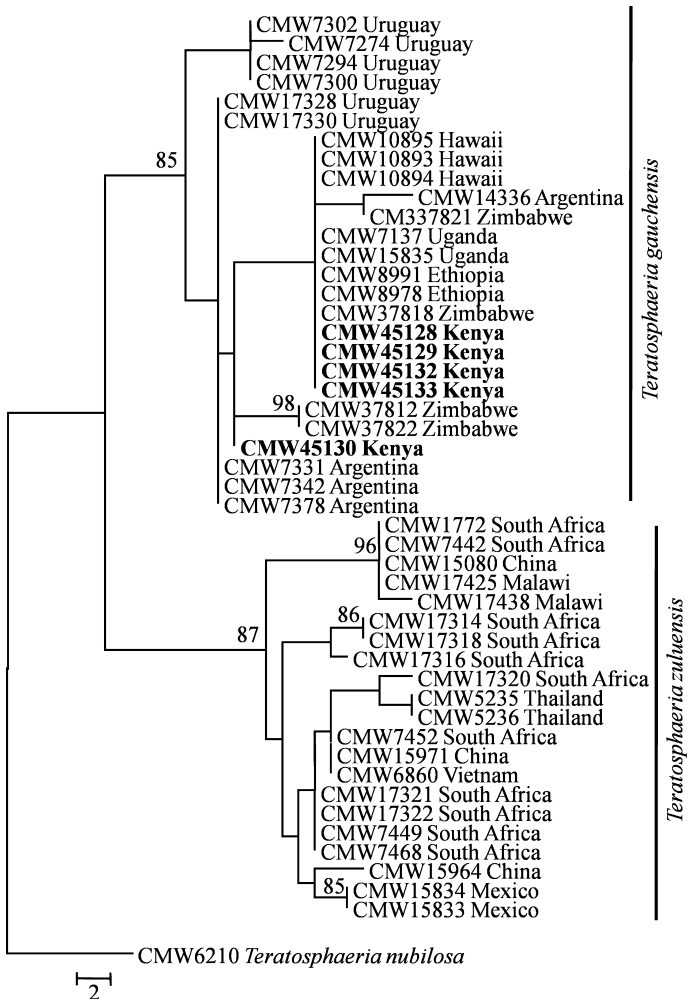

Fig. 2. A parsimonious tree obtained from a heuristic search with 48 random taxon additions of combined ITS and β-tubulin sequences alignment using PAUP v4.0b10. Bootstrap support values greater than 85 after 1000 replicates are shown at the nodes. Isolates from Kenya are shown in bold. *Teratosphaeria nubilosa* was used as an out-group.

internal transcribed spacer regions (ITS1, ITS2) (including the complete 5.8S) of the nuclear rDNA, as well as exons 3–6 and the respective introns of the β-tubulin 2 (BT2) gene regions, were sequenced as described by Jimu et al. (2015a).

2.3 Sequence analyses

DNA sequences were subjected to Blast searches in GenBank [National Centre for Biotechnology Information (NCBI), USA National Institute of Health Bethesda (http:/www.ncbi.nlm.nih.gov/BLAST)] and then compared with published sequences of closely related species in phylogenetic analyses. Sequence data alignment was conducted online using MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/). Parsimony analyses of separate and combined gene region data sets were conducted using PAUP v. 4.0b10. *Teratosphaeria nubilosa* was used as an out-group.

3 Results and discussion

A total of five isolates resembling the pathogens that cause Teratosphaeria stem canker were obtained from five diseased *Eucalyptus* trees in Kenya. The isolates were identified, based on DNA sequence (GenBank accession numbers KU052593–KU052597 for ITS and KU052598–KU052602 for BT) comparisons (Fig. 2), as *T. gauchensis*. This study represents the first report of this important stem canker disease and its associated causal agent, in Kenya. Discovery of *T. gauchensis* in Kenya, rather than *T. zuluensis,* is not surprising because the pathogen has been known in east Africa for more than a decade. This is in contrast to *T. zuluensis*, which was only recently reported in the region, from Uganda (Jimu et al. 2014), and which is the dominant species in Southern African countries (Cortinas et al. 2006).

The outbreak of Teratosphaeria stem canker in Kenya appears to be as a result of a recent introduction. This view is based on the fact that previous disease surveys in the region failed to detect the disease (Roux et al. 2005), even in the area where it was found during the current study. Furthermore, the disease currently has a limited known distribution in the country. During this study, disease surveys were conducted on multiple *Eucalyptus* species and clones along the coast of Kenya, as well as in the Highlands. However, the disease was only observed on a limited number of trees, and in a limited number of compartments in the Highlands. *Teratosphaeria gauchensis* could have entered the country via natural spread, from neighbouring Ethiopia or Uganda, or via the introduction into Kenya of contaminated plant material (Jimu et al. 2015b).

New disease and pest problems are emerging increasingly commonly in planted forests, and these provide significant challenges for forest industries. This report of Teratosphaeria stem canker in Kenya emphasizes the fact that great care should be taken to avoid further introduction of new *T. gauchensis* genotypes that could exacerbate the current disease problem. The recent appearance of *T. gauchensis* in the country also suggests that *T. zuluensis* could easily be introduced, if it is not already present. The consequences of a co-occurrence of these two closely related species are difficult to predict but hybridization between them could result in more serious disease problems in future. It is, therefore, important that more extensive surveys be conducted in other areas to determine the occurrence of *T. gauchensis* and possibly *T. zuluensis* in Kenya.

Acknowledgements

This work is based on research supported by the National Research Foundation of South Africa (Grant specific unique reference number (UID83924). The grant holders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the authors and that the NRF accepts no liability whatsoever in this regard.

We thank the members of the Tree Protection Co-operative Program (TPCP), the THRIP initiative of the Department of Trade and Industry, the DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and the Kenya Forestry Research Institute (KEFRI) for financial support to undertake this work. Forestry companies in Kenya are thanked for assistance and permission to conduct surveys in their plantations.

References

Chungu, D.; Muimba-Kankolongo, A.; Wingfield, M. J.; Roux, J., 2010: Plantation forestry diseases in Zambia: contributing factors and management options. Ann. For. Sci. **67**, 1–9.

Cortinas, M. N.; Crous, P. W.; Wingfield, B. D.; Wingfield, M. J., 2006: Multi-gene phylogenies and phenotypic characters distinguish two species within the *Colletogloeopsis zuluensis* complex associated with *Eucalyptus* stem cankers. Stud. Mycol. **55**, 133–146.

Jimu, L.; Wingfield, M. J.; Mwenje, E.; Roux, J., 2014: First report of *Teratosphaeria zuluensis* causing stem canker of *Eucalyptus grandis* in Uganda. Forest Pathol. **44**, 242–245.

Jimu, L.; Wingfield, M. J.; Mwenje, E.; Roux, J., 2015a: Diseases of *Eucalyptus* species in Zimbabwean plantations and woodlots. South. For. **77**, 221–230.

Jimu, L.; Kemler, M.; Wingfield, M. J.; Mwenje, E.; Roux, J., 2015b: The *Eucalyptus* stem canker pathogen *Teratosphaeria zuluensis* detected in seed samples. Forestry. doi:10.1093/forestry/cpv037.

Roux, J.; Meke, G.; Kanyi, B.; Mwangi, L.; Mbaga, A.; Hunter, G. C.; Nakabonge, G.; Heath, R. N.; Wingfield, M. J., 2005: Diseases of plantation forestry tree species in eastern and southern Africa. S. Afr. J. Sci. **101**, 409–413.

Silva, M. R. C.; Diogo, E.; Bragança, H.; Machado, H.; Phillips, A. J. L., 2014: *Teratosphaeria gauchensis* associated with trunk, stem and foliar lesions of *Eucalyptus globulus* in Portugal. Forest Pathol. **45**, 224–234.

Wingfield, M. J.; Crous, P. W.; Coutinho, T. A., 1997: A serious canker disease of *Eucalyptus* in South Africa caused by a new species of Coniothyrium. Mycopathologia **136**, 139–145.