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* x *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 1980’s
(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, Vietnam, Uganda and Zambia (Roux et al. 2005; Cortinas et al. 2006; Chungu et al. 2010). *Teratosphaeria gauchensis* is known from Argentina, Ethiopia, Hawaii, Portugal, Uganda, Uruguay, and Zimbabwe (Cortinas et al. 2006; Silva et al. 2014, Jimu et al. 2015b).

The symptoms of *Teratosphaeria* stem canker (Figure 1) include dark brown circular lesions that sometimes coalesce to give rise to larger lesions; sub-epidermal dark brown pycnidia on the lesions; kino exudation and epicormic shoots (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.
Figure 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.

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

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
Figure 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 after 1000 replicates are shown at the nodes. Isolates from Kenya are shown in bold. *Teratosphaeria nubilosa* was used as an out-group.
DNA sequence (Genbank accession numbers KU052593-KU052597 for ITS, and KU052598-KU052602 for BT) comparisons (Figure 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. 2015a).

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 emphasises 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.*
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 hybridisation
between them could result in more serious disease problems in the 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.

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References
diseases in Zambia: Contributing factors and management options. Annals of Forest
Science 67, 1–9.
phylogenies and phenotypic characters distinguish two species within the
Colletogloeopsis zuluensis complex associated with Eucalyptus stem cankers. Studies in Mycology 55, 133–146.


