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SHORT COMMUNICATION

# First report of *Teratosphaeria zuluensis* causing stem canker of *Eucalyptus grandis* in Uganda

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## Summary

Teratosphaeria stem canker is one of the most important diseases to have emerged on non-native plantation-grown *Eucalyptus* trees globally. In 2012, *Eucalyptus grandis* trees with typical Teratosphaeria stem canker symptoms were observed in Uganda. Multigene sequence analyses of isolates from these cankers led to the identification of *T. gauchensis*, previously recorded in Uganda, and *T. zuluensis*. This study represents the first report of *T. zuluensis* in Uganda. Furthermore, this is the first report of the co-occurrence of *T. zuluensis* and *T. gauchensis* in a single area.

## 1 Introduction

Teratosphaeria stem canker, previously known as Coniothyrium stem canker, is one of the most damaging diseases of non-native plantation-grown *Eucalyptus* species, globally. The disease was first discovered in the Zululand region of South Africa in 1989, and the pathogen was described as *Coniothyrium zuluensis* M.J. Wingf., Crous and T.A. Cout (Wingfield et al. 1997). The disease is characterized by the formation of discrete sunken lesions which may merge to form large necrotic cankers on susceptible trees (Wingfield et al. 1997; van Zyl et al. 2002; Gezahgne et al. 2003). In some cases, the lesions have characteristic parallel cracks which give them a typical 'cat's eye' appearance. Pycnidia can often be seen on the dead bark between the two cracks (Wingfield et al. 1997). Kino periodically exudes from resulting kino pockets in the wood of infected trees, staining stems and branches. Epicormic shoots may develop on the stems of severely infected trees, and trees may develop brushlike, flattened crowns (Wingfield et al. 1997; van Zyl et al. 2002; Gezahgne et al. 2003). Although infected trees may still be used for the production of pulp, infected timber is brittle and unsightly, making trees unsuitable for construction and sawn timber.

For many years, Teratosphaeria stem canker was thought to be caused by a single pathogen species. Multigene phylogenetic studies by Cortinas et al. (2006), however, led to the identification of a second species, closely related to *C. zuluensis,* causing a disease with the same symptoms in Uruguay. The pathogens at that time were described as *Colletogloeopsis gauchensis* M.N. Cortinas, Crous and M.J. Wingf. and *C. zuluensis* (Cortinas et al. 2006). The two pathogens have undergone several name changes due to the applications of new technologies to this group of fungi, with the names *Teratosphaeria zuluensis* and *T. gauchensis* currently accepted (Crous et al. 2009). *Teratosphaeria zuluensis* has been reported from Africa, Asia and Central America (Wingfield et al. 1997; Roux et al. 2002, 2005; van Zyl et al. 2002; Gezahgne et al. 2004; Cortinas et al. 2006; Chungu et al. 2010). *Teratosphaeria gauchensis* is known from Africa, North and South America (Gezahgne et al. 2003, 2004; Roux et al. 2005; Cortinas et al. 2006). In Africa, the pathogen has been reported from Ethiopia and Uganda on *E. camaldulensis* Dehn. and *E. grandis* (Hill) Maiden, respectively (Gezahgne et al. 2003; Roux et al. 2005). There are no instances where the two pathogens have both been encountered in a single country.

In previous disease surveys of Uganda, Teratosphaeria stem canker was reported to be widespread but concentrated in the Fort Portal area where it affected mainly young trees of about 2 years of age (Roux and Slippers 2007). In 2010, symptoms of Teratosphaeria stem canker on Eucalypts were identified in areas of Uganda where the disease was not previously known (J. Roux, personal communication). The aim of this study was to characterize the cause of Teratosphaeria stem canker of *Eucalyptus* in these new regions using multigene sequence data.

## 2 Materials and methods

### 2.1 Sampling and isolation

Typical symptoms of Teratosphaeria stem canker of *Eucalyptus* species (Figure 1) were observed in the Fort Portal and Jinja areas (Figure 2) of Uganda in 2012 and material collected for characterization of the causal agent. Bark samples with symptoms of Teratosphaeria stem canker were collected from a 5-year-old compartment in Fort Portal and a coppice-regenerated compartment in Jinja. The samples were placed in moist chambers to induce sporulation, after which spore masses were lifted from the pycnidia and transferred to Petri dishes containing MEA (20 g/l malt extract, 15 g/l agar

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*Fig. 1.* Typical symptoms of Teratosphaeria stem canker. a: Teratosphaeria lesions on a *Eucalyptus* stem showing parallel cracks and measles like spots; b: lesion on a young branch; c: kino pockets in the sapwood of an infected tree.

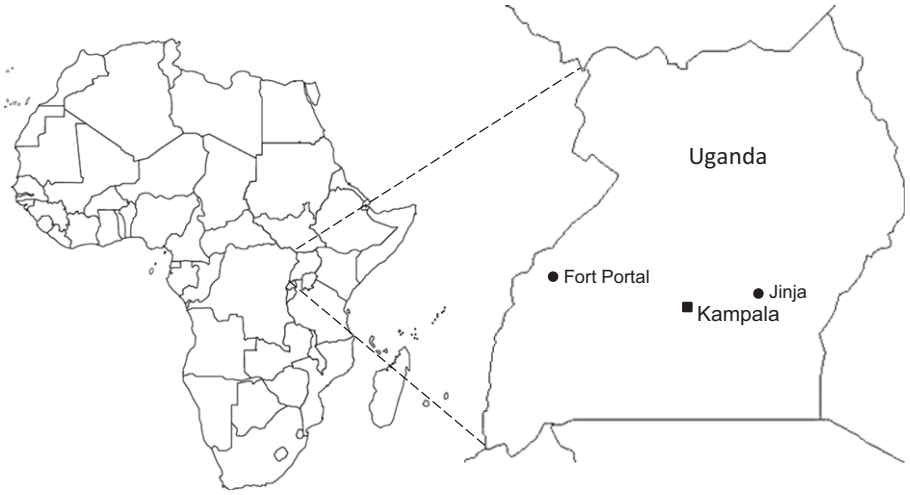


*Fig. 2.* Map of Uganda showing the geographical locations of Fort Portal and Jinja, the two areas from which bark samples with Teratosphaeria stem canker symptoms were collected.

Biolab, Midrand, South Africa, and 1 l deionized water). After 5–7 days, cultures were purified by transferring hyphal tips to fresh MEA plates. The plates were incubated at 25°C for 4 weeks. Cultures from Uganda are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

### 2.2 DNA extraction, PCR and sequence analyses

Actively growing mycelium was scraped from cultures using a sterilized surgical blade and transferred into 2-ml Eppendorf tubes. Mycelium was freeze dried and ground to a fine powder using a Retsch Mixer MM 301. The method described by Möller et al. (1992) was used to extract DNA from the mycelium. Each sample was treated with 3 µl of RNAse (1 mg/ml) and left overnight to digest RNA. DNA concentrations were measured the following morning using a NanoDrop® ND-1000

*Table 1.* List of representative isolates of *Teratosphaeria gauchensis* and *T. zuluensis* collected from Uganda.

| Species | Isolate number | Gene region/GenBank accession no | | Site |
|---|---|---|---|---|
| | | ITS | TEF | |
| *T. gauchensis* | CMW40384 | KF891414 | KF891419 | Jinja |
| | CMW40385 | KF891415 | KF891420 | Fort Portal |
| | CMW40386 | KF891416 | KF891421 | Jinja |
| | CMW40387 | KF891417 | KF891422 | Jinja |
| | CMW40388 | KF891418 | KF891423 | Fort Portal |
| *T. zuluensis* | CMW39044 | KF690139 | KF647563 | Jinja |
| | CMW39045 | KF690141 | KF647565 | Jinja |
| | CMW39046 | KF690138 | KF647562 | Jinja |
| | CMW39047 | KF690140 | KF647564 | Jinja |
| | CMW39048 | KF690142 | KF647566 | Jinja |

ITS, internal transcribed spacer region; and TFF, translation elongation factor.

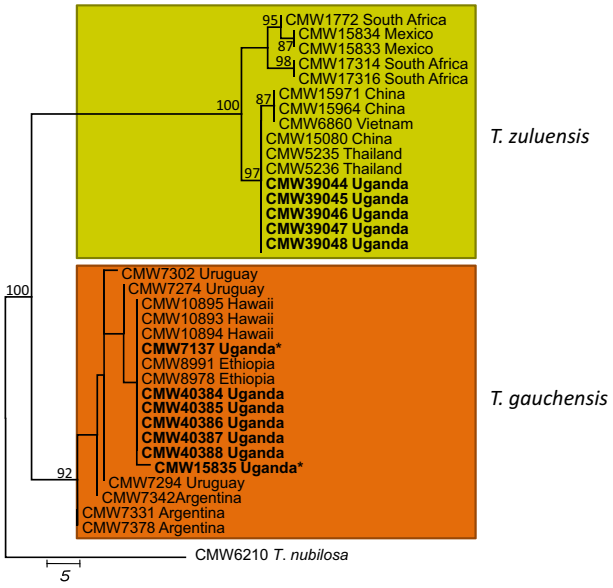


*Fig. 3.* First of 1 000 equally most parsimonious trees obtained from a heuristic search with 30 random taxon additions of combined ITS and TEF1-a sequences aligned using PAUP v4.0b10. Bootstrap support values (after 1 000 replicates) above 75% are shown at the nodes. Isolates collected from Uganda in this study are shown in bold, and those of *T. gauchensis* reported previously from Uganda are shown in bold and marked with an asterix (*). *Teratosphaeria gauchensis* was used as an out-group.

spectrophotometer (NanoDrop Technologies, Montchanin, Delaware). The oligonucleotide primer pairs ITS 1 (5′ TCC GTA GGT GAA CCT GCG G) and ITS 4 (5′ GCT GCG TTC TTC ATC GAT GC) (White et al. 1990) as well as EF1-728F (5′ CAT CGA GAA GTT CGA GAA GG) and EF1-986R (5′ TAC TTG AAG GAA CCC TTA CC) (Carbone and Kohn 1999) were used to amplify and sequence the internal transcribed spacer regions (ITS1 and ITS2) (including the complete 5.8S) and translation elongation factor 1-α (TEF1α) gene regions, respectively.

DNA sequences for isolates collected from Uganda were compared with previously published *T. zuluensis* and *T. gauchensis* sequences obtained from GenBank [National Centre for Biotechnology Information (NCBI), USA National Institute of Health Bethesda (http:/www.ncbi.nlm.nih.gov/BLAST)]. Alignments of sequence data were made online using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/software/) after which the program MEGA v.5.1 (Tamura et al. 2011) was used to check the alignments. Parsimony analyses were conducted considering the individual and combined gene sequences. Most parsimonious trees were generated using PAUP v. 4.0b10 (Swofford 2002). *Teratosphaeria nubilosa* was used as an out-group.

## 3 Results and discussion

Teratosphaeria stem canker of *Eucalyptus* trees was observed in two regions of Uganda (Figure 2). Samples were obtained from *E. grandis* trees in Jinja, eastern Uganda and Fort Portal in Western Uganda. A total of 59 isolates, typical of *Teratosphaeria* species were obtained from 53 trees in Jinja and six trees in Fort Portal. BLAST searches on the NCBI database

showed that the stem canker pathogens in Uganda were most similar to *T. gauchensis* and *T. zuluensis*. Using ITS and TEF gene sequences, isolates from Uganda were compared to those of previously identified *T. gauchensis* and *T. zuluensis* (Cortinas et al. 2006) using maximum parsimony in PAUP v. 4.0b10 (Swofford 2002). Five representatives of each species (Table 1) from the 2012 collections were used to construct the phylogenetic tree for representation in this paper (Figure 3). A total of 18 isolates from Jinja were identified as *T. zuluensis* and 35 as *T. gauchensis*. All six isolates from Fort Portal were identified as *T. gauchensis*.

Previously, in Africa, *T. zuluensis* was known to cause stem canker of *Eucalyptus* in Southern Africa (Wingfield et al. 1997; Chungu et al. 2010) while *T. gauchensis* was known to cause the same disease in Uganda and Ethiopia in East Africa (Gezahgne et al. 2003; Roux et al. 2005). This is, therefore, the first report of *T. zuluensis* causing stem canker of *Eucalyptus* in Uganda and East Africa. Although the two pathogens had previously been known to cause Teratosphaeria stem canker in geographically isolated regions, this is the first report of the coexistence of *T. zuluensis* and *T. gauchensis*, in a single plantation compartment and country.

This report raises important questions regarding the possibilities of horizontal gene and/or chromosome transfer and hybridization between the two closely related fungal pathogens. This would be consistent with the fact that a number of tree pathogens have emerged as a result of hybridization and introgression of genes between or among phylogenetically related allopatric fungal species (Brasier 2001; Woolhouse et al. 2005; Giraud et al. 2010). Furthermore, co-occurrence of *T. gauchensis* and *T. zuluensis* could lead to increased disease incidence and severity in Uganda and it will have implications on disease management in the country.

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