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

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Abstract
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. Multi-gene sequence analyses of isolates made from these cankers led to the identification of two species of *Teratosphaeria* on these trees. These were *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.

Keywords: Ascomycete, Coniothyrium stem canker, *Coniothyrium zuluense*, *Teratosphaeria gauchensis*

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 & T.A. Cout (Wingfield *et al.* 1997). Since then, the disease has been reported from several countries in Africa, the Americas and Asia (Roux *et al.* 2002; van Zyl *et al*. 2002; Gezahgne *et al*. 2003; Old *et al*. 2003; Cortinas *et al*. 2004; Gezahgne *et al*. 2004; Cortinas *et al*. 2006; Chungu *et al*. 2010; Chen *et al*. 2011).

For many years, Teratosphaeria stem canker was thought to be caused by a single species of fungal pathogen. 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 pathogen at that time was described as *Colletogloeopsis gauchensis* M.N. Cortinas, Crous & M.J. Wingf. (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 *Teratosphaeria zuluensis* has been reported from Africa, Asia and Central America (Wingfield *et al*. 1997; Van Zyl *et al*. 2002; Roux *et al*. 2002; Gezahgne *et al*. 2003; Roux *et al*. 2005). *Teratosphaeria gauchensis* is known from Africa, North and South America (Cortinas *et al*. 2006). There are no instances where the two pathogens have been encountered in any one country.

Little is known regarding the origins of *T. gauchensis* and *T. zuluensis*. Neither species has been observed in Australasia, the centre of origin of eucalypts. Due to a high level of genetic diversity amongst isolates, it was originally believed that *T. zuluensis* had originated in South Africa, possibly on native Myrtaceae (Wingfield *et al*. 1997; Van Zyl *et al*. 2002; Old *et al*. 2003). However, recent genetic studies of populations from Africa and China have suggested

that the centre of origin of the pathogen could be Asia (Cortinas *et al*. 2010; Chen *et al*. 2011). Similarly, studies of *T. gauchensis* populations from Argentina and Uruguay suggest that this species is most likely native to South America (Cortinas *et al*. 2011).

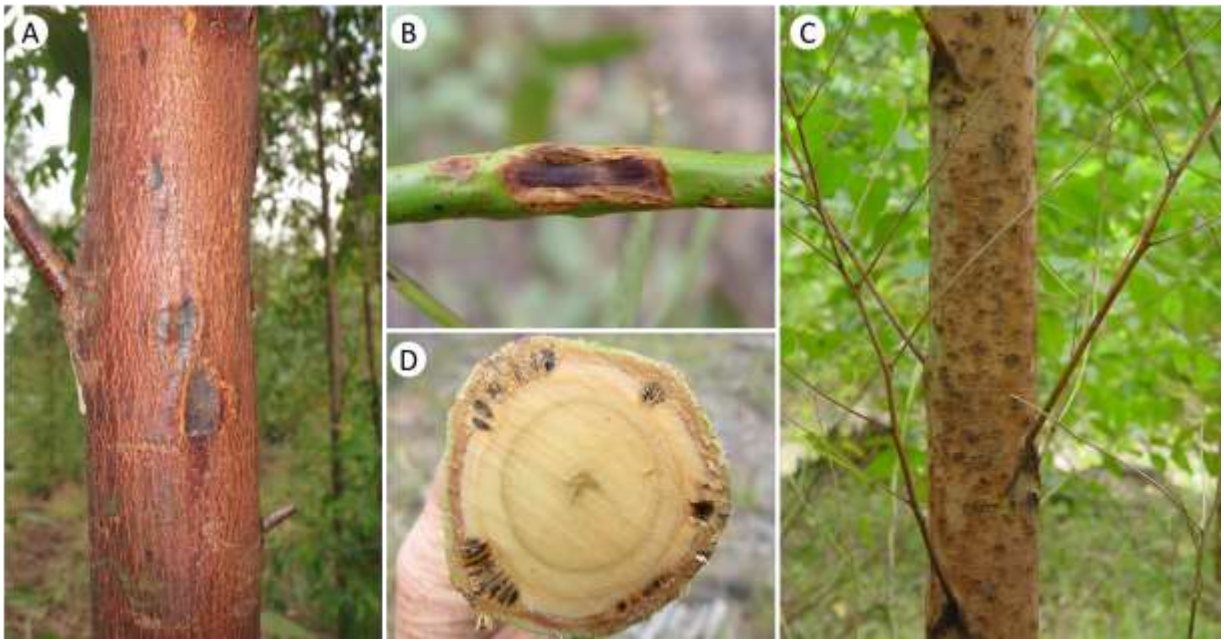

Figure 1A: Teratosphaeria lesions on a *Eucalyptus* stem; B: A lesion on a young branch; C: Stem with lesions and epicormic shoots caused by Teratosphaeria stem canker and D: Lesions and kino pockets deep in the wood.

Teratosphaeria gauchensis and *T. zuluensis* cause discrete sunken lesions (Figure 1) that can merge to form large necrotic cankers on susceptible trees (Wingfield *et al*. 1997; Van Zyl *et al*. 2002; Gezahgne *et al*. 2003; Old *et al*. 2003). In some cases, the lesions have characteristic parallel cracks that 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 exudes from resulting kino "pockets" in the wood of infected trees, resulting in blackened stems and branches. Epicormic shoots often develop on the stems of severely infected trees (Figure 1) and due to multiple branching, tree can develop brush-like, flattened crowns (Wingfield *et al*. 1997; Van Zyl *et al*. 2002; Gezahgne *et al*. 2003; Old *et al*. 2003). Although infected trees may still be used for the production of pulp, the bark of infected trees is recalcitrant and difficult to strip. In addition, infected timber is brittle and unsightly, making trees unsuitable for construction and sawn timber.

During a survey in 2012, conducted to obtain isolates of *T. gauchensis* from Uganda, bark pieces with symptoms of Teratosphaeria stem canker were sampled from *Eucalyptus grandis* trees in Jinja, eastern Uganda. The bark samples were placed in moist chambers to induce sporulation after which spore masses were lifted from the pycnida 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). After 5-7 days, cultures were purified by transferring hyphal tips to fresh MEA plates. The plates were incubated at 25^{o}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.

To identify Teratosphaeria isolates obtained from Uganda, actively growing mycelium was scraped from cultures using a sterilised 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 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), Bt2A (5' GGT AAC CAA ATC GGT GCT GCT TTC) and Bt2B (5' AAC CTC AGT GTA GTG ACC CTT GGC) (Glass & Donaldson 1995), and EF1-728F (5' CAT CGA GAA GTT CGA GAA GG) and EF1-986R (5' TAC TTG AAG GAA CCC TTA CC) (Carbone & Kohn 1999) were used to amplify and sequence the internal transcribed spacer(ITS) (including the complete 5.8S), exons 3 to 6 and the respective introns of the β tubulin 2 and translation elongation factor 1-α (TEF1α)gene regions respectively.

DNA sequences for isolates collected from Uganda were compared with *T. zuluensis* and *T. gauchensis* sequences obtained from GenBank [National Centre for Biotechnology

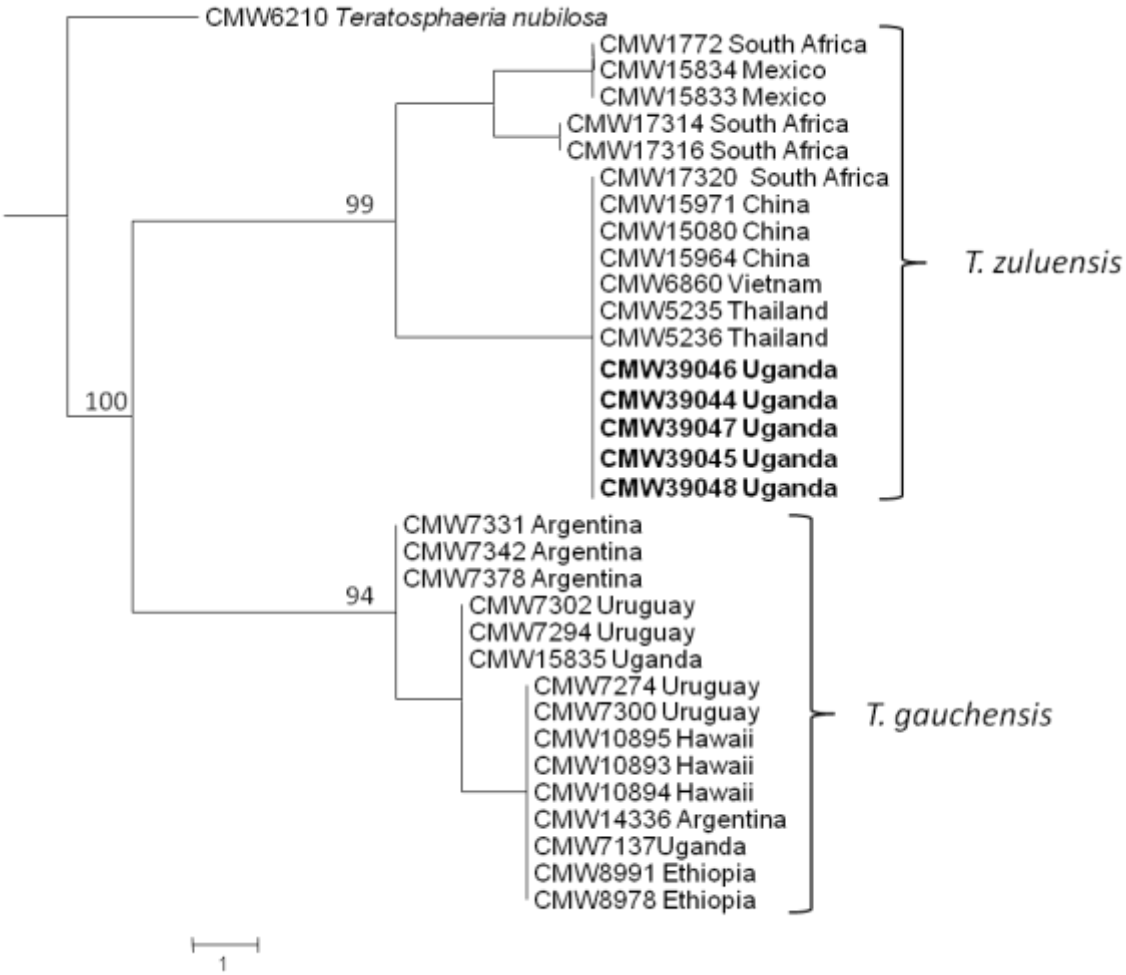

Figure 2 First of 1 000 equally most parsimonious trees obtained from a heuristic search with 34 random taxon additions of elongation factor 1-α sequence alignment using PAUP v4.0b10. Bootstrap support values 1 000 replicates are shown at the nodes. *Teratosphaeria nubilosa* was used as an out-group.

Information (NCBI), USA National Institute of Health Bethesda (http:/www.ncbi.nlm.nih.gov/BLAST)] after BLAST searches. Phylogenetic analyses of sequences obtained from stem cankers in Uganda led to the identification of both *T. zuluensis* and *T. gauchensis* (Figure 2). This is the first report of *T. zuluensis* causing stem cankers of eucalypts in Uganda.

Previous studies have shown that *T. gauchensis* causes stem cankers in Uganda (Roux *et al*. 2005). 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 country.

This report raises important questions regarding the possibility of 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 hybridisation and introgression of genes between or among phylogenetically related allopatric fungal species (Brasier 2001; Woolhouse *et al*. 2005; Desprez-Loustau *et al*. 2007).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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