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Pathogenicity of *Teratosphaeria* species on *Eucalyptus grandis* and selected hybrids 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 cultivated globally. The study assessed the pathogenicity of two *Teratosphaeria* species associated with Teratosphaeria stem canker and the susceptibility of *Eucalyptus grandis* and six commonly grown *Eucalyptus* hybrids to these fungal species in Uganda. The inoculation trials indicated that *Eucalyptus* species and hybrids were susceptible to fungal pathogens used. A comparison of *Eucalyptus* species and hybrids for each fungal species showed that GC 540 and GC 796/2 are the most susceptible hybrids to *T. zuluensis* and *T. gauchensis* respectively while hybrid clone GU 8 had a higher tolerance to both fungal species. GC 540 was also tolerant to *T. gauchensis* while F2 and GC 550 were tolerant to *T. zuluensis. Eucalyptus grandis* sourced from Australia (F2) showed a higher tolerance than *Eucalyptus grandis* from South Africa (F1). The results further revealed that both *Teratosphaeria zuluensis* and *Teratosphaeria gauchensis* are pathogenic to *Eucalyptus* species and hybrids. The generated information indicates that there is an opportunity to sustainably manage *Teratosphaeria* stem canker disease if tolerant species are grown in areas with high disease impact.

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Susceptibility; stem canker; plantation forestry; fungi

Introduction

The demand for fuel-wood and wood products has tremendously increased in East Africa as a result of increased population growth and the over exploitation of natural forests (Trust 2011). Natural forests and woodlands across the region cannot keep pace with this increasing demand and the resulting deforestation is degrading the environment (FAO 2005). In Uganda, natural forest cover for instance reduced from 24% in 1990 to 8% in 2016 (Nabanyumya 2017). According to National Forest Authority (NFA) estimates and projections, by 2030 the annual demand for sawn timber will be about 879,500 m^3 (Kaboggoza 2011). The government of Uganda therefore is advocating for investment in plantation forestry in an effort to mitigate the shortage of wood and wood products and increase forest cover (FAO 2005). *Pinus* and *Eucalyptus* species have been identified as the major plantation species mainly because of their fast growth rates, higher productivity, and adaptability to a range of site conditions (Chungu et al. 2010). Although *Eucalyptus* species are well known for growing faster than any other species, their use in plantation forestry may not provide an immediate solution to the current wood crisis (Jacovelli 2009; Rezende et al. 2014). This is because of the many challenges facing *Eucalyptus* species related to pests and diseases, increased variability and relatively low growth rate compared to current demand (Trust 2011).

Clonal hybrid resources were introduced to Uganda in 2002, to overcome the challenges facing *Eucalyptus* plantations since they are fast growing, and have better disease resistance (Jacovelli 2009). The introduced clones included 5 hybrids of *Eucalyptus grandis*× *Eucalyptus urophylla* (GUs; GU 7, GU 8, GU 21, GU 607 and GU 609), 6 hybrids of *Eucalyptus grandis*× *Eucalyptus camaldulensis* (GCs; GC 796, GC 784, GC 578, GC 540, GC 514 and GC 550) and one pure *Eucalyptus grandis* (Epila-otara 2004; Jacovelli 2009; Turinawe et al. 2014).

Teratosphaeria stem canker previously known as Coniothyrium canker (Wingfield et al. 1996) is one of the most damaging diseases of grown *Eucalyptus* species and hybrids in Uganda and globally (Jimu et al. 2014). The disease was first reported in Kwazulu-Natal, South Africa where it damaged large areas of planted *Eucalyptus* stands (Wingfield et al. 1996). There are two closely related species of *Teratosphaeria* (*Teratosphaeria zuluensis* and *Teratosphaeria gauchensis*) that are associated with Teratosphaeria stem canker. The species belong to the class Dothideomycetes, family Teratosphaeriaceae and genus *Teratosphaeria* (Jimu et al. 2016).

Disease surveys carried out in Uganda reported Teratosphaeria stem canker disease to be widespread but mainly concentrated in Fort portal where it affected young *Eucalyptus* trees of about 2 years (Roux and Slippers 2007). Subsequent reports of the disease have then followed in Uganda identifying the disease in areas where it was not previously known and the cause of the disease was confirmed to be *Teratosphaeria zuluensis* and *Teratosphaeria gauchensis* (Jimu et al. 2014). However, there have been no controlled experiments conducted to investigate the pathogenicity of species associated with the disease on *Eucalyptus* species and hybrids grown in Uganda. Thus, the objectives of this study were; (1) to determine the pathogenicity of *Teratosphaeria* species associated with Teratosphaeria stem canker disease on *Eucalyptus* species and selected hybrids and (2) to determine the susceptibility of *Eucalyptus* species and selected

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hybrids to Teratosphaeria disease. With the pathogenicity of *Teratosphaeria* species investigated, management ideas on how to contain the pathogens that cause the disease can be sought over. Understanding the susceptibility of *Eucalyptus* species and hybrids is essential as tolerant *Eucalyptus* planting material can be recommended for planting in areas of high disease pressures.

Materials and methods

Experimental design

The experiment evaluated pathogenicity of *Teratosphaeria gauchensis and T. zuluensis* (Table 1) from Jinja and Tororo that had previously been collected and characterized (Jimu et al. 2014). *E. grandis* species from South Africa (F1) and Australia (F2) and six commonly grown hybrids in Uganda including; GC 540, GC 550, GC 796, GC 796/2, GU 7 and GU 8 were also evaluated for susceptibility. These *Eucalyptus* species and hybrids were chosen for the study because they were recommended hence commonly grown in different regions of the country (Epila-otara 2004). The experiment was a completely randomized block design with factorial treatment. The tree seedlings were grown in one-liter buckets for 6 months while the fungal isolates were grown on 2% malt extract agar (MEA) at 20°C before inoculation. Each of the eight *Eucalyptus* species and hybrids was inoculated with five fungal isolates and sterile MEA that acted as the controls. The buckets were arranged in a completely randomized design (Figure S1) and watered daily.

Inoculation tests

Three seedlings of each *Eucalyptus* species and hybrids were inoculated on the stem approximately 10 cm above the base. A 4 mm diameter cork borer was used to remove the bark and expose the cambium. A 4 mm diameter mycelial plug from each actively growing fungal culture was then placed under the bark of each of the *Eucalyptus* hybrids and species using a scalpel blade with the mycelium facing the cambium. After inoculation, the wounds were wrapped and sealed off immediately with Parafilm (Bemis company, Inc United States) to prevent desiccation and contamination (Chen et al. 2011). As control, three seedlings of each *Eucalyptus* species and hybrids were inoculated with sterile MEA plug. The inoculated plants were observed and monitored for a period of 2 months with daily watering. Assessment was done by identifying whether the wounds were callused over 2 months after inoculation. The bark of the inoculated plants was removed to expose lesions by cutting off the parafilm and exposing the cambium to allow measurements (lesion length and width) to be taken (Figure 1).

Stem lesion length and width data were analyzed by One-way ANOVA to compare the virulence of isolates to *Eucalyptus* species and hybrids. Thereafter, isolates were grouped into species and data were re-analyzed to compare their virulence to *Eucalyptus* species and hybrids. Another analysis of variance (Two-way analysis) to determine susceptibility of *Eucalyptus* species and hybrids to *Teratosphaeria* species was also performed. Mean values were compared by LSD test at 5% significance level. Microsoft office excel was used to generate graphs for the comparison of all means. The inoculated fungi were re-isolated by cutting small pieces of wood from the edges of lesions and plating them on 2% MEA at 25°C to confirm that the inoculated fungi are responsible for development of symptoms seen on inoculated trees.

Table 1. Isolates used in inoculation tests.

Isolate number	Origin	Species	Author
CBS11726	Tororo, Uganda	*T. gauchensis*	Aylward et al. 2019
CMW39045	Jinja, Uganda	*T. zuluensis*	Jimu et al. 2014
CMW39046	Jinja, Uganda	*T. zuluensis*	Jimu et al. 2014
CMW40386	Jinja, Uganda	*T. gauchensis*	Jimu et al. 2014
CMW40387	Jinja, Uganda	*T. gauchensis*	Jimu et al. 2014

Results

Pathogenicity of *Teratosphaeria* isolates

Two months after inoculation, the trees had developed lesions that were measured, recorded, and used in the analysis. Summary statistics of mean lesion length and width caused by each *Teratosphaeria* species revealed that *T. zuluensis* produced longer lesions compared to *T. gauchensis* (Table S1). Lesion width and length of isolates were compared and plotted on the same axis (Figure 2). Isolate CMW39045 (*T. zuluensis*) showed the highest mean width, while isolate CBS11726 (*T. gauchensis*), the lowest. The widths caused were significantly different among the inocula including the control by ANOVA test ($F = 7.825$; $df = 5$; $P = 0.000$). The LSD test however showed that the width caused by the isolates didn't differ from each other but differed from the control, meaning all isolates were pathogenic. The fungal isolate which caused the longest mean length was CMW40387 (*T. gauchensis*) followed by CMW39045 (*T. zuluensis*) (Figure 2). Lesion lengths also significantly differed among the inocula including the control by ANOVA test ($F = 22.540$; $df = 5$; $P = 0.000$). The lesion length caused by the fungal species used in the study was significantly larger than the control ($F = 17.84$; $df = 2$; $P = 0.000$), further confirming the pathogenicity of fungal species to *Eucalyptus* species and hybrids used in this study (Figure S2).

Susceptibility of Eucalyptus *species and hybrids to* Teratosphaeria *species*

Eucalyptus hybrid clone GC 540 exhibited the longest lesions after *T. zuluensis* inoculation, followed in order by GC 796/2, GU 7, F1, GC 796, GU 8, F2 and GC 550 (Figure 3). Meanwhile, GC796/2 exhibited the longest lesions after inoculation with *T. gauchensis*, followed in order by GC 796, F1, F2, GU 7, GU 8, GC 550 and GC 540. There was a significant difference in susceptibility of *Eucalyptus* species and hybrids to both *T. zuluensis* ($F = 2.93$; $df = 7$; $P = 0.010$) and *T. gauchensis* ($F = 2.59$; $df = 7$; $P = 0.018$). A Tukey pairwise comparison of *Eucalyptus* species and hybrids for each fungal species further showed that GC 540 and GC 796/2 are the most susceptible hybrids to *T. zuluensis* and *T. gauchensis* respectively (Table S2). Meanwhile, GU 8 and GC 540 were tolerant to *T. gauchensis* while GU 8, F2, and GC 550 were tolerant to *T. zuluensis*. A two-way Analysis of Variance showed an interaction between *Eucalyptus* species and hybrids and *Teratosphaeria* species causing diseases was also significant ($F = 1.96$; $df = 14$; $P = 0.024$) implying that the lesion length varied depending on the combination of both fungal species and *Eucalyptus* species and hybrids (Table 2).

Figure 1. Developed lesion on *Eucalyptus* Eight weeks after inoculation with *Teratosphaeria* fungi.

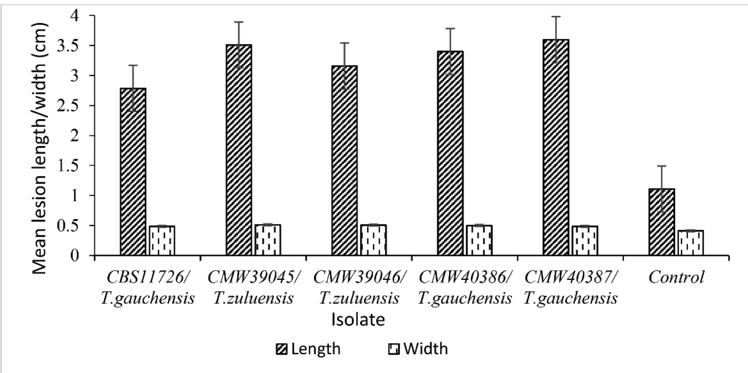

Figure 2. Comparison of mean width and mean length of lesions formed following inoculation with five Teratosphaeria isolates. Error bar is SE.

Discussion

The results obtained in the study indicate that all *Eucalyptus* trees were susceptible to the inoculated fungal isolates. *Teratosphaeria* species are virulent pathogens of *Eucalyptus* capable of causing tremendous damage to the wood and death of the trees. Variation in susceptibility in the planting stock is the only effective strategy that can be exploited in Teratosphaeria canker disease management. The study was the first of its kind to evaluate pathogenicity of *Teratosphaeria* species on *Eucalyptus* and its hybrids grown in Uganda.

T. zuluensis was the most virulent on the four *Eucalyptus* species/hybrids used in the study, supporting previous studies conducted in South Africa (Wingfield et al. 1996). *T. zuluensis* is a virulent fungal pathogen that was first reported in South Africa on a single clone of *E. grandis* (Wingfield et al. 1996). Since then, the pathogen has been reported to cause disease on *Eucalyptus* in various parts of the world both tropical and

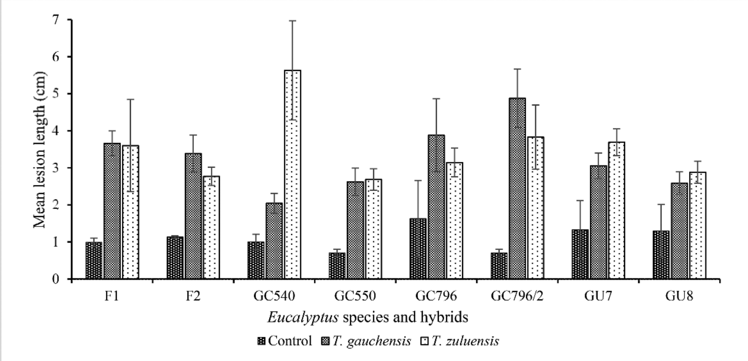

Figure 3. A comparison of susceptibility of *Eucalyptus* species and hybrids to *Teratosphaeria* fungal species used in the study. Error bar is SE.

Table 2. Differences caused by *Teratosphaeria* species on *Eucalyptus* species and hybrids using two-way ANOVA.

Source	Type III sum of squares	df	Mean square	F	Sig.
Model	1919.206	24	79.967	30.609	0.000
Eucalyptus species and hybrids	17.818	7	2.545	0.974	0.452
Fungal species	107.409	2	53.705	20.557	0.000*
Eucalyptus species and hybrids *Fungal species	71.685	14	5.120	1.960	0.024*
Error	433.674	166	2.612		
Total	2352.880	190			

Values followed by * are significant ($p < 0.05$).

temperate such as; Thailand on *E. camaldulensis* (Van Zyl et al. 2002), Mexico on *E. grandis* (Roux et al. 2002), Vietnam and Argentina on *E. globulus* and *E. grandis* respectively (Gezahgne et al. 2003), Hawaii on *E. grandis* (Cortinas et al. 2004), Ethiopia on *E. camaldulensis* (Gezahgne et al. 2005), China on *E. urophylla* (Cortinas et al. 2006a) and Uganda on *Eucalyptus grandis* (Jimu et al. 2014).

Teratosphaeria gauchensis was first described from Uruguay and Argentina (Cortinas et al. 2006b). The pathogen has been reported to cause disease in different parts of the world for example Portugal on *E. globulus* (Silva et al. 2015), Kenya on *E. grandis* and a GC hybrid clone (Machua et al. 2016), Uganda on *E. grandis* (Jimu et al. 2014) and Southern Africa (Jimu et al. 2015). *Teratosphaeria* species have not been reported causing disease to any host genus other than *Eucalyptus* (Cortinas et al. 2011; Aylward et al. 2019).

Generally, all *Eucalyptus* species and hybrids developed larger lesions at the point of fungal inoculations compared to the controls, indicating that they are susceptible to *Teratosphaeria* species. The level of susceptibility however differed among the *Eucalyptus* species and hybrids. From multiple comparison test, the most susceptible hybrid clones were GC 540 and GC 796/2 while F1 and F2 had moderate susceptibilities. The findings do not support the notion that most *Eucalyptus* hybrids are more tolerant to disease compared to *Eucalyptus* species (Jacovelli 2009), which was one of the justifications for their introduction to East Africa.

The variation in susceptibility of *Eucalyptus* species/hybrids has been reported previously towards other diseases such as Powderly Mildew (Bovolini et al. 2018), *Botryosphaeria* canker (Nakabonge et al. 2019) and *Chryphonetria cubensis* (Heerden et al. 2005). The difference in susceptibility shown in the study may be attributed to pathogen–genotype interaction and provides a promising strategy towards management of the disease (Wingfield et al. 1996; Roux et al. 1999).

The findings from this study should be exploited as the first step to select material for planting in areas with high disease pressure, since this is known to be the most effective strategy in the management of canker diseases (Nakabonge et al. 2019). Additionally, field evaluations should be conducted in different agro-ecological zones to confirm the findings of the current study.

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Disclosure statement

The authors report no conflict of interest.

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