Susceptibility of *Eucalyptus* hybrid clones to Botryosphaeria canker in Uganda

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**ABSTRACT**

The study assessed the susceptibility of the nine commonly grown *Eucalyptus* clones to *Neofusicoccum* species associated with Botryosphaeria canker in Uganda. The inoculation trials indicated that susceptibility of *Eucalyptus* hybrids differed significantly (*p = .000*), clones GU609, GU7, GC578, and GC796 exhibiting a higher tolerance than GC784, GC550, GU8, GC514, and GC540. The results further revealed that *N. parvum* was more pathogenic than *N. kwambonambiense*. The generated information can be exploited in sustainable forest management by expanding the growing of tolerant hybrids in areas with high Botryosphaeria canker disease pressure.

**KEYWORDS**

Botryosphaeriaceae; *Neofusicoccum*; pathogenicity

**Introduction**

Botryosphaeria canker is associated with fungi in the family *Botryosphaeriaceae* which include species that are most commonly known to cause die-back and canker diseases on twigs, branches and trunks of trees including *Eucalyptus* species and hybrids (Slippers et al., 2007). *Neofusicoccum* species (formerly known as *Botryosphaeria* species) belong to the *Botryosphaeriaceae* and have been reported worldwide in countries such as Uganda, Australia, Chile, China, Ethiopia, Indonesia, South Africa, Uruguay, Venezuela to mention but few (Iturritxa, Slippers, Mesanza, & Wingfield, 2011; Mohali, Slippers, & Wingfield, 2009; Slippers et al., 2007). *Neofusicoccum* species are saprophytic or endophytic organisms which infect a wide range of monocotyledonous, dicotyledonous and gymnosperm hosts (Burgess, Barber, & Hardy, 2005; Burgess & Wingfield, 2002; Slippers & Wingfield, 2007). Symptom development in the hosts normally occurs when exposed to unsuitable environmental conditions such as drought, freezing, extreme temperatures, defoliation, hail, and wounds caused by insects or other pathogens (Roux et al., 2005).

Clonal propagation is known to be of great value in plantation forestry worldwide as it facilitates the development of uniformly fast-growing genotypes with homogeneous physical properties and disease resistance (Dehon, Rezende, Resende, & Assis, 2014; Wingfield et al., 2013). *Eucalyptus* clonal hybrids were introduced to E. Africa and Uganda in 1997, from Mondi Forests Ltd, in South Africa, suitable for a range of wood products and for planting by smallholder farmers (Epila-Otara & Ndhokero, 2010; Kilimo Trust, 2011). The clones were generated by cross-breeding *Eucalyptus* species and produced three main hybrid clone groups. GC clones (grandis-camaldulensis) that are a cross between
Eucalyptus grandis and Eucalyptus camaldulensis; GU clones (grandis-uroplylla) that are a cross between Eucalyptus grandis and Eucalyptus uroplylla; and GT clones (grandis-tereticornis) that are a cross between Eucalyptus grandis and Eucalyptus tereticornis. The most popular clones grown in Uganda are GC and GUs that are grown throughout the country (Epila-Otara and Ndhokero 2010, Kilimo Trust, 2011).

It’s anticipated that the successful use of Eucalyptus hybrid clones in plantation forestry is capable of reducing timber and wood shortage worldwide (Dehon et al., 2014; Wingfield et al., 2013). However, diseases such as Botryosphaeria canker, may threaten their successful establishment. The genetic uniformity of clonal hybrids, exposes them more to disease if susceptible ones are grown under favorable environmental conditions as compared to Eucalyptus species (Guimarães et al., 2010). Previous nationwide surveys in Uganda reported Botryosphaeria canker being the most widely distributed disease (Roux and Slippers 2007; Nyeko & Nakabonge, 2008). Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips) and Neofusicoccum kwambonambiense Pavlic, Slippers, M.J. Wingfield, have similarly been reported as the most predominant and pathogenic Botryosphaeriaceae on Eucalyptus species in many parts of the world (Pillay, Slippers, Wingfield, & Gryzenhout, 2013) and previous studies reported their presence in Uganda (Nakabonge, 2002; Toljander, Nyeko, Stenström, Ihrmark, & Barklund, 2007). In spite of this, no studies have been conducted to determine the susceptibility of the grown Eucalyptus hybrid clones to the disease. In this study, we assessed the susceptibility of nine Eucalyptus hybrid clones to Botryosphaeria canker caused by Neofusicoccum species. Understanding the susceptibility of Eucalyptus hybrids to Botryosphaeria canker is essential in an effort to identify resistant hybrid clones, since the alternative mechanisms such as the use of fungicides have not recorded success in management of the disease (Slippers et al., 2007).

Materials and methods

Isolation of fungi

Samples were collected from symptomatic Eucalyptus hybrid clones GU and GCs from Serere in Serere district Eastern Uganda, Mukungwe, Masaka District central Uganda, Ntungamo sub-country Ntungamo District in western Uganda and Kifu, Mukono District Central Uganda (Figure 1). The areas were selected based on previous reports of occurrence of Botryosphaeria canker (Nyeko & Nakabonge, 2008). For isolations, perithecia or pycnidia were picked from the Eucalyptus twigs and plated directly onto sterile, 2% malt extract agar (MEA), Fisher Scientific UK Limited. The MEA cultures were incubated for 10 days at 36°C until mycelia with fruiting structures grew. Single conidia from isolates resembling Botryosphaeriaceae (e.g., brownish white and fluffy mycelia) were sub-cultured until pure cultures were obtained.

Molecular phylogenetic characterization

For each single conidial isolate culture, actively growing mycelium were scraped off the surface a MEA plate and used for DNA extraction using the method described by Roux, Coutinho, Byabashaija, and Wingfield (2001). DNA concentrations were estimated
visually on a 1% agarose gel using known concentrations of lambda (λ) DNA after dying with ethidium bromide and photographed under UV illumination.

Amplification of the ITS region was performed with the primers ITS1F (Gardes & Bruns, 1993) and ITS4 (Farris, Kallersjo, Kluge, & Bult, 1995). The PCR reaction mixture (25 μL), PCR conditions and visualization of products were as described by Slippers et al. (2004). Five μL of the PCR reaction mixture was loaded onto a 2% agarose gel, also containing 1% ethidium bromide. This was exposed to UV light to visualize the PCR products. Sanger sequencing was performed using both forward and reverse primers at INQABA Biotech, Pretoria, South Africa.

Homology searches were done from the GenBank/EMBL databases using the BLAST program (National Center for Biotechnology Information, U. S. National Institute of Health, Bethesda. http://www.ncbi.nlm.nih.gov/BLAST). Eight sequences with homologies >80%, known to be of *Botryosphaeria* spp. (Table 1), were selected and co-aligned with those from the Ugandan sequences obtained in this study (Table 1) using the program ClustalW (Thompson, Higgins, & Gibson, 1994) in MEGA Version X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018).

Phylogenetic analysis was performed in MEGA Version X software (Kumar et al., 2018). The evolutionary history was inferred using the UPGMA (Unweighted Pair Group Method with Arithmetic mean) method (Sneath & Sokal, 1973). The analysis involved 14 nucleotide sequences. The evolutionary distances were computed using the Maximum Composite
Likelihood method (Tamura, Nei, & Kumar, 2004). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 bootstraps) was generated (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The fungus Guignardia mangiferae A.J. Roy, known to be related to Botryosphaeria spp. as a Botryosphaeriaceae was used as the out-group to root the trees (Slippers et al., 2013) (Table 1). The identified species where used for pathogenicity tests.

**Pathogenicity trial**

A total of eight (GU 7, GU 8, GU 609, GC 540, GC 796, GC 784, GC 578, and GC 550) 7-months old clones were used in the pathogenicity tests. The clonal cuttings were grown in pots under screen house conditions at Makerere University, Uganda with day/night temperature of approximately 20-25°C. Each clone consisted of 35 trees and these included 7 replicates (trees) for each of the 4 isolates. In the same clone were included seven replicates to represent the control. This made the total number of trees per clone 35 (5x7) and the number of trees in the experiment 315 (35x9).

Using a 5 mm cork borer, agar discs were made from 2 week old MEA plate cultures of each isolate and from sterile MEA plates for the controls. The same size wounds were made in the bark of the ~ 2 m tall clones to expose the cambium at a height of about ~40 cm from the soil level. Trees were immediately inoculated by placing an agar disk, with the mycelium side facing the cambium into each wound and the inoculation sites were rapidly sealed with parafilm (Pechiney, Chicago, USA) to prevent desiccation and contamination. Controls were inoculated in the same way using non-colonized agar plugs. Six weeks following inoculation the lengths and widths of the resulting lesions were taken. The fungus was re-isolated from the lesions by cutting small pieces of wood from the leading edges of lesion margins that were surface-sterilized in 70% ethanol and plated directly onto MEA. The whole experiment was repeated to improve the precision and reliability of the results.

Data from the two experiments were merged. One way ANOVA was used to determine the variation in the effects of isolates on individual clones. Isolates were grouped into

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**Table 1. Fungal isolates included in this study.**

| Species identity | Alternative isolate no. | Origin       | Host               | Collector          | ITS              |
|------------------|-------------------------|--------------|--------------------|--------------------|------------------|
| N. parvum        | GN1                     | Uganda       | Eucalyptus sp.     | G. Nakabonge       |                 |
| N. parvum        | GN2                     | *            | *                  | *                  |                 |
| N. parvum        | GN3                     | *            | *                  | *                  |                 |
| Neofusicoccum species | GN4                 | *            | Eucalyptus sp.     | *                  |                 |
| N. kwambonambiense | GN5                | *            | *                  | *                  |                 |
| N. kwambonambiense | CMW14023           | S. Africa    | Syzygium cordatum  | D. Pavlic          | EU821900        |
|                  | CMW14024               | S. Africa    | *                  | *                  |                 |
| N. parvum        | CMW9079                | New Zealand  | Actinidia delicosa | S.R. Pennicook     | AY236940        |
| N. parvum        | CMW8081                | New Zealand  | Populus nigra L    | G.J. Samuels       | AY236943        |
| N. rhibs         | USA                     | Ribes sp     | B. Slippers/G.Hudler | AY236935        |
| N. rhibs         | USA                     | Ribes sp     | *                  | *                  | AY236936        |
| Neofusicoccum batangarum | CMW28315 | Cameroon     | Terminalia catappa | D. Begoude/J.Roux | FJ900606        |
|                  | CMW28363               | Cameroon     | T. catappa         | D. Begoude/J.Roux  | FJ900607        |
| L. pseudotheobromae | CMW26716          | S. Africa    | T. catappa         | D. Begoude/J. Roux | FJ900598        |
| L. pseudotheobromae | CMW26721          | S. Africa    | T. catappa         | D. Begoude/J. Roux | FJ900599        |
species and data were re-analyzed using one way ANOVA to determine the differences in the effects of Botryosphaeria species on individual clones. An overall analysis of data was done to determine the overall pathogenicity of isolates using two-way ANOVA.

Results

Isolation and molecular characterization

A total of 58 Botrosphariaceae samples were collected and isolated from symptomatic Eucalyptus trees. From the 58 isolates, 5 were selected based on cultural characteristics, and used for DNA sequencing. Alignment of the 14 total isolates including those from the genebank were successfully conducted. Phylogenetic analysis of the aligned 14 sequences generated an optimal tree with the sum of branch length = 0.19079930. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are indicated next to the branches (Figure 2). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. All ambiguous positions were removed for each sequence pair. There were a total of 624 positions in the final dataset.

Phylogenetic analysis revealed three groups representing three species. Where, isolates GN1, GN2 and GN3 represented N. parvum, and GN5 represented N. kwambonambiense (Figure 2) and GN4 represented Neofusicoccum species an unresolved isolate that will require further efforts to identify to species level.

Pathogenicity of Botryosphaeria isolates

Nine weeks after inoculation, all the inoculated trees had developed lesions. Clear brown discolorations stretching from the point of inoculation were observed and all controls were overgrown with callus tissue (Figures 3 and 4).

Figure 2. The most parsimonious phylogenetic tree obtained from a heuristic search of the ITS rDNA data of Neofusicoccum isolates from Uganda (GN1, GN2, GN3, GN4, GN5) compared to other known isolates.
Trees inoculated with GN2 had the longest lesions followed by GN3, GN1, GN4, and GN5, respectively. The widths varied slightly although appeared to be significantly different (F = 18.73; df = 5; P = .000). Lesion lengths differed significantly among isolates (F = 45.76; df = 5; P = .000). There was also a significant isolate x clone interaction (F = 2.35; df = 40; P = .000). *Neofusicoccum parvum* (GN1, GN2, and GN3) had the highest lesion dimensions, followed by *Neofusicoccum* species (GN4) and lastly *N. kwambonambiense* (GN5). The mean widths varied slightly among the isolates.

**Susceptibility of eucalyptus clones to *Neofusicoccum sp***

*Eucalyptus* clones GC 784, GC 550, GU 8, GC 514, and GC 540 exhibited higher lesion lengths than clones GU 609, GU 7, GC 578 and GC 796. However, the width of lesions indicated GU8 and GU7 as the highest while the rest of the clones varied slightly. Lesion dimensions differed significantly (F = 4.79; df = 8; P = 0.000) and (F = 5.83; df = 8; P = .05) for length and width, respectively. Length of lesions indicated no significant difference in clone by species interaction (P = .000). On the contrary, the lesion width showed a significant (P = .000) interaction between clones and species.
Discussion

We evaluated the relative susceptibility of *Eucalyptus* hybrid clones that are currently grown as commercial plantation forest trees in Uganda to *Neofusicoccum* species and assessed the pathogenicity of the fungal species to the hybrid clones. There was varying susceptibility within the *Eucalyptus* hybrid clones and pathogenicity between the two *Neofusicoccum* species. The results indicate that *Eucalyptus* hybrid clones could be used in the sustainable management of Botryosphaeria canker in areas where the disease is prevalent.

DNA sequences of the ITS regions of five (5) *Botryosphaeriaceae* isolates yielded *Neofusicoccum parvum* and *Neofusicoccum kwambonambiense* species. *Neofusicoccum parvum* was first described from Kiwifruit and *Populus* spp. in New Zealand as *B. parva* (Pennycook, & Samuels, 1985). Other studies, identified *Neofusicoccum parvum* causing disease on *Eucalyptus* and other woody species in Uganda, South Africa and Venezuela (Heath et al. 2011, Nakabonge, 2002; Mohali, Slippers, & Wingfield, 2007; Mohali et al., 2009; Pavlic, Slippers, Coutinho, & Wingfield, 2009). *N. kwambonambiense* is a closely related species to *N. parvum* and was isolated from *Eucalyptus* and from symptomless branches and leaves, dying branches and pulp of ripe fruits of *Syzygium cordatum* in Kwambonambi South Africa thus its name (Pavlic, Slippers, Coutinho, Gryzenhout, & Wingfield, 2007; Pavlic et al., 2009). The isolation of *N. parvum* and *N. kwambonambiense* from samples collected during the current study confirms earlier reports of the occurrence of the pathogens in Uganda and their association to clonal *Eucalyptus* hybrids (Nakabonge, 2002).

Trees inoculated with sterile media were able to recover from wounds by developing callus tissue unlike fungal-inoculated trees which had growing lesions, confirming that *Neofusicoccum* species which were used are pathogenic to all the inoculated *Eucalyptus* clones. The endophytic nature of *Neofusicoccum* species enables them to live within healthy plant tissues (Smith, Wingfield, & Petrini, 1996) and this can make their introduction into new environments on germplasm easily possible (Slippers et al., 2004). Although *Eucalyptus* hybrid clones grown in Uganda have their origin in South Africa, the disease had already been reported in the country on *E. grandis* as far back as 2001 (Nakabonge, 2002), before the introduction of hybrid clones and their nationwide planting.

A significant difference in the levels of pathogenicity among *Neofusicoccum* species were recorded. *N. parvum* was the most aggressive to all *Eucalyptus* hybrid clones used in the study (Figure 3). *N. parvum* is known as an aggressive canker pathogen, possessing a high potential of causing large lesions, cracks in the bark and black kino exudation on *Eucalyptus* hybrid clones (Mohali et al., 2009). The fungus has a wide host range and global distribution and it has been recorded as the most pathogenic on woody plants of all *Botryosphaeriales* (Jami, Wingfield, Gryzenhout, & Slippers, 2017; Li et al., 2014; Slippers & Wingfield, 2007). *Neofusicoccum kwambonambiense* was the less pathogenic, in this study but significant differences were observed from the control (P = .000) (Figure 3). In a study conducted by Pavlic et al. (2009), however, *N. kwambonambiense* was reported to be more pathogenic, than *N. parvum*, to *Syzygium cordatum* in South Africa. Earlier studies though had reported *N. parvum* and *N. kwambonambiense* being more pathogenic to *Eucalyptus* than *S. cordatum* (Pavlic et al., 2007). The presence of these species in Uganda and their confirmed ability to cause disease on *Eucalyptus* clones grown in the
region is an indication that monitoring campaigns should be established to avert any losses to the commercial forestry sector. Exploring the varying resistance to diseases in the *Eucalyptus* planting stock, could contribute to the sustainable management of plantation forests.

Generally, lesions which developed on inoculated trees were relatively small. This may give an impression that all clones tested in this study have a certain degree of tolerance to *Neofusicoccum* infection. This tolerance however differed significantly among the clones, with GU609, GU7, GC578, and GC796 exhibiting a higher tolerance than GC784, GC550 GU8, GC514 and GC540 (Figure 4). The variation in susceptibility of *Eucalyptus* hybrid clones corroborates previous studies by van Heerden, Amerson, Preisig, Wingfield, and Wingfield (2005) and Guimarães et al. (2010) toward *Chryphonetria cubensis*.

In conclusion, the varying responses reported in this study indicate that there is an opportunity to sustainably manage the disease by growing clones that are tolerant to Botryosphaeria canker in places where the disease is most prevalent. Since, *Neofusicoccum* species are known to be stress-related and opportunistic pathogens of *Eucalyptus* spp. (Smith et al., 1996), there is a need to avoid the situation that may trigger development of disease in rather tolerant clones. The study could also be repeated under field conditions and in various agro-ecological zones to identify genotypes suitable for various conditions. Meanwhile, the more tolerant clones (i.e., GU609, GU7, GC578, and GC796) can be planted in the most affected areas.

**Limitations**

The study has generated information which is the first of its kind toward understanding the resistance of the grown *Eucalyptus* clones to the most prevalent disease in Uganda. However, it could have been interesting to evaluate the pathogenicity of Botryosphaeriaceae from other hosts on *Eucalyptus* clones grown in Uganda. Further studies should address this important aspect that will contribute to ecological sustainability in plantation forestry disease management.

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**Disclosure of interest**

The authors report no conflict of interest.

**References**

Burgess, T., & Wingfield, M. J. (2002). Quarantine is important in restricting the spread of exotic pathogens in the Southern Hemisphere. *International Forestry Review*, 4, 56–65.

Burgess, T. I., Barber, P. A., & Hardy, G. E. (2005). *Botryosphaeria* sp. associated with *Eucalyptus* in Western Australia, including the description of *Fusicoccum macroclavatum* sp.nov. *Australasian Plant Pathology*, 34, 557–567. doi:10.1071/AP05073
Dehon, S. P., Rezende, G., Resende, M. D. V., & Assis, T. (2014). Eucalyptus Breeding for Clonal Forestry. Challenges and Opportunities for the World’s Forests in the 21st Century, 81, 393–424. doi:10.1007/978-94-007-7076-8_16

Epila-Otara, J. S., & Ndlokero, J. (2010). Selection and site matching of Eucalyptus clones in Uganda. Journal of East African Natural Resources Management, 3(1), 237–248.

Farris, J., Kallersjo, M., Kluge, A., & Bult, C. (1995). Testing significance of incongruence. Cladistics, 10, 315–319. doi:10.1111/j.1096-0031.1994.tb00181.x

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39, 783–791. doi:10.1111/j.1558-5646.1985.tb00438.x

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes—Application to the identification of morchellae and rusts. Molecular Ecology, 2, 113–118. doi:10.1111/j.1365-294X.1993.tb00005.x

Guimarães, L. M. S., Resende, M. D. V., Lau, D., Rosse, L. N., Alves, A. A., & Alfenas, A. C. (2010). Genetic control of Eucalyptus urophylla and E. grandis resistance to canker caused by Chrysoporthe cubensis. Genetics and Molecular Biology, 33, 525–531. doi:10.1590/S1415-475720100005000054

Heath, R.N., & Roux, R., Slippers, B., Drenth, A., Pennycook, S.R., Wingfield, B.D. & Wingfield M.J. (2011). Occurrence and pathogenicity of Neofusicoccum parvum and N. mangiferae on ornamental Tibouchina species. Forest Pathology, 41, 48–51.

Iturritixa, E., Slippers, B., Mesanza, N., & Wingfield, M. J. (2011). First report of Neofusicoccum parvum causing canker and die-back of Eucalyptus in Spain. Australasian Plant Disease Notes, 6, 57–59. doi:10.1007/s13314-011-0019-5

Jami, F., Wingfield, M. J., Gryzenhout, M., & Slippers, B. (2017). Diversity of tree-infecting Botryosphaeriales on native and non-native trees in South Africa and Namibia. Australasian Plant Pathology, 46(6), 529–545. doi:10.1007/s13313-017-0516-x

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution, 35, 1547–1549. doi:10.1093/molbev/msy096

Li, Q. L., Deng, T. J., Huang, S. P., Guo, T. X., Mo, J. Y., & Hsiang, T. (2014). First report of gummosis of Mango trees caused by Neofusicoccum parvum in Sichuan, Southwest China. Journal of Plant Pathology, 96, 113–131.

Mohali, S. R., Slippers, B., & Wingfield, M. J. (2007). Identification of Botryosphaeriaceae from Eucalyptus, Acacia and Pinus in Venezuela. Fungal Diversity, 25, 103–125.

Mohali, S. R., Slippers, B., & Wingfield, M. J. (2009). Pathogenicity of seven species of the Botryosphaeria on Eucalyptus clones in Venezuela. Australia Plant Pathology, 38(2), 135–140. doi:10.1071/AP08085

Nakabonge, G. (2002). Diseases associated with plantation forestry in Uganda. (MSc Thesis) Retrieved from https://repository.up.ac.za/bitstream/handle/2263/25938/Complete.pdf?sequence=4

Nyeko, P., & Nakabonge, G. (2008). Occurrence of pests and diseases in tree nurseries and plantations in Uganda. A study commissioned by the sawlog production grant scheme (SPGS). Retrieved from www.sawlog.ug/downloads/Pests%2520

Pavlic, D., Slippers, B., Coutinho, T. A., Gryzenhout, M., & Wingfield, M. J. (2007). Botryosphaeriaceae occurring on native Syzygium cordatum in South Africa and their potential threat to Eucalyptus. Plant Pathology, 56, 624–636. doi:10.1111/j.1365-3059.2007.01608.x

Pavlic, D., Slippers, B., Coutinho, T. A., & Wingfield, M. J. (2009). Molecular and phenotypic characterisation of three phylogenetic species discovered within the Neofusicoccum parvum/N. ribis complex. Mycologia, 101(5), 103–193. doi:10.3852/08-193

Pennycook, S. R., & Samuels, G. J. (1985). Botryosphaeria and fusccicocum species associated with ripe fruit rot of actinidia deliciosa (kiwifruit) in new zealand. Mycotaxonom, 24, 445–458.

Pillay, K., Slippers, B., Wingfield, M. J., & Gryzenhout, M. (2013). Diversity and distribution of co-infecting Botryosphaeriaceae from Eucalyptus grandis and Syzygium cordatum in South Africa. South African Journal of Botany, 84, 38–43. doi:10.1016/j.sajb.2012.09.003
Roux, J., Coutinho, T. A., Byabashaija, D. M., & Wingfield, M. J. (2001). Diseases of plantation Eucalyptus in Uganda. South African Journal of Science, 97, 16–18.

Roux, J., Meke, G., Kanyi, B., Mwangi, L., Mbaga, A., Hunter, G. C., … Wingfield, M. J. (2005). Diseases of plantation forestry trees in eastern and Southern Africa. South African Journal of Science, 101, 409–413.

Roux, J., & Slipper, B. (2007). Entomology and pathology survey with particular reference to leptocybe invasa. Saw Log Production Grant Scheme, 23–26 (July).

Slippers, B., Boissin, E., Phillips, A. J. L., Groenewald, J. Z., Lombard, L., Wingfield, M. J., … Crous, P. W. (2013). Phylogenetic lineages in the Botryosphaeriales: A systematic and evolutionary framework. Studies in Mycology, 76(1), 31–49. doi:10.3114/sim0020

Slippers, B., Crous, P. W., Denman, S., Coutinho, T. A., Wingfield, B. D., & Wingfield, M. J. (2004). Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as Botryosphaeria dothidea. Mycologia, 96(1), 83–101.

Slippers, B., Smith, W. A., Crous, P. W., Coutinho, T. A., Wingfield, B. D., & Wingfield, M. J. (2007). Taxonomy, phylogeny and identification of Botryosphaeriaceae associated with pome and stone fruit trees in South Africa and other regions of the world. Plant Pathology, 56, 128–139. doi:10.1111/ppa.2007.56.issue-1

Slippers, B., & Wingfield, M. J. (2007). Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. Fungal Biology Reviews, 21, 90–106. doi:10.1016/j.fbr.2007.06.002

Smith, H., Wingfield, M. J., & Petrini, O. (1996). Botryosphaeria dothidea endophytic in Eucalyptus grandis and Eucalyptus nitens in South Africa. Forest Ecology and Management, 89, 189–195. doi:10.1016/S0378-1127(96)03847-9

Sneath, P.H. and Sokal, R.R. (1973) Numerical Taxonomy: The Principles and Practice of Numerical Classification. 1st Edition, W. H. Freeman, San Francisco, USA.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA), 101, 11030–11035.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22(22), 4673–4680. doi:10.1093/nar/22.22.4673

Toljander, Y. K., Nyeko, P., Stenström, E., Ihrmark, K., & Barklund, P. (2007). First Report of Canker and Dieback Disease of Grevillea robusta in East Africa Caused by Botryosphaeria spp. Plant Disease, 91(6), 773. doi:10.1094/PDIS-91-6-0773B

Trust, K. (2011). Eucalyptus Hybrid Clones in East Africa; Meeting the Demand for Wood through Clonal Forestry Technology. Occasional Paper No.1

van Heerden, S. W., Amerson, H. V., Preisig, O., Wingfield, B. D., & Wingfield, M. J. (2005). Relative pathogenicity of Cryphonectria cubensis on Eucalyptus clones differing in the tolerance to C. cubensis. Plant Diseases, 89(6), 659–662. doi:10.1094/PD-89-0659

Wingfield, M. J., Roux, J., Slippers, B., Hurley, B. P., Garnas, J., Myburg, A. A., & Wingfield, B. D. (2013). Established and new technologies reduce increasing pest and pathogen threats to Eucalypt plantations. Forest Ecology and Management, 301, 35–42. doi:10.1016/j.foreco.2012.09.002