Chrysosporthe zambiensis detected on native *Syzygium* in Zimbabwe

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Received: 8 May 2017 / Accepted: 11 December 2017 / Published online: 19 December 2017
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Abstract

Fruiting bodies similar to those of *Chrysosporthe* were observed on *Syzygium guineense* in Bindura, Zimbabwe. The objective of this study was to identify the fungus and to test its pathogenicity on *Eucalyptus*. BLAST searches and phylogenetic analyses of the ITS, BT1 and BT2 gene regions identified the fungus as *Chrysosporthe zambiensis*. This study represents the first report of this fungus in Zimbabwe and on native *Syzygium*. The presence and high pathogenicity of *C. zambiensis* presents a potential threat on *Eucalyptus* plantations in Zimbabwe.

Keywords Cryphonectriaceae · *Eucalyptus* · Host range expansion · Pathogenicity · Stem pathogen · Tree disease

Myrtaceae are widespread in Africa, where some species are native while others were introduced as ornamentals, fruits and plantations. The most dominant and widespread native Myrtaceae are *Syzygium* species for example *S. cordatum* and *S. guineense* (e.g. Maroyi 2008). Introduced species are dominated by eucalypts that have been domesticated in most countries in Africa. As such, eucalypt plantations are established in areas that were previously occupied by native forests, sharing boundaries with native Myrtaceae. Where there is poor management, stumps left when land is cleared sometimes coppice, leading to a mixture of native Myrtaceae and eucalypt species in a plantation.

Establishment of eucalypt plantations in areas that were previously occupied or adjacent to native Myrtaceae increases the risk of enemies such as pathogens and pests extending their hosts that may end up detrimental to either species. Host range expansions by such enemies are highly likely because native and introduced hosts share a similar internal environment that makes either of them susceptible to enemies of their relatives (De Vienne et al. 2009). Some of the most serious pathogens of eucalypts worldwide are thought to have emerged from related native hosts in areas where they were domesticated. Highly cited examples of such pathogens are

Puccinia psidii and *Chrysosporthe* species, the causal pathogens of Myrtle rust and stem cankers, respectively (e.g. Slippers et al. 2005; Graça et al. 2013). A good example is *C. austroafricana*, a fungus that is thought to be native on *Syzygium* species in southern Africa where it is thought to have extended its host range to cause stem cankers on eucalypts (Slippers et al. 2005). During a disease survey in Zimbabwe's *Eucalyptus* plantations and native *Syzygium*, a fungus resembling those in the genus *Chrysosporthe* was observed on *S. guineense*. The aim of this study was to identify this fungus and to assess its potential threat to *Eucalyptus* plantations in Zimbabwe.

Disease surveys on native Myrtaceae were conducted from November to December 2016 in Bindura, Zimbabwe. Fruiting bodies similar to those of *Chrysosporthe* were observed on dying stems and branches of *S. guineense*. Diseased trees were observed along rivers and in wetlands. Surveys conducted on *S. cordatum* did not show any disease symptoms. Diseased bark and branch material were sampled, placed in sampling bags and taken to the laboratory for isolation. Diseased plant materials were placed in moist chambers to induce sporulation after which the isolation was done by lifting spore drops from fruiting structures with a sterile needle and transferring these to MEA (20 g/l malt extract, 15 g/l agar). Single spore cultures were incubated at 25 °C for six days to obtain sufficient mycelium for DNA extraction. Isolates are maintained in the culture collection (PPRI) of the National Collection of Fungi, South Africa. Mycelia were scrapped from the cultures and macerated under liquid nitrogen. DNA was extracted using the modified CTAB protocol (Doyle and Doyle 1987). About 200 mg mycelia were ground using liquid nitrogen in

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Fig. 1 Maximum likelihood phylogenetic trees of ITS (**a**), β -tubulin 1 (BT1) and β -tubulin 2 (BT2) sequence data showing identity of Zimbabwean *Chrysophorthe* isolates. Numbers below branches indicate bootstrap support values

pestle and mortar. The extraction buffer (100 mM Tris-Cl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 M NaCl, 3% CTAB) was used to break up the cells. DNA concentrations were quantified using a BioDrop μ Lite Micro-Volume UV/Vis Spectrophotometer.

Amplification of the ITS-rDNA was performed using primers ITS1 and ITS 4 (White et al. 1990). Primers β t1a and β t1b (Glass and Donaldson 1995) were used for amplification of the β -tubulin 1 (BT1) gene region. The β -tubulin gene (BT2) region of the isolates was amplified using primers β t2a and β t2b (Glass and Donaldson 1995). Amplification was conducted in 25 μ l reaction mixtures containing 4 ng of template DNA; 250 mM each dATP, dTTP, dGTP, and dCTP (Fermentas); 10 $\times$ PCR reaction buffer, consisting of 160 mM

(NH₄)₂SO₄, 670 mM Tris-HCl at pH 8.8, and 100 mM KCl (GeneDireX); 0.25 U of *Taq* DNA polymerase (GeneDireX) and 0.2 mM each primer (Inqaba Biotechnical Industries, South Africa). Amplification of the ITS gene region was carried out in a thermal cycler (9700 GeneAmp) with the following conditions: an initial step at 95 $^{\circ}$ C for 3 min; followed by 35 cycles at 95 $^{\circ}$ C for 30 s, 55 $^{\circ}$ C for 30 s, and 72 $^{\circ}$ C for 45 s; and a final extension of 72 $^{\circ}$ C for 5 min. The PCR reactions for the BT gene regions were as follows: 3 min at 96 $^{\circ}$ C followed by cycles of 30 s at 95 $^{\circ}$ C, 45 s at 57 $^{\circ}$ C, 45 s at 72 $^{\circ}$ C, repeated 40 times.

A 5 μ l aliquot of each polymerase chain reaction (PCR) product was separated by electrophoresis on 1.5% (wt/vol) agarose (Lonza) stained with Ethidium bromide solution

(0.1 mg/l) and visualized using a UV transilluminator (UVitec U.K.). When the bands of the appropriate size were observed, the remaining PCR product was purified using Sephadex spin column (Sigma Aldrich Co.) (5 g of Sephadex G-50 powder dissolved in 75 ml of sterile water) and the resulting amplicons were submitted to Inqaba Biotechnical Industries (South Africa) for sequencing. The DNA sequences obtained were edited and consensus sequences created from both the forward and reverse sequences using BioEdit v 7.1.3 (Hall 1999). Sequences generated in the present study have been deposited in GenBank (ITS: MF115972-MF115974, BT1: MG593146-MG593148 and BT2: MG593149-MG593151). Maximum likelihood analyses were conducted using the programme MEGA v.7.1 (Tamura et al. 2016).

For pathogenicity tests, three isolates of the fungus isolated from *Syzygium* were cultured in MEA for seven days. For each isolate, ten six-month old *E. camaldulensis* trees grown at Bindura University of Science Education farm were used for the pathogenicity tests. An additional ten trees were inoculated with a sterile MEA plug to serve as control. An 8 mm diameter cork-borer was used to punch the bark to expose the cambium. To avoid desiccation, wounds were sealed with parafilm 'M' (American National Can™ Chicago, USA).

Lesion lengths and depths were measured six weeks after inoculation. The fungus was re-isolated from the lesions and was identified using the fruiting body and culture morphologies. Data on lesion lengths and depths were tested for normality and homogeneity of variance using the Kolmogorov-Sminov test and were analysed using multivariate ANOVA (MANOVA) to reduce problems with Type I error because we examined the three dependent variables simultaneously. Tukey's post hoc test was used for mean comparisons of lesion lengths and depths at 95% confidence level.

A total of 28 fungal isolates were obtained from 16 trees. The cultures were grouped into three morphotypes. Three isolates, representing each morphotype were sequenced. BLAST searches on the NCBI GenBank using ITS, BT1 and BT2 sequences identified the fungus as *Chrysosporthe zambiensis*. The identity of the fungus was verified by phylogenetic reconstructions that grouped sequences from this study with those of *C. zambiensis* deposited in GenBank (Fig. 1a–c).

Eucalyptus stems inoculated with *C. zambiensis* developed some lesions compared to the control (Fig. 2). All isolates caused significantly longer outer-lesions than the control treatment ($P < 0.001$) (Fig. 3). PPRI24125 had significantly longer outer-lesions compared to PPRI24126 ($P = 0.001$) and

Fig. 2 Outer (a) and inner (b) bark of the control inoculation on *E. camaldulensis* stem, outer (c) and inner (d) lesions of a stem inoculated with *C. zambiensis* (PPRI24125). Fruiting structures of *C. zambiensis* on inoculated *E. camaldulensis* stem (c)

Fig. 3 Comparison of the outer and inner bark lesion lengths on *E. camaldulensis* inoculated with *C. zambiensis* isolates and the control. Error bars represent standard deviation at 95% confidence level

PPR124127 ($P = 0.001$). Outer lesions due to PPR124126 were moderately longer than those caused by PPR124125 ($P = 0.097$). For the inner lesions, the control treatment had shorter lesions compared to PPR124125 ($P = 0.003$), PPR124126 ($P < 0.001$) and PPR124125 ($P < 0.001$). The inner lesions were significantly longer than the outer lesions for PPR124125 and PPR124126 isolates. In terms of lesion depth, the control treatment had significantly shallow ($P < 0.001$) lesions compared to all isolates (Fig. 4). PPR124125 and PPR124126 had significantly deeper ($P = 0.016$ and $P = 0.002$ respectively) lesions compared to PPR124127 (Fig. 4).

This study reports for the first time, the presence of *C. zambiensis* on *Syzygium* in Zimbabwe. This result was not

Fig. 4 Comparison of lesion depths on *E. camaldulensis* inoculated with *C. zambiensis* isolates and the control. Error bars represent standard deviation at 95% confidence level

surprising given that the pathogen was first isolated and described from *E. grandis* in neighbouring Zambia (Chungu et al. 2010). However, this study represents the first report of the pathogen on native *Syzygium*. The origin of *C. zambiensis* is not known. However, the fact that it was first described from Zambia and that it is being reported in this study from native Myrtaceae, raises the possibility that it might be native to Africa, similar to *C. austroafricana* (Slippers et al. 2005; Wingfield et al. 2008). Furthermore, other *Chrysosporthe* species such as *C. cubensis* are thought to be native on Melastomataceae in South America and South-east Asia (Hodges et al. 1986; Rodas et al. 2005).

Chrysosporthe zambiensis was found on native *Syzygium* and not on *E. camaldulensis* that is commonly grown in the Bindura area. This could be due to the fact that *E. camaldulensis* might be relatively more resistant to *Chrysosporthe* species than other species for example *E. grandis* (van Heerden et al. 2005). However, pathogenicity tests showed that *C. zambiensis* is pathogenic on *Eucalyptus*, similar to findings from Zambia (Chungu et al. 2010). This presents a potential threat on *Eucalyptus* plantations that are established in areas previously occupied by or adjacent to *Syzygium* species in Zimbabwe. Regular disease surveys should be conducted to enable early detection should the pathogen expand its host range onto *Eucalyptus*. This will enable corrective measures to be implemented. For example, it has been shown that *Chrysosporthe* can be controlled by selection for resistance and clonal propagation techniques of the resistant individuals (e.g. Wingfield et al. 2012).

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