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Overlap of latent pathogens in the *Botryosphaeriaceae* on a native and agricultural host

James W. M. MEHL[a], *Bernard SLIPPERS*[b,*], *Jolanda ROUX*[c], *Michael J. WINGFIELD*[a]

[a]*Department of Microbiology and Plant Pathology, DST-NRF Centre of Excellence in Tree Health Biotechnology (CTHB), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa*

[b]*Department of Genetics, DST-NRF Centre of Excellence in Tree Health Biotechnology (CTHB), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa*

[c]*Department of Plant Science, DST-NRF Centre of Excellence in Tree Health Biotechnology (CTHB), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa*

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ABSTRACT

Some species of the *Botryosphaeriaceae* are capable of infecting a broad range of host plants. We studied the species diversity of *Botryosphaeriaceae* associated with marula (*Sclerocarya birrea* subsp. *caffra*, *Anacardiaceae*) trees in South Africa over two seasons, as well as species common to both *S. birrea* and adjacent mango (*Mangifera indica*, *Anacardiaceae*) trees in a subset of sites. Gene flow amongst populations of *Botryosphaeriaceae* shared on these tree species was tested using microsatellite markers. Twelve species were identified from *S. birrea* and eleven species were found on *M. indica* trees. From isolations done in 2006, the dominant species on *S. birrea* was *Neofusicoccum vitifusiforme*, while *N. parvum* was the dominant species isolated from *M. indica*. *Neofusicoccum parvum* was dominant in isolations from both hosts in 2012. Isolates of *Botryosphaeria fabicerciana*, *Lasiodiplodia mahajangana*, *L. pseudotheobromae*, *L. theobromae*, *N. mediterraneum*, and *N. umdonicola* were also collected from both hosts. Population genetic analyses on isolates of *N. parvum* suggested that three populations were present, each comprising isolates from both hosts. There was significant gene flow between *N. parvum* populations on these hosts. This ability to infect multiple hosts and to migrate amongst them facilitates the establishment and spread of species and genotypes of the *Botryosphaeriaceae*, such as *N. parvum*, in new areas.

Introduction

Fungi in the *Botryosphaeriaceae* are well known as endophytic and opportunistic pathogens of woody plants. These fungi infect plants via wounds or through natural plant openings such as buds, lenticels, and stomata (Slippers & Wingfield 2007). Many species in the family have a wide range of plant hosts, including commercial fruit crops (van Niekerk *et al.*

* *Corresponding author*. Department of Genetics, DST-NRF Centre of Excellence in Tree Health Biotechnology (CTHB), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa. Tel.: +27 12 420 3938; fax: +27 12 420 3960.
E-mail address: bernard.slippers@fabi.up.ac.za (B. Slippers).

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2004; Slippers et al. 2005; Chen et al. 2014), forest trees (Burgess et al. 2006b; Slippers et al. 2009), and plants in native woody ecosystems (Pavlic et al. 2007; Mehl et al. 2011; Jami et al. 2014). These fungi occur in healthy plant tissues as latent pathogens and persist endophytically until stress occurs, after which disease symptoms can manifest (Slippers & Wingfield 2007).

The spores (sexual and asexual) of *Botryosphaeriaceae* are principally dispersed by wind or rain splash (Swart et al. 1987; Mehl et al. 2013). Since many of the *Botryosphaeriaceae* have broad host ranges (Slippers & Wingfield 2007; Jami et al. 2014), these fungi can spread to and infect both related and unrelated plants. There are many examples of inter-host exchanges of the *Botryosphaeriaceae*, and these include those amongst and between native and non-native trees. For example, species of the *Botryosphaeriaceae* have been shown to move between trees in native stands of *Eucalyptus* (*Myrtaceae*) and adjacent plantations of these trees (Burgess et al. 2006b), between native waterberry trees (*Syzygium cordatum*; *Myrtaceae*) and related eucalypt plantations (*Myrtaceae*) (Pavlic et al. 2007), from *Pinus resinosa* windbreaks to pine nurseries (Stanosz et al. 2007), among various tree hosts in the *Casuarinaceae*, *Cupressaceae*, *Fabaceae*, *Myrtaceae*, *Proteaceae*, *Santalaceae* (Sakalidis et al. 2011), and among native *Terminalia* spp. (*Combretaceae*) and between these trees and *Theobroma cacao* (*Malvaceae*) (Begoude et al. 2012), amongst others.

The ability of fungi such as the *Botryosphaeriaceae* to infect multiple hosts, increases the threat that they pose as potential economic and ecological important pathogens of native and cultivated trees globally. In South Africa, two related tree species, the native *Sclerocarya birrea* subsp. *caffra* known locally as marula, and non-native mango (*Mangifera indica*), in the *Anacardiaceae* commonly occur in close proximity to each other.

Mangifera indica is native to India and is an important subtropical crop cultivated in various countries, including South Africa (Snyman 1998). Species of the *Botryosphaeriaceae* are associated with two important diseases on *M. indica* globally. These include stem-end rot on fruit which occurs when these fungi gain entrance via the peduncle (Johnson & Kotzé 1994) causing disease when fruits ripen or are harvested (Menge & Ploetz 2003). The *Botryosphaeriaceae* can also infect *M. indica* via wounds that occur during fruit abscission, pruning or hand-picking, or via lenticels on the fruit surface (Menge & Ploetz 2003). Another important disease known as blossom blight occurs when *Botryosphaeriaceae* infect the *M. indica* inflorescences (Ploetz 2003).

Sclerocarya birrea is an iconic native African tree with a broad geographic range that extends from Senegal through Ethiopia to South Africa and into Angola and Namibia (Peters 1988). It is extensively used by local communities and is prominent in the production of well-known liqueur (Shackleton et al. 2002). Little is known regarding the diseases of *S. birrea* but a few fungi (7 species) have been recorded, and none of these include the *Botryosphaeriaceae*. This is likely due to a very limited number of studies that have considered the fungi associated with this tree species (Doidge 1950; Crous et al. 2000; Farr & Rossman 2016).

The aims of this study were to determine which species of the *Botryosphaeriaceae* infect *S. birrea* trees in South Africa. Since *S. birrea* and *M. indica* trees are taxonomically related and grow in close proximity to each other, *M. indica* trees were also sampled. This was principally to determine whether species of the *Botryosphaeriaceae* might be common to both trees. A subsequent aim was to seek evidence of gene flow in specific species of the *Botryosphaeriaceae* that occur on both *S. birrea* and *M. indica*.

Materials and methods

Sample collections and isolations

Two sample collections were made in 2006 and 2012. In 2006, branches from *Sclerocarya birrea* trees were sampled at three locations: Skukuza/Pretoriuskop area in the Kruger National Park (Mpumalanga Province), Hans Merensky estate close to Hoedspruit (Limpopo Province), and Lakelands, Mfolozi Village in the KwaZulu-Natal Province. One hundred and forty four branches from 130 *Mangifera indica* trees were also sampled at the Hans Merensky estate from two orchards; one an orchard where trees were chemically treated and a second where trees were organically grown. For the former, 15 branches were sampled from the central tree and then four trees in a 10 m diagonal to this tree were sampled (one branch each). This was followed by sampling one branch from 15 trees in the vicinity of each of the four trees, making up 79 branches from 65 trees. In the organic orchard, the same strategy was used except that a single branch was sampled from the central tree, resulting in 65 branches sampled. In 2012, three to five branches per tree were collected from neighbouring *S. birrea* and nearby *M. indica* trees alongside the road between Hoedspruit and Klaserie (Limpopo Province). Two sites along this road, less than 10 km apart, were sampled and these included six *M. indica* trees and three *S. birrea* trees at the first site, and 13 *M. indica* trees and 14 *S. birrea* trees at the second site.

Isolations were made from discoloured pith tissue, leaf samples, edges of visible lesions, and from asymptomatic twigs following the method described by Pavlic et al. (2004). Isolations were made one and four weeks after sampling for the 2006 samples and two, four, six, and eight weeks after sampling for the 2012 samples. Resulting cultures were purified and isolates resembling the *Botryosphaeriaceae* retained for further study.

Isolates from the 2006 collections were transferred to 2 % water agar (Biolab, South Africa) overlaid with sterile pine needles and incubated under near ultraviolet light (Smith et al. 1996) at 25 °C. Fruiting structures were sectioned and spores examined microscopically to group isolates into genera. Isolates collected in 2012 were purified using single hyphal tip transfers (Mehl et al. 2011). Cultures used in this study have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, Pretoria, South Africa.

DNA extractions

DNA was extracted from all isolates collected in both 2006 and 2012 for identification using DNA sequence data comparisons. For the 2006 isolates, DNA was extracted using the method of