

Future danger posed by fungi in the Ophiostomatales when encountering new hosts

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Abstract

The Ophiostomatales contain pathogens that threaten forests world-wide. Global trade increases encounters with new hosts, with potential devastating consequences. We assessed the danger posed by the movement of Ophiostomatales between different host trees in South Africa. We tested the pathogenicity of five fungal species from native South African trees, and three from exotic trees, on various native and exotic trees. To evaluate the potential of fungi to move to new hosts, we investigated the strength of their associations with arthropod vectors. Results indicate that many fungal species are pathogens of newly encountered and distantly-related hosts. Encounters of pathogens with new hosts are less likely when host plants are distantly related, and outside the host range of boring beetle vectors, which also

reduces the chances of vectoring by phoretic mite associates. However, pathogens associated with numerous mite species and wounds are more likely to encounter new hosts and pose future threats.

Key words: Afromontane forest, *Graphilbum*, *Ophiostoma*, *Raffaelea*, Scolytinae

Introduction

Ophiostomatoid fungi (Ophiostomatales) are best known as associates of beetles (Coleoptera: Curculionidae, Scolytinae) (Harrington, 1987; Jacobs and Wingfield, 2001; Jacobs et al. 2003). In many cases these associations are mutualistic, whereby the fungi are believed to provide nutritional benefits to the beetles (Klepzig et al. 2001) and the beetles transport fungi to new host plants (Paine et al. 1997; Six, 2003). However, some members are not associated with Scolytinae beetles and have been isolated from diverse habitats including wounds on trees, soil, other fungi and wood of dead trees (de Meyer et al. 2008; Kamgan et al. 2008; Cruywagen et al. 2010; Musvuugwa, 2014) and may be associated with many other arthropods such as Cerambycidae beetles, mites and Nitidulide beetles (Bridges and Moser, 1983; Moser, 1985; Jacobs and Wingfield, 2001; Klepzig et al. 2001; Lombardero et al. 2003; Roets et al. 2007). In fact, all members of the Ophiostomatales are specifically adapted for arthropod mediated spore dispersal as they produce sticky spores at the tips of elongated structures that are not easily distributed via other mechanisms (Malloch and Blackwell, 1993). The dispersal biology of the non-Scolytinae associated taxa is far less well-studied, but it is becoming clear that mites may be central to the dispersal of many of these. For example, mites are known to have associations with Ophiostomatales in environments such as *Protea* flower heads (Roets et al. 2007) and wounds on trees (Musvuugwa, 2014).

Mites also play a very important role in the dispersal of some Ophiostomatales associated with Scolytinae beetles (Hofstetter et al. 2013). For example, *Tarsonemus ips* and *T. krantzii* are associated with the southern pine beetle, *Dendroctonus frontalis* (Klepzig et al. 2001; Price et al. 1992; Smiley and Moser, 1974) and both are responsible for vectoring spores of *Ophiostoma minus* (Klepzig et al. 2001). As in the case of some beetles that have specialised spore carrying structures (mycangia), mites may have mutualistic relationships with the fungi they carry and may also carry fungi in special structures (sporothecae) (Bridges and Moser, 1983; Moser et al. 1995). These relationships can be just as specialised as with the beetles, as some mites feed and reproduce exclusively on their fungal associates (Klepzig and Six, 2004; Roets et al. 2007).

Many Ophiostomatales are well-known pathogens of both native and exotic trees world-wide (Klepzig et al. 2001; Six and Wingfield, 2011). They have caused major financial losses in many forestry and agricultural sectors. For example, *Raffaelea quercivora* and its associated beetle, *Platypus quercivorus*, were responsible for the catastrophic mass mortality and die-back of Japanese oak trees (Kubono and Ito, 2002). Numerous taxa can also cause substantial financial losses as they contribute to blue-staining of conifer timber (Harrington, 2005). The blue-stain fungi *Ophiostoma minus* and *O. pluriannulatum* are, for example, known to reduce the commercial value of timber (Harrington, 2005).

In many cases, accidental introductions of fungal species and their vectors into new environments can cause disease outbreaks with serious consequences (Brasier, 2008; Loo, 2009). These accidental introductions happen due to increased globalisation of trade in plants, and inadequate international plant biosecurity protocols (Brasier and Webber, 2010). In some cases the introduced fungi can move from one host species to another, often aided by their

vectors (Wingfield, 2003; Woolhouse et al. 2005). The limited resistance offered by the new hosts may lead to excessive aggressiveness by the introduced pathogen (Brasier and Buck, 2001). Well-known examples include the Dutch elm disease epidemic that was responsible for the catastrophic losses of American elm trees, *Ulmus americana* (Brasier and Buck, 2001; Heybroek, 1993; Lamb, 1979). This was due to the introduction of *O. ulmi* and *O. novo-ulmi*, along with their vector the European elm bark beetle (*Scolytus multistriatus*) (Webber, 1990), from their European host, through the importation of infested elm timber to America (Brasier, 1990; Peace, 1960). *Raffaelea lauricola* along with its invasive exotic vector beetle, the red bay ambrosia beetle *Xyleborus glabratus*, were introduced into southeastern USA from Asia on solid wood packing material (Harrington et al. 2008). The fungus moved onto native members of the Lauraceae, including avocado trees, causing Laurel wilt disease that often kill these new hosts (Harrington et al. 2008). Even when introduced fungi do not necessarily kill trees, these may degrade tree defence systems and contribute to successful bark beetle attack which indirectly increases tree mortality (DiGuistini et al. 2011; Hammerbacker et al. 2013).

Like other ecosystems around the world, the Cape Floristic Region (CFR) of South Africa is under increased threat from anthropogenic influences. Numerous economically important exotic plant species and their associated organisms have been introduced and are now invasive within natural CFR habitats. This is especially true for some of the most important South African plantation forestry taxa such as *Acacia mearnsii*, *Eucalyptus* spp. and *Pinus* spp. (Moran and Hoffman, 2012). These taxa invaded vast areas of natural CFR vegetation and are ideal organisms to study the possibility and consequences of fungi moving to newly encountered hosts, as they are in close contact with many native tree taxa. The aim of this study was firstly to identify possible consequences when members of the Ophiostomatales move to newly encountered hosts by testing their pathogenicity on various usual hosts and

possible newly encountered hosts. The likelihood that fungi would move to newly encountered hosts was assessed by identifying known host range of the fungi, and also by testing the specificity of various mites towards their natural beetle vectors and those that they would not normally associate with.

Materials and methods

Selection of study organisms

We focussed on three important plantation forestry tree species (*Acacia mearnsii*, *Eucalyptus grandis* and *Pinus radiata*) that are invasive in natural CFR vegetation (Moran and Hoffman, 2012). These taxa often grow in dense stands and are often in close proximity to native trees found in the Afromontane forest patches scattered throughout the CFR. Recent surveys of members of the Ophiostomatales from the CFR revealed that there are many undescribed or newly described taxa present on native trees within Afromontane forests (Musvuugwa, 2014, Musvuugwa et al. 2015). Some of these appeared to be host specific, while others had very wide host ranges. These surveys also revealed the presence of members of the Ophiostomatales on exotic species. Numerous taxa were associated with sub-cortical beetles, their phoretic mites and/or mites from wounds on storm damaged trees (Musvuugwa, 2014). For the present study we selected fungal species that likely differ in their dispersal ecology in order to assess the possible consequences and likelihood of moving to new hosts (Table 1).

Our study was prompted by recent problems caused by a native ophiostomatoid fungus (Microascales: *Ceratocystis albifundus*) on exotic *A. mearnsii* plantation trees (De Beer, 1994; Roux and Wingfield, 1997) and then our own recent collection of an exotic phoretic mite (*Dendrolaelaps quadrisetus*) on both a non-native sub-cortical beetle (*Orthotomicus erosus*) from *P. radiata* and a native species (*Lanurgus* sp. 1) from a native host, *Olea*

Table 1. Study organisms and their associated host tree species (from Musvuugwa, 2014; Musvuugwa et al. 2015) in the CFR that were used in this study. CMW = culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa.

Fungal species and isolates	Host trees	Beetle associates	Mite associates
<i>Ceratocystiopsis</i> sp. 1 (CMW40346, CMW40347, CMW40348)	<i>Pinus radiata</i>	<i>Orthotomicus erosus</i>	<i>Dendrolaelaps quadrisetus</i>
<i>Graphilbum roseus</i> (CMW40349, CMW40350, CMW40351)	<i>Curtisia dentata</i> , <i>Halleria lucida</i> , <i>Olea capensis ssp. Macrocarpa</i> , <i>Pterocelastrus tricuspidatus</i> , <i>Trichocladus crinitus</i>	None known	<i>Lasioseius</i> sp. 1 Mesostigmata sp. 1 Oribatida sp. 2
<i>Ophiostoma ips</i> (CMW40322, CMW40337, CMW40338)	<i>Pinus radiata</i>	<i>Orthotomicus erosus</i>	<i>Dendrolaelaps quadrisetus</i>
<i>Ophiostoma pluriannulatum</i> - like (CMW40333, CMW40334, CMW40335)	<i>Acacia mearnsii</i> , <i>Curtisia dentata</i> , <i>Olea capensis</i> , <i>Pterocelastrus sp.</i> , <i>Rapanea melanophloeos</i>	None known	Acaridae sp. 1 Mesostigmata sp.1 <i>Uroobovella</i> sp. 1
<i>Raffaelea rapanae</i> (CMW40357, CMW40358, CMW40359)	<i>Rapanea melanophloeos</i>	Platypodinae sp. 1	None known
<i>Sporothrix pallida</i> (CMW40330, CMW40331, CMW40332)	<i>Olea capensis ssp. macrocarpa</i>	<i>Ctonoxylon</i> sp. 1 <i>Lanurgus</i> sp. 1,	None known
<i>Sporothrix rapanae</i> (CMW40367, CMW40368, CMW40369)	<i>Rapanea melanophloeos</i>	None known	None known
<i>Sporothrix</i> sp. 1 (CMW40378, CMW40379, CMW40380)	<i>Pinus radiata</i>	<i>Orthotomicus erosus</i>	<i>Dendrolaelaps quadrisetus</i>

capensis ssp. macrocarpa (Musvuugwa, 2014). *Dendrolaelaps quadrisetus* was found to be associated with three exotic Ophiostomatales species (*Ophiostoma ips*, *Sporothrix* sp. 1 and *Ceratocystiopsis* sp. 1) on *P. radiata* (all associated with exclusively pine-associated bark-

beetles) and *Sporothrix pallida* that was isolated from *Olea capensis* spp. *macrocarpa* (Musvuugwa, 2014). *Sporothrix pallida* was also isolated from the beetle *Lanurgus* sp. 1, the native phoretic partner of *D. quadrisetus* (Musvuugwa, 2014). This led us to suspect that the fungi associated with this mite can move between host plants and that the mite is not very specific towards its vectors. *Sporothrix pallida* was also found to be associated with the native subcortical beetle *Ctonoxylon* sp. 1 (Musvuugwa, 2014) and is known from many other environments including water sediments, soil, the sporophore of a slime-mould (De Meyer et al. 2008) and from a corneal ulcer of a human patient (Morrison et al. 2013). This fungus is, therefore, not very host and/or vector specific and may easily move from one environment to the next.

Ophiostoma pluriannulatum and *Graphilbum* proposed sp. nov. *roseus* (*G. roseus* from hereon, Musvuugwa, 2014) are associated with numerous mite taxa (none known to be phoretic) and with wounds on diverse native trees (Musvuugwa, 2014). These species are expected to easily move between various hosts using these mites as primary vectors. These fungi have, however, not yet been isolated from wounds on exotic trees, which raises the question of whether they are able to grow on these hosts. *Sporothrix* proposed sp. nov. *rapanae* (*S. rapanae* from hereon, Musvuugwa, 2014) is currently only known from wounds on one host tree species (*Rapanea melanophloes*). Its associated organisms are unknown, but most likely include various mites. Like with *O. pluriannulatum* and *G. roseus*, it may therefore be able to move easily between different hosts. *Raffaelea rapanae* is an ambrosial fungus associated with a Platypodinae beetle (Musvuugwa et al. 2015). The nature of this association is probably very specific (Henriques et al. 2006) and the dispersal of this species to tree taxa other than *R. melanophloeos* is therefore less likely.

Growth studies

The growth of various members of the Ophiostomatales from the CFR was tested by inoculating them onto various hosts including those that they are not usually associated with (Table 1). Three native tree species (*Curtisia dentata*, *Olea capensis* ssp. *macrocarpa* and *Rapanea melanophloeos* and the three exotic tree species (*A. mearnsii*, *E. grandis*, *P. radiata*) were used in these experiments. Our aim was to determine how host specific these fungi are (i.e. can they grow on possible newly encountered hosts) and if so, whether they could be pathogenic to these newly encountered hosts. Three isolates of each of the eight fungal species assessed were used for inoculation experiments. A cork borer (8 mm diameter) was used to make wounds on branches (*ca.* 15 mm diameter) of trees by opening the cambium. Mycelial plugs of the same size were taken from edges of actively growing fungal colonies (1-2 weeks old) on Malt Extract Agar (MEA; Biolab, Midrand, South Africa) and inserted onto the wounds with mycelium facing the cambium. The experiment was replicated five times for each of the three isolates per fungal species tested on each of the six tree species evaluated. Only a single inoculation was done per individual tree, whereafter wounds were sealed with masking tape to prevent desiccation. As a control, wounds were inoculated with sterile MEA plugs. After 6 weeks all inoculated branches were removed and lengths of lesions resulting from the inoculations were measured. Re-isolations were made from the front of the lesions in order to confirm the identity of the lesion-causing fungi. Re-isolations were made by surface sterilising wounds with 70% ethanol and removing pieces of inner wood (2 mm²). These were placed on MEA in Petri dishes and incubated in the dark at room temperature (*ca.* 23°C). Pure cultures of the fungi were identified based on morphological characters.

Differences in mean lesion length caused by the various fungal isolates and that of the control on each of the host trees were determined using One-way ANOVA in Statistica 11 (Statsoft Corporation, USA). The data were tested for normality using a Shapiro-Wilk test. Data were normally distributed and no transformation was required before analyses. Means were separated by a Dunnett Post-hoc test in Statistica 11. We assumed that when the mean lesion length for an isolate was higher than the upper standard error of the control, the fungal isolate was able to grow on that particular host. We further assumed that a particular isolate was pathogenic to the host when its mean lesion length was statistically longer than that of the control.

Ability of phoretic mites to distinguish between vector and non-vector beetles

To determine the level of specificity between phoretic mites and their beetle vectors, we tested the ability of mites to distinguish between vector and non-vector beetles. Three mites that are known to be vectored by beetles were selected based on availability. These were *Dendrolaelaps quadrisetus* known from bark beetles on *Pinus* spp. and *Olea capensis* ssp. *macrocarpa* (Table 1), and two species (*Proctolaelaps vandenbergi* and *Trichouropoda* sp. 3) that are only known as associates of native beetles (*Genuchus hottentottus* and *Trichostetha fascicularis*, both in the Scarabaeidae, collected from *Protea* spp. (Roets et al. 2009). *Dendrolaelaps quadrisetus* is a predatory mite that feeds on eggs and larvae of its phoront beetle (Kinn, 1967) and also in some cases feeds on nematodes, but it often carries spores of members of the Ophiostomatales (Cardoza et al. 2008; Musvuugwa, 2014). *Trichouropoda* sp. 3 has a close mutualistic association with members of the Ophiostomatales (Roets et al. 2007), while *Proctolaelaps vandenbergi* is a pollen and nectar feeder that also often carries spores of the Ophiostomatales (Roets et al. 2007). To account for possible learned behavior (or experience), mites used in experiments were not collected from vector beetles. Rather,

individuals of *D. quadrisetus* were collected from under the bark of *O. capensis* ssp. *macrocarpa*, while those of *P. vanderbergi* and *Trichauropoda* sp. 3 were collected from *Protea* infructescences. Each mite individual was only used once in any experiment.

Mites were allowed to associate with three other beetle taxa besides their natural vector. *Dendrolaelaps quadrisetus* was allowed to associate with one of its normal vectors (*Lanurgus* sp. 1 from host *O. capensis* ssp. *macrocarpa*) and the non-usual vectors *Lasioderma serricorne* (Anobiidae) (from contaminated dried *Gonimbrasia belina* used in stews in southern Africa), Cryphalinae sp. 1 (from *Virgilia oroboides*) and *Ctonoxylon* sp. 2 (from *Olea* sp.). *Lasioderma serricorne* was chosen as this beetle has no known mite associates and is not found in natural areas in the CFR. Both *P. vanderbergi* and *Trichauropoda* sp. 3 were allowed to associate with their normal vector (*G. hottentottus*) and the non-usual vectors *Lanurgus* sp. 1, *L. serricorne* and Cryphalinae sp. 1. Vector studies were carried out testing two different scenarios, a no-choice experiment and a choice experiment.

In the no-choice experiments, five individuals of each of the three tested mite species were placed in a Petri dish (60 mm diameter) containing a single individual of the beetle species tested as possible associate (as outlined above). The experiment was replicated five times for each of the tree mite species on each of the different beetle species tested. After 24 hr the total number of mites found on the individual beetles was counted. In the choice experiment, test arenas were again set up in Petri dishes (60 mm diameter). One individual of each of the four beetle species tested for association with a particular mite species (as outlined above) was placed in the dish. Hereafter 20 individuals of the particular mite species assessed were added to the arena. The experiment was replicated five times for each of the three mite species tested. The total number of mites found on each individual beetle per arena was again

recorded after 24 hr. Differences in medians for number of mites that associated with the various beetles were determined using Kruskal-Wallis ANOVA in Statistica 11.

Results

Growth studies

After 6 weeks all fungal species had at least two isolates that were able to grow on *P. radiata* (Fig. 1A) and there were no differences between mean lesion lengths caused by the various fungal isolates and that of the control (df = 24; f = 1.4; p = 0.12) (Fig. 1A). Therefore, according to our definition, none of the fungal species used proved to be pathogenic to *P. radiata*. All fungal taxa tested were able to grow on *A. mearnsii*, except for the pine associated *O. ips* (Fig. 1B). Mean lesion length varied significantly on this host (df = 24; f = 6.8; p < 0.001) with *G. roseus* and *O. pluriannulatum*, producing distinct lesions compared to the control (Fig. 1B). Four of the fungal taxa tested were not able to grow on *E. grandis* including *S. rapanae*, *S. pallida*, *O. pluriannulatum* and *Ceratocystiopsis* sp. 1 (Fig. 1C). However, mean lesion length varied significantly on this host (df = 24; f = 3.7; p < 0.001), with the native fungi *R. rapanae* and *G. roseus*, and the non-native *Sporothrix* sp. 1 and *O. ips* causing significantly larger lesions than the controls (Fig. 1C).

All isolates of all tested fungal species were able to grow on *R. melanophloeos* (Fig 1D) and mean lesion lengths varied significantly (df = 24; f = 4.1; p < 0.001). Taxa that produced significantly larger lesions than the control on this species included *S. rapanae*, *G. roseus*, *S. pallida*, *O. pluriannulatum* and the non-native *O. ips* (Fig 1D). Very few isolates tested were able to grow on *O. capensis* ssp. *macrocarpa* and there were no differences in mean lesion lengths caused by the various fungal isolates and that of the control (df = 24; f = 0.81; p = 0.72) (Fig. 1E). All fungal species tested on *C. dentata* were able to grow on this tree (Fig.

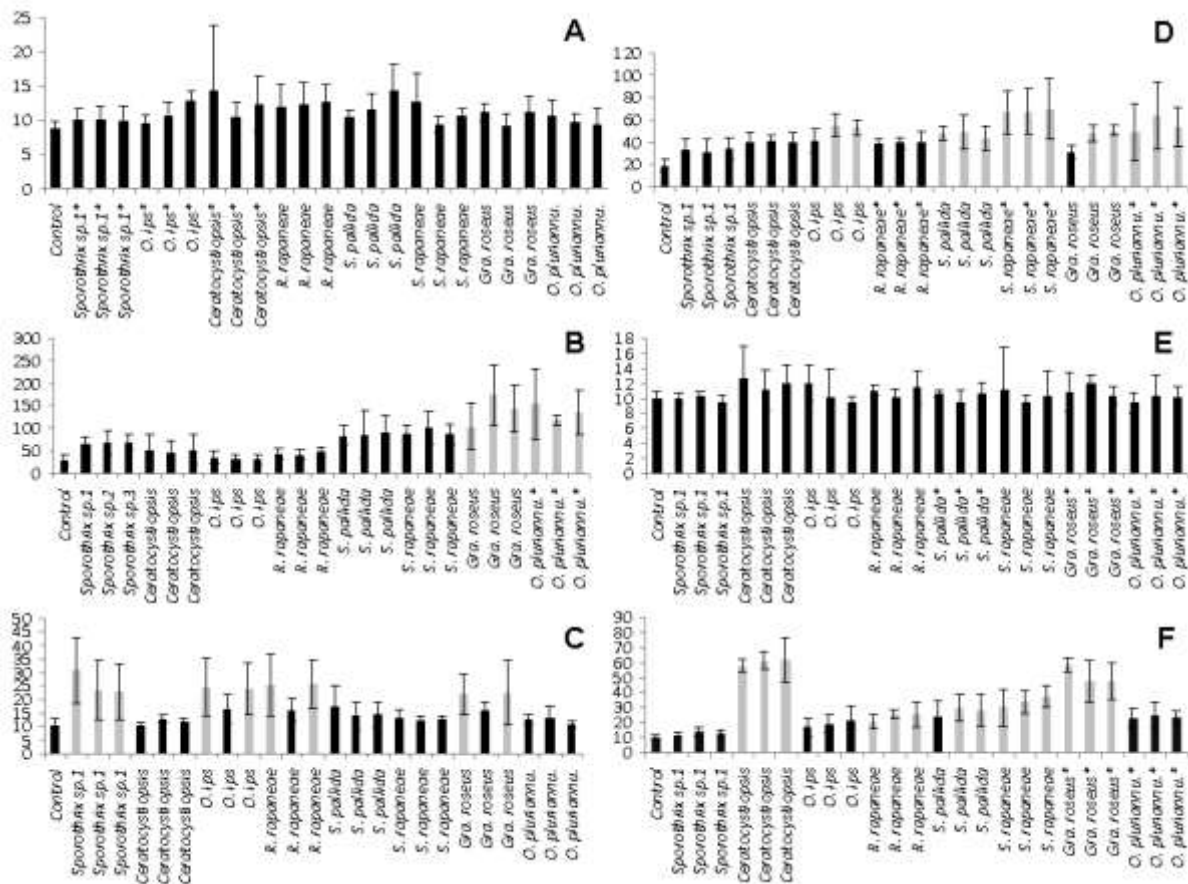


Figure 1. Mean lesion lengths (+/- SE) caused by various members of the Ophiostomatales on the non-native host tree taxa (A) *Pinus radiata*, (B) *Acacia mearnsii* and (C) *Eucalyptus grandis* and the native host tree species (D) *Rapanea melanophloeos*, (E) *Olea capensis* ssp. *macrocarpa* and (F) *Curtisia dentata*. Isolates indicated by grey bars differed significantly from the controls. The first three fungal species on the graphs (*Sporothrix* sp. 1, *Ophiostoma ips* and *Ceratocystiopsis* sp.) are presumably exotic to South Africa while the rest are likely native to the country. All but the last three fungal species (*Sporothrix rapanaeae*, *Graphilbum roseus*, *Ophiostoma pliriannulatum*) are known as Scolytinae beetle associates. These three taxa are known only from wounds on trees. Species indicated by asterisks (*) are known from the particular host depicted in the graph.

1F). Results from the One-way ANOVA indicated significant variance in mean lesion length (df = 24; f = 20.2; p < 0.001) with five fungal species producing significantly longer lesions than controls on *C. dentata*. These were *S. rapanaeae*, *R. rapanaeae*, *G. roseus*, *S. pallida* and the non-native *Ceratocystiopsis* sp.1 (Fig. 1F).

Ability of mites to distinguish between vector and non-vector beetles

In the no-choice experiments, all the phoretic mite species only associated with their natural vector beetles. *Dendrolaelaps quadrisetus* only associated with its vector beetle, *Lanurgus* sp.

1 (df = 3, H = 18.5547, $p < 0.001$), *Trichouropoda* sp. 3 only chose to climb onto their native *Protea* beetle vector, *G. hottentottus* (df = 3, H = 18.6275, $p < 0.001$), while the *Protea*-associated *P. vanderbergi* were also specific to their native vector beetle, *G. hottentottus* (df = 3, H = 18.6031, $p < 0.001$). Similarly, in the choice experiments, all the phoretic mite species only associated with their natural vector beetles. The *Olea*-associated *D. quadrisetus* only associated with *Lanurgus* sp. 1 (df = 3, H = 18.5547, $p < 0.001$), *Trichouropoda* sp. 3 only associated with their natural phoront, *G. hottentottus* (df = 3, H = 18.7500, $p < 0.001$), and *P. vanderbergi* only climbed onto *Genuchus hottentottus* (df = 3, H = 18.6275, $p < 0.001$).

Discussion

Results from this study reveal the possible effects and dangers posed by various members of the Ophiostomatales when they encounter new host trees in the CFR. This is important, as it has been shown that when fungal species encounter new environments, they can cause disease outbreaks with serious consequences (Brasier, 2008; Loo, 2009). When they move from one host species to another, usually through their vectors (Wingfield, 2003; Woolhouse et al. 2005), they can experience limited resistance by the new hosts, which can lead to excessive aggressiveness by the introduced pathogen (Brasier and Buck, 2001). At least two fungal species were able to grow on each of the host trees they were tested on and at least two fungal species were pathogenic on all the tree species they were tested on except on *O. capensis* ssp. *macrocarpa* and *P. radiata*. Importantly these hosts do not necessarily have to be closely related. This implies that future dangers posed by movement of Ophiostomatales species between native and non-native hosts cannot be readily predicted.

According to the Food and Agriculture Forest Organisation of the United Nations (FAO; www.fao.org/forestry/pests) a disease can be defined as a condition caused by living organisms or environmental changes that impair the normal functions of another living organism. A pathogen is a micro-organism (such as a fungus) that causes a disease. Although at least two fungal species were identified as pathogenic on most tree species, it is important to note that the definition of pathogenicity in this paper refers to an isolate that caused a lesion significantly larger than the wound only controls. We, therefore, assume that that a particular isolate could cause an infection and may interfere with the normal functioning of the plant if left untreated for extended periods of time. Plant defences may, however, stop these infections from causing harm in the future and we may, therefore, have overestimated pathogenicity here. In contrast, some isolates that grow slowly may still prove to cause longer lesions and be classified as pathogenic given more time to develop. However, many studies rely on this method of testing pathogenicity (e.g. Chen et al. 2013; Machingambi et al. 2015) as monitoring disease development after inoculation will have to be carried out over extended periods of time on long-lived organisms such as trees to verify whether the fungi could impair normal functioning.

Also presented in this study is the relationship between phoretic mites, their natural beetle vectors and other beetles they are not likely to encounter in the field. Results showed that there is a high level of specificity between the mites and their natural vectors, at least when the beetle taxa are phylogenetically distantly related. However, it is well known that mites tend to move between vector beetles, especially when these occur on the same hosts. In fact, commonly a single mite species is often phoretic on many species of bark and other beetles including the predators and competitors of Scolytinae (Hofstetter et al. 2013). These predators may provide phoretic mites opportunities to switch between distantly related plant

hosts in the search for food and it is, therefore, possible that the fungi studied here could encounter new hosts via insect taxa not evaluated in the current study. However, Ophiostomatales species typically do not rely only on mites for vectoring, but rather mites complement their movement via beetles (e.g. Hofstetter et al. 2013). Fungal species associated with boring beetles are not likely to easily encounter new, distantly related, hosts as the beetles tend to be specific towards certain hosts at the plant family or plant genus level (Smiley and Moser, 1974; Price et al. 1992; Klepzig et al. 2001; Walter et al. 2010; Hofstetter et al. 2013). However, chances of encountering new hosts by boring-beetle associated Ophiostomatales may be enhanced if the hosts are phylogenetically closely related. For example in the case of the Dutch elm disease, the causative agents *O. ulmi* and *O. novo-ulmi* together with their vector, the European elm bark beetle, successfully invaded a new host, the American elm tree, which is closely related to their natural host, the European elm tree (Peace, 1960; Lamb, 1979; Brasier, 1990; Webber, 1990; Heybroek, 1993; Brasier and Buck, 2001). Similarly *Raffaelela lauricola* along with its vector beetle *Xyleborus glabratus* that were introduced into the southeastern USA successfully moved onto native species that belong to the same family (Lauraceae) as that of its natural *Persea borbonia* host. (Harrington et al. 2008). Importantly, native bark beetles commonly acquire introduced Ophiostomatales species especially when the hosts are fairly closely related (e.g. Jacobs et al. 2004). Some of the beetle-associated fungi tested in this study were from tree species that are relatively closely related to tree species that are of economic and ecological importance in the CFR. The phylogenetic relatedness of these hosts may enhance their chances of getting infected by beetle-associated Ophiostomatales. For example, *Pinus radiata* is fairly closely related to some ecologically and economically important native trees such as *Podocarpus* species and *Widdringtonia* species in the order Pinales. *Olea capensis* ssp. *macrocarpa* is closely related to the economically very important domestic olive tree (*O. europea*). It is

possible that the beetle-associated Ophiostomatales from *P. radiata* and *O. capensis* ssp. *macrocarpa* can encounter these close relatives where these grow in close proximity in the CFR and cause major damage. There is, therefore, a need to assess the pathogenicity of the fungi tested in this study on other, more closely related tree taxa in future studies.

None of the fungi tested was pathogenic on *P. radiata* or *O. capensis* ssp. *macrocarpa*. It is possible that these tree species are highly resistant to attack by many pathogens. *O. capensis* ssp. *macrocarpa* has never before been recorded to be attacked by, or associated with, any pathogenic fungus. However, major losses of *P. radiata* caused by non-ophiostomatoid fungi have been recorded in South Africa. Examples include *Arnylostereum areolatum* and *A. chailletii* vectored by the woodwasp, *Sirex noctilio* (Madden, 1988; Hurley et al. 2007). *P. radiata* is also associated with members of the Ophiostomatales that can cause sapstain (Reay et al. 2002). The mere ability of all species tested in this study to grow on this host may indicate that some native Ophiostomatales may well be of future economic importance if these prove to cause similar wood defects.

The wound-associated species *G. roseus* and *O. pluriannulatum*-like were pathogenic on several tree species. *G. roseus* was pathogenic on the non-native *A. mearnsii* and *E. grandis* as well as on the native *R. melanophloes* and *C. dentata*. This species was recently isolated from several native hosts, including *Curtisia dentata*, *Halleria lucida*, *Pterocelastrus tricuspidatus*, *Trichocladus crinitus* and *O. capensis* ssp. *macrocarpa* (Musvuugwa, 2014). Interestingly it is also associated with several wound-associated mites such as Oribatida sp. 2, *Lasioseius* sp. 1 and Mesostigmata sp. 1 (Musvuugwa, 2014). These mites may aid the transport of this fungus to many hosts, especially since these hosts grow in sympatry. Since it is possible that this fungus is vectored by its associated mites, it is likely that it can at some

point be transported to these exotic hosts and pose a danger to South Africa's forestry industry. This scenario has occurred at least once before in South Africa, when *C. albifundus* shifted hosts from native taxa to the non-native *A. mearnsii*, causing a serious wilt disease that led to financial losses in the forestry industry (De Beer, 1994; Roux and Wingfield, 1997; Roux et al. 1999). Similarly, *O. pluriannulatum*-like is also known from many native hosts and the exotic *A. mearnsii*, and is also associated with several mite species (Musvuugwa, 2014). It has now been confirmed to be pathogenic to *A. mearnsii* and may therefore pose a significant threat should it move into plantation forestry areas. The current distribution of this fungus is unknown, but as *A. mearnsii* has invaded much of coastal South Africa, it may well be able to extend its range rapidly eastwards to where *A. mearnsii* is commercially planted. The other wound-associated fungus, *S. rapanae*, was pathogenic on its host *R. melanophloeos* and on *C. dentata*. Currently the fungus is only known from *R. melanophloeos* and its associated arthropods are unknown. These arthropods are highly likely to be mites, as mites have been observed on *S. rapanae* fungal mats under the bark of this tree (pers. observ.). We suspect that the vector species is probably rather specific to this host, as this fungus has thus far been only collected from this host tree, despite focussed collection effort (Musvuugwa, 2014).

Three of the fungal species from pine trees, associated with *D. quadrisetus* and *O. erosus*, were also pathogenic on some of the tree species tested. Although these fungi were able to grow on almost all the tree species they were tested on, they were only pathogenic on a few. *Sporothrix* sp. 1 was pathogenic on *E. grandis*, the *Ceratocystiopsis* species was pathogenic on *C. dentata*, and *O. ips* was pathogenic on *E. grandis* and *R. melanophloeos*. These species may be able to move to *E. grandis* and native hosts in areas where these occur in close proximity using *D. quadrisetus* as a carrier. This notion is aided by the recent collection of

this mite from both native and exotic bark beetles and hosts (Musvuugwa, 2014). A possible reason why none of these fungi have been found on native hosts yet is that this mite has moved from pine-associated beetles to a beetle associated with *O. capensis* ssp. *macrocarpa* (Musvuugwa, 2014), a host that seems to be very resistant to attack by members of the Ophiostomatales. However, this beetle may not be very host specific as it is also known from *Gonioma kamassi* (Musvuugwa, 2014). Future exchanges of these fungi between usual and non-usual hosts therefore cannot be ruled out.

The beetle-associated fungi from native hosts, *S. pallida* and *R. rapanae*, were able to grow on all tree species and were pathogenic on some. Both species were pathogenic on *C. dentata*, while *S. pallida* was also pathogenic on *R. melanophloeos* and *R. rapanae* was also pathogenic on *E. grandis*. *Sporothrix pallida* is associated with two beetle species, *Lanurgus* sp. 1 and *Ctonoxylon* sp. 1, as well as with the phoretic mite of *Lanurgus* sp. 1 (*D. quadrisetus*) (Musvuugwa, 2014). The fact that this fungus is associated with three different arthropods may aid its dispersal to new hosts on which it may be pathogenic. In fact, this species may not be very vector specific at all, as it is known from many different environments (de Meyer et al. 2008). Conversely, *R. rapanae* is currently only known as an associate of one Platypodinae beetle species and is likely ambrosial (Musvuugwa et al. 2015). It may therefore be highly specific to its beetle associate, which would reduce the chances of it being vectored to another host tree.

Conclusions

Even though efforts are made to restrict the movement of exotic species across the borders of countries, many Ophiostomatales species continuously invade new regions (e.g. Kubono and Ito, 2002; Brasier, 2008; Loo, 2009; Brasier and Webber, 2010). Preventing introductions

especially in developing countries that are under pressure for increased growth in international trade, is therefore extremely difficult. This is due to the cryptic nature of the fungi and their vector organisms and a lack of appropriately experienced scientists to perform risk analyses (Plant Health (Phytosanitary) Policy for South Africa 2014). A shift in focus to mitigating the spread of exotic species within countries and the facilitation of their eradication and/or control is, therefore, often needed. For fungi in the Ophiostomatales, eradication and/or control are not attainable when these fungi enter natural ecosystems due to their close integration into these systems. Focus should, therefore, be on restricting movement of affected exotic plant materials (such as logs used for making fires) within countries to prevent these from coming in contact with indigenous ecosystems as much as possible. The eradication of exotic invasive species (e.g. *Acacia mearnsii*, *Eucalyptus* spp. and *Pinus* spp. in South Africa), many of which are hosts to both native and non-native Ophiostomatales (Musvuugwa, 2014), should also be targeted as these can act as conduits for the movement of associated exotic and indigenous organisms across the invaded ranges (Van der Colff et al. 2015). This should especially be prioritised in areas where these grow in close proximity to natural forests where indigenous and exotic hosts often occur in sympatry (Moran and Hoffman, 2012).

Many of the fungal species assessed in this study may threaten new hosts that they may encounter. This is especially true for those species found on wounds and those associated with several arthropod taxa. These threats are less likely for fungal species associated with sub-cortical beetles, unless newly encountered hosts are phylogenetically closely related to the usual hosts. The virulence of the pathogenic fungi identified here is not yet known, but some taxa may be able to kill newly encountered hosts. However, even if these cannot kill

their hosts, they could still pose a great ecological (when exotic fungi compete with native fungi) or economic threat (e.g. blue staining of lumber).

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