FUNGAL BIOLOGY 120 (2016) 827–835

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/funbio

Endophytic Cryphonectriaceae on native Myrtales: Possible origin of Chrysoporthe canker on plantation-grown *Eucalyptus*

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ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form
14 March 2016
Accepted 15 March 2016
Available online 22 March 2016
Corresponding Editor:
Nabla Kennedy

Keywords:
Canker pathogens
Forestry
Melastomataceae
Myrtaceae
Plants for planting
Quarantine

ABSTRACT

Chrysoporthe austroafricana (Cryphonectriaceae) is a damaging canker pathogen on *Eucalyptus* species in Southern Africa. Recent studies have shown that the fungus occurs on native *Syzygium* species and that it has apparently undergone a host range expansion from these native trees to infect non-native *Eucalyptus*. The aim of this study was to consider whether *Chr. austroafricana* and other Cryphonectriaceae might exist as endophytes in native Myrtaceae, providing a source of inoculum to infect non-native Myrtales. Healthy branches were collected from Myrtaceae in Mozambique, incubated in florist foam, allowed to dry gradually and monitored for the appearance of fruiting bodies resembling species in the Cryphonectriaceae. Isolates were identified based on DNA sequence data. Two species in the Cryphonectriaceae were obtained, representing the first evidence that species in the Cryphonectriaceae occur as endophytes on native Myrtales, thus providing a source of inoculum to infect non-native and susceptible trees. This has important implications regarding the movement of planting stock used by ornamental tree and forestry enterprises.

Introduction

Fungi in the Cryphonectriaceae include a number of important tree pathogens globally, both in native and commercial plantation ecosystems (Gryzenhout *et al.* 2009). The best known of these is *Cryphonectria parasitica*, the cause of chestnut blight, that has lead to the near extinction of American and European chestnut trees in their respective native ranges (Anagnostakis 1987). Related species in the genus *Chrysoporthe* (previously known as species of *Cryphonectria*) gained notoriety in the 1970''s when they were identified as important pathogens of commercially grown *Eucalyptus* species in Brazil (Hodges *et al.* 1976; Wingfield 2003).

Chrysoporthe (Cryphonectriaceae) includes a number of important eucalypt pathogens (Wingfield 2003; Gryzenhout *et al.* 2009), including *Chrysoporthe austroafricana* in Africa

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http://dx.doi.org/10.1016/j.funbio.2016.03.005
1878-6146/

(Gryzenhout et al. 2004; Nakabonge et al. 2006), *Chrysoporthe cubensis* in Latin America (Hodges et al. 1976; Gryzenhout et al. 2004) and Africa (Roux & Apetorgbor 2010), and *Chrysoporthe deuterocubensis* in Asia (Sharma et al. 1985; Van der Merwe et al. 2010) and Africa (Nakabonge et al. 2007). Infections by *Chrysoporthe* species result in stem and root collar cankers after colonization of the bark, cambium and woody tissues at the bases of *Eucalyptus* trees (Hodges et al. 1976; Sharma et al. 1985; Wingfield et al. 1989). Infection of young trees results in death, while stem cankers on older trees make the stems prone to wind breakage (Sharma et al. 1985; Wingfield 2003; Nakabonge et al. 2006).

Chrysoporthe species have a host range restricted to plants in the family Myrtales. Host genera include *Lagerstroemia* (Gryzenhout et al. 2006), *Miconia* (Rodas et al. 2005), *Psidium* (Hodges 1988), *Syzygium* (Hodges et al. 1986), *Tibouchina* (Wingfield et al. 2001), and a number of others (Seixas et al. 2004; Barreto et al. 2006; Gryzenhout et al. 2006). In most countries where *Eucalyptus* species are grown as non-natives, they occur in close proximity to related, native plants in the Myrtales (Wingfield et al. 2001; Seixas et al. 2004). The occurrence of similar fungal species on both the native and non-native hosts suggests that some *Chrysoporthe* species have undergone host shifts (Slippers et al. 2005) from the native Myrtales, e.g. *Miconia*, *Syzygium*, *Tibouchina* species, to infect non-native *Eucalyptus* spp. (Heath et al. 2006; Van der Merwe et al. 2010, 2012). Evidence from population genetic studies suggests that *Chr. cubensis* is native to Latin America (Gryzenhout et al. 2009), where it underwent a host shift from native Myrtales to infect non-native *Eucalyptus* species (Van der Merwe et al. 2012). Similarly, *Chr. austroafricana* is an African fungus that has undergone a host shift from native African Myrtales (Heath et al. 2006) to infect Australian *Eucalyptus* species grown as non-natives in plantations.

At least two of the *Eucalyptus* pathogens, *Chr. cubensis*, and *Chr. deuterocubensis* have moved beyond their purported regions of origin. *Chr. cubensis*, believed to be native in South and Central America, has been found in Central and West Africa (Gibson 1981; Roux et al. 2003; Roux & Apetorgbor 2010). Likewise, *Chr. deuterocubensis*, which is believed to be native to Asia (Myburg et al. 2002; Pegg et al. 2010; Van der Merwe et al. 2010), has been found in East and Southern Africa (Nakabonge et al. 2006; Van der Merwe et al. 2010). These important pathogens have been recorded only from non-native *Eucalyptus* species and *Syzygium aromaticum* (clove) in Africa. The limited distribution of *Chr. deuterocubensis* outside East Africa, together with a low population diversity (Nakabonge et al. 2007), strongly supports the hypothesis that it was introduced to the African continent, most likely from Asia with the trade in cloves (Roux et al. 2003; Gryzenhout et al. 2006).

The accidental movement of fungi to new environments, and the disease epidemics that have subsequently arisen in some cases, has raised increasing concern as the incidence and impact of these introductions has increased (Desprez-Loustau et al. 2007; Brasier 2008; Liebhold et al. 2012; Wingfield et al. 2015). The trade in life plants, sometimes also referred to as 'plants for planting', and timber have been identified as two of the main pathways of pathogen introductions into new regions (Brasier 2008; Liebhold et al. 2012). It has for example been suggested that the chestnut blight pathogen, *C. parasitica*, was introduced into the United States of America with living plants (Milgroom et al. 1992; Dutech et al. 2012), while the most likely route of movement of *Chrysoporthe* species is still not well understood. A pathway of spread that has not received attention for fungi in the Cryphonectriaceae, is where they might have been carried as symptomless endophytes. This would be in seemingly healthy plants or commercially traded plant tissue such as that used for floral arrangements.

Endophytes are microorganisms living within plant tissues, for all or part of their life cycle, without causing any apparent or detectable symptoms of disease (Petrini et al. 1993; Bacon & White 2000; Arnold et al. 2003). These organisms can be latent or opportunistic pathogens, causing disease when infected plants are exposed to unsuitable environmental conditions (Bacon & White 2000). Some endophytic microorganisms have also been reported to benefit their host plants by providing protection from herbivores or insect infestation (Siegel & Latch, 1985; Clay 1986; Arnold & Lewis 2005), by enhancing growth (Ren et al. 2011), improving drought tolerance (Hubbard et al. 2012) and protection against pathogens (Arnold et al. 2003). Endophytes probably occur in all plant species and plant parts (Sturz et al. 2000; Rosenblueth & Martínez Romero 2006) and while they contribute significantly to the hyperdiversity of fungi, they typically go unnoticed (Hawksworth 2001; Arnold 2008).

Despite the fact that *Chrysoporthe* species are important pathogens of *Eucalyptus* species, very little is known regarding their origin or how they have emerged as important pathogens on non-native, commercially propagated trees. The fact that *Chr. austroafricana* is found sporulating on bark and dead branches of native Myrtaceae in areas where the fungus occurs as a pathogen of *Eucalyptus* suggests that the fungus and its relatives possibly could occur as non-damaging endophytes in asymptomatic trees. The aim of this study was to test this hypothesis by making isolations from asymptomatic tissues of Myrtales growing in a native environment and to identify the resulting fungi. Because fungi in the Cryphonectriaceae are likely to develop and sporulate gradually as plant tissue dies, a novel technique to detect possible infections by them was applied.

Materials and methods

Endophyte isolations

During the course of two field surveys in Mozambique in July 2010 and August 2011, segments (~30 cm length, ~1 cm diameter) were cut from healthy branches of various native and non-native Myrtales in eucalypt-growing areas of the country. All leaves were removed from the samples at the time of collection. Trees sampled included native species of *Dissotis* and *Syzygium*, *Eugenia capensis*, and non-native *Psidium guajava* in the Central, Northern, and Southern Provinces of Mozambique (Table 1). A total of 89 trees, collected in six provinces of Mozambique, were sampled. Six trees were from Inhambane (*Syzygium guineense*), seven were from Gaza (four of *E. capensis* and three of *Syzygium cordatum*), 23 from Nampula (20 *S.*