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Ecology and population structure of a tree wound-infecting fungus in a native South African forest environment

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ABSTRACT

Ceratocystis tsitsikammensis was first isolated from bark harvesting wounds on two indigenous tree species in the Afromontane forests of the Western Cape Province of South Africa. Inoculation studies indicated that it is a potential pathogen of native *Rapanea melanophloeos* trees. In this study, we investigated the distribution, ecology and biology of *C. tsitsikammensis* in the Garden Route National Park of South Africa. Isolates were obtained from wounds on *R. melanophloeos,* three non-native hosts as well as from nitidulid and staphylinid beetles visiting wounds on these trees. The genetic diversity and population biology of the fungus was examined using microsatellite markers. Its mating strategy was also determined by amplifying its mating type genes and the fungus was shown to be homothallic. Despite the homothallic nature of the fungus, high levels of random mating and absence of genetic structure was found in the investigated population, suggesting a strong effect of gene flow, probably linked to insect dispersal. The gene diversity of *C. tsitsikammensis* was similar to that of a related fungus, *Ceratocystis albifundus*, that is known to be native in Africa. This, together with the fact that *C. tsitiskamensis* is not known elsewhere, within or outside South Africa, suggests that it is native and endemic to the Cape Afromontane region.

Introduction

The genus *Ceratocystis* (Microascales, Ceratocystidaceae), as recently redefined by de Beer *et al.* (2014), includes pathogens of economically important trees and root crops worldwide (Upadhyay 1981; Wingfield *et al.* 1993; Roux & Wingfield 2009). These pathogens induce various disease symptoms, including stem cankers, wilting and root rot resulting in tree/plant death (Kile 1993; Seifert *et al.* 2013). The type species, *Ceratocystis fimbriata*, was described as a pathogen of sweet

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potato (*Ipomea batata*) in the United States of America (USA), (Halsted 1890). Since then, numerous congener species have been found associated with diseases on various plants on all continents, excluding Antarctica. Some species that have caused significant economic losses include *Ceratocystis manginecans*, the causal agent of mango sudden decline in Oman and Pakistan (van Wyk *et al.* 2007) and the wilt and die-back of *Acacia mangium* in Indonesia (Tarigan *et al.* 2011; Fourie *et al.* 2016), *Ceratocystis cacaofunesta* causing wilt, canker and death of cacao (*Theobroma cacao*) in South and Central America and the Caribbean islands (Engelbrecht & Harrington 2005) and *Ceratocystis platani*, the cause of a canker stain disease on plane trees (*Platanus* sp.) in the USA and Europe (Gibbs 1981; Panconesi 1999; Baker *et al.* 2003; Ocasio-Morales *et al.* 2007).

Ceratocystis species colonize their hosts principally through wounds, which may result from animal browsing, insects, wind and hail damage, as well as human management activities (DeVay *et al.* 1968; Roux & Wingfield 1997; Roux *et al.* 2004; Mbenoun *et al.* 2014). Infection can also occur via root grafts between adjacent plants, but this has been ascertained only for *Ceratocystis fagacearum* (Gibbs & French 1980; Blaedow & Juzwik 2010) and *C. platani* (Mutto 1986). Natural overland transmission is generally provided by insects, facilitated by adaptations in these fungi for insect dispersal (Moller & DeVay 1968; Gibbs & French 1980; Redfern *et al.* 1987; Hayslett *et al.* 2008; Heath *et al.* 2009a). These include the production of globoid ascomata with elongated necks exuding sticky ascospore masses at their apices (Ingold 1961; Upadhyay 1981; Malloch & Blackwell 1993). These sticky spores easily attach to the bodies of cohabiting insects (Malloch & Blackwell 1993) and are also adapted for endozoic transfer (Juzwik & French 1986). All species, with the exception of *Ceratocystis caryae*, produce fruity volatiles that are attractive to insects (Hanssen 1993; Kile 1993; Johnson *et al.* 2005). Nitidulid beetles (Coleoptera: Nitidulidae) are the most common vectors of *Ceratocystis* species (Hinds 1972; Appel *et al.* 1990; Heath *et al.* 2009a; Kamgan Nkuekam *et al.* 2012; Mbenoun *et al.* 2014). Other insects that appear to contribute to the dispersal of these fungi include drosophilid flies (Diptera: Drosophilidae) (Moller & DeVay 1968; Hinds 1972), staphylinid beetles (Coleoptera: Staphylinidae) (Hinds 1972; Kamgan Nkuekam *et al.* 2012) and bark beetles (Coleoptera: Scolytidae) (Juzwik *et al.* 2008).

Agricultural activities have contributed to broaden the distribution of many *Ceratocystis* species via the trade and movement of infected plant material and products (Ferreira *et al.* 2011; Wingfield *et al.* 2013). These include important pathogens such as *C. platani*, *C. manginecans*, and *C. eucalypticola*, which have been introduced to new regions where they have initiated new disease outbreaks on exotic as well as native host plants (Roux & Wingfield 2009). Emergence of new diseases caused by native *Ceratocystis* species on non-native hosts has also been reported. One vivid example is the wattle wilt disease affecting Australian *Acacia* trees (*Acacia mearnsii*) commercially grown in Africa (Morris *et al.* 1993; Wingfield *et al.* 1996; Roux *et al.* 1999; Roux & Wingfield 2009). This disease is caused by *Ceratocystis albifundus*, an indigenous African fungus commonly occurring as a saprobe on wounds on various trees in natural South African woodlands (Morris *et al.* 1993; Wingfield *et al.* 1996; Roux *et al.* 1999; Roux & Wingfield 2009).

The emergence of wattle wilt disease has prompted an interest in the diversity of *Ceratocystis* species in natural as well as human-modified ecosystems in Africa as a potential source of new pathogens of agricultural and forestry tree crops. This has resulted in the discovery of several previously unknown species, most of which were new to science (Barnes *et al.* 2003; Kamgan Nkuekam *et al.* 2008; Heath *et al.* 2009b; van Wyk *et al.* 2012; Mbenoun *et al.* 2014). There is evidence that some of these fungi found in plantation forest environments, including *Ceratocystis eucalypticola* and *Ceratocystis pirilliformis*, may have originated outside Africa (Mbenoun *et al.* 2014). This is also supported by population genetic studies suggesting their probable recent introduction into South Africa (van Wyk *et al.* 2006; Kamgan Nkuekam *et al.* 2009). Another group of species, mostly from native ecosystems have, along with *C. albifundus* been shown to form an African lineage within the genus *Ceratocystis* (Mbenoun *et al.* 2014). However, unlike *C. albifundus* for which abundant information has been generated regarding its genetic diversity (Roux *et al.* 2001; Barnes *et al.* 2005; Lee *et al.* 2016), distribution and insect associations (Heath *et al.* 2009 a,b), almost nothing is known regarding the biology or origins of its closest relatives.

Ceratocystis tsitsikammensis was described from the Garden Route National Park (GRNP), in the Western Cape Province of South Africa (Kamgan Nkuekam *et al.* 2008). It was first isolated from bark harvesting wounds on native *Rapanea melanophloeos* and *Ocotea bullata* trees (Kamgan Nkuekam *et al.* 2008). Inoculation experiments on *R. melanophloeos* resulted in significant lesions, highlighting the need to monitor this fungus as a potentially important pathogen. Until recently, *C. tsitsikammensis* was known only from GRNP and several collections undertaken in natural and plantation ecosystems across and outside South Africa have failed to recover this fungus. In 2014, *C. tsitsikammensis* was isolated from *Virgilia divaricata* and *A. mearnsii* in the GRNP (Van der Colff 2014), suggesting that it may have a wider host range and, more importantly, this host range includes commercially planted non-native trees.

The objective of this study was to increase our knowledge regarding *C. tsitsikammensis*. We determined the mating strategy of the fungus and investigated its geographic distribution, biology, ecology and possible impact on *R. melanophloeos* in the GRNP. The genetic diversity and structure of *C. tsitsikammensis* was studied using microsatellite markers, developed for congener species, to determine whether it is an indigenous species that readily disperses between native and non-native plants. In addition, the role of insects associated with tree wounds as potential dispersal agents was considered.

Materials and methods

Study area

This study was conducted in the GRNP of the Western Cape Province of South Africa. The GRNP forest covers about 35 765 ha stretching from sea level to altitudes of more than 1000 m. Its floristic composition spans eight types of