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Inheritance of phenotypic traits in the progeny of a *Ceratocystis* interspecific cross

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ABSTRACT

Ceratocystis fimbriata is a fungal plant pathogen that causes black rot on *Ipomoea batatas*. Based on inoculation studies on numerous tree species, the pathogen is known to be host specific. The closely related species, *Ceratocystis manginecans*, causes severe wilt on a broad range of tree hosts, including *Mangifera indica*, *Acacia mangium* and other leguminous tree species. The genetic factors underlying the pathogenicity and host specificity of *Ceratocystis* species have rarely been investigated. In this study, an F_1 population of 70 recombinant progeny from a cross between *C. fimbriata* and *C. manginecans* was generated and the inheritance of various phenotypic traits was investigated. Results showed that colony colour, growth rate, asexual spore production and aggressiveness to *I. batatas* and *A. mangium* are all quantitative traits with high levels of heritability. However, conidia production and aggressiveness appeared to be regulated by a small number of genes. No correlation could be found between aggressiveness and other phenotypic traits, suggesting that these are inherited independently. This is the first study to consider genetic inheritance of pathogenicity and host specificity in *Ceratocystis* species and the results will contribute, in future, to the identification of quantitative trait loci and candidate genes associated with the traits investigated.

1. Introduction

The effective control of fungal plant pathogens is dependent on our understanding of the biology and life history traits of the pathogen. In fungal plant pathogens, most traits associated with pathogenicity and host specificity are quantitative and thus regulated by multiple genes. Examples of such traits in fungi include mycotoxin production (Cumagun et al., 2004; Talas et al., 2016), melanisation (Lendenmann et al., 2014), mycelial/hyphal growth rate (De Vos et al., 2011; Lee et al., 2015), hyphal length (Lin et al., 2006) and spore production (Lannou, 2011; Milus et al., 2008). In some species, different mating types that differ in growth rate, have been shown to indirectly influence aggressiveness (Lee et al., 2015; Lin et al., 2006). However, to understand how these phenotypes are regulated, the genomic regions associated with each trait must be identified.

Sexual crosses between different strains or closely related species provide an effective approach to determine whether a trait is quantitative. This is achieved by investigating the segregation of traits in the progeny (Cumagun et al., 2004; Stewart and McDonald, 2014). In *Ophiostoma ulmi*, for example, fitness traits such as host-colonising ability, toxin production and growth rate were all determined to be complex, based on the non-Mendelian segregation in the progeny arising from a sexual cross (Kile and Brasier, 1990). Interspecific crosses provide more divergent traits for comparison than intraspecific crosses and have been used to investigate the inheritance of traits associated with host specificity (De Vos et al., 2011; Lind et al., 2007; Olson et al., 2005). Although only established recently in fungi, quantitative studies have also been undertaken in natural populations, based on genome-wide association studies (GWAS), where genetic factors associated with virulence traits have been identified in several fungi (Dalman et al., 2013; Gao et al., 2016; Talas et al., 2016).

The genus *Ceratocystis* includes 36 species, most of which are plant pathogens (De Beer et al., 2014; Marin-Felix et al., 2017), that

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infect hosts such as forest and fruit trees (De Beer et al., 2014; Van Wyk et al., 2013) and various root crops (Harrington et al., 2014; Wingfield et al., 2013). Species in this genus infect their hosts through wounds, after which they occupy the xylem vessels that results in symptoms such as stem cankers, xylem staining, wilt and in many cases tree death (Van Wyk et al., 2013).

Knowledge of the factors associated with host specificity and aggressiveness in *Ceratocystis* species has been limited to studies focusing on host response during infection (Al-Sadi et al., 2010; Kojima and Uritani, 1976; Trang et al., 2017) and studies on enzymes produced by *Ceratocystis* for the breakdown of host phenolics (Wadke et al., 2016). A recent *Ceratocystis* genome comparison identified potential pathogenicity genes in *Ceratocystis cacaofunesta* and *Ceratocystis fimbriata*, including putative effectors and a highly expanded phosphatidylinositol-specific phospholipase-C gene family (Molano et al., 2018). This provides various candidates for future functional studies.

Host specificity has been demonstrated in a number of *Ceratocystis* species. For example, *C. cacaofunesta* is pathogenic only on *Theobroma cacao* and *C. platani* only on *Platanus* spp. (Baker et al., 2003; Tsopelas et al., 2017). Similarly, the type species of *Ceratocystis*, *C. fimbriata sensu stricto*, has been reported only to cause black rot of *Ipomoea batatas* (sweet potato) (Halsted, 1890). The host specificity of this species has been confirmed from inoculations on *T. cacao*, *Platanus* spp (Baker et al., 2003) and *Acacia mangium* trees (Rauf M.R., personal communication 2016).

Contrary to the host specific *C. fimbriata*, the closely related species, *Ceratocystis manginecans*, has a broader host range and has resulted in significant losses in *A. mangium* plantations (Brawner et al., 2015; Fourie et al., 2016; Tarigan et al., 2011) and *Mangifera indica* (mango) orchards (Al Adawi et al., 2006). Other reported hosts include *Eucalyptus* (Chen et al., 2013), *Punica granatum* (Harrington et al., 2014; Huang et al., 2003) and the leguminous trees, *Dalbergia sissoo* and *Prosopis cineraria* (Al Adawi et al., 2013). The pathogenicity of *C. manginecans* on *I. batatas* has not been determined.

C. fimbriata s.s. and *C. manginecans* are morphologically very similar but phylogenetically distinct species (Fourie et al., 2014). They can, however, be induced to mate in a controlled laboratory environment (Ferreira et al., 2010; Li et al., 2016; Oliveira et al., 2015b). Mating studies have been used extensively in the Ceratocystidaceae to understand mating systems and the inheritance of mating type genes (Kile et al., 1996; Webster and Butler, 1967), colony colour (Webster, 1967), mycelial phenotypes, cycloheximide resistance (Harrington and McNew, 1997) and microsatellite marker regions (Ferreira et al., 2010) within the family. This has established an ideal system to perform an interspecific cross that can be used to investigate the inheritance of other phenotypic traits in the progeny (De Vos et al., 2011; Lind et al., 2007).

Ceratocystis species are haploid and homothallic, with a unidirectional mating-type switching system that results in both self-fertile (MAT2) and self-sterile (MAT1) progeny (Webster and Butler, 1967; Wilken et al., 2014; Witthuhn et al., 2000). In MAT1 isolates one of the three *MAT* genes (*MAT1-2-1*) is absent. These isolates are used in sexual crosses in combination with self-sterile MAT2 isolates (Engelbrecht and Harrington, 2005; Johnson et al., 2005). Isolates of the latter type have been produced only in laboratory conditions, they contain all three *MAT* genes but ascomata (sexual structures) and protoperithecia are absent, likely due to a mutation influencing protoperithecia formation (Ferreira et al., 2010; Webster, 1967).

The aim of this study was to perform an interspecific cross between an isolate of *C. manginecans* and *C. fimbriata* and to investigate the inheritance of traits associated with host specificity and aggressiveness in the progeny. For this purpose, the recombinant progeny from the cross were used to determine the frequency distribution and heritability of phenotypic traits. The primary trait investigated was lesion lengths induced on the respective plant hosts, *I. batatas* and *A. mangium*, after reciprocal inoculation. In addition, because colony pigmentation (Lendenmann et al., 2014), growth rate in culture (Zhan et al., 2016) and asexual spore production (Lannou, 2011) have been associated with aggressiveness in other fungal pathogens, these traits were also considered in the current study.

2. Materials and methods

2.1. Parental isolates

Three *C. fimbriata s.s.* isolates (CMW14799 from USA and CMW42704 and CMW42705 from Malaysia) and two *C. manginecans* isolates (CMW46461 and CMW48940 from Malaysia) were used for mating experiments between the two species (Table 1). The parental isolates were selected, based on their specific pathogenicity on *I. batatas* and *A. mangium*, respectively. Cultures were grown on 2 % malt extract agar (MEA), supplemented with 150 mg/L Streptomycin sulfate salt (Sigma–Aldrich, Germany) and 1 mg/L Thiamine hydrochloride (Sigma–Aldrich, Germany), at room temperature (22 °C) for 14 d.

2.2. *Mating between* C. fimbriata *and* C. manginecans *to establish an* F_1 *population*

For each parental isolate used in the study, both MAT1 and MAT2 self-sterile cultures were generated. MAT1 individuals were produced from the self-fertile MAT2 parental isolates by dispersing spores from a single ascospore drop on MEA media, using Soltrol® 130 isoparaffin solvent (Chevron Phillips Chemical Company LP, Texas, US) as described by Wilken et al. (2014). Germinating single ascospores were isolated and cultures failing to produce ascomata were selected. MAT2 self-sterile isolates were produced by continuous sub-culturing of aerial mycelia of MAT2 self-fertile isolates until no ascomata were produced. The presence/absence of the *MAT1-2-1* gene in the putative MAT1 and MAT2 self-sterile isolates were confirmed using the primers MAT-121F and

Table 1
Information on the host and location of *Ceratocystis* isolates used in this study.

Species	CMW no.[a]	CBS no.[b]	Host	Location	Collector and date collected
C. fimbriata	14799	CBS114723	*Ipomoea batatas*	North Carolina, USA	D. McNew, 1998
	42704		"	Malaysia	R. Rauf, 2014
	42705		"	Malaysia	R. Rauf, 2014
C. manginecans	46461	CBS143454	*Acacia mangium*	Sabah, Malaysia	R. Rauf, 2014
	48940		"	Malaysia	R. Rauf, 2014

[a] Fungal culture collection at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.
[b] Culture Collection (CBS) of Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

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