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First report of *Fusarium boothii* from pecan (*Carya illinoinensis*) and camel thorn (*Vachellia erioloba*) trees in South Africa

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ABSTRACT

Fusarium boothii forms part of the *Fusarium graminearum* species complex (FGSC), the important grain pathogen group that causes Gibberella ear rot of maize and Fusarium head blight of wheat. It is known to infect many grain crops such as maize, wheat and barley. Moreover, this pathogen is a 15-ADON mycotoxin producer and thus of concern for stored grains and grain products. During endophyte isolations in the Hoopstad area of the two unrelated trees, pecan and camel thorn, isolates of the FGSC constituted a dominant part of the *Fusarium* isolates obtained. Pecan (*Carya illinoinensis*) is a rapidly developing industry in the semi-arid to arid regions of South Africa, while camel thorn (*Vachellia erioloba*) is a dominant native tree in the same areas. DNA sequence comparisons of the translation elongation factor 1-α and β-tubulin gene regions clearly showed that these isolates were *F. boothii*. This study thus represents the first report of this grain pathogen from unlikely tree hosts, and only the second report of a species in the FGSC from trees, the other being that of *Fusarium acacia-mearnsii*. The unexpected occurrence of *F. boothii* in hosts other than grain could represent an important gap in our understanding of the epidemiology, geographical occurrence and movement, and genetic pool of this important pathogen. It also raises the question of whether other species in the FGSC could have unexpected host associations.

1. Introduction

Fusarium head blight (FHB) is a disease of wheat (*Triticum aestivum* L.) where florets or entire spikes of the wheat plant are infected, giving them a bleached appearance or discolouration at the base of the head (Goswami and Kistler, 2004). Individual kernels may become shriveled and white (Goswami and Kistler, 2004). This disease already had several serious outbreaks globally, and causes yield loss and low test weights, low seed germination and contamination of grain with mycotoxins (Goswami and Kistler, 2004). It is a major research focus for the international wheat industry, and also affects barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) (Goswami and Kistler, 2004). It is caused by several spp. of *Fusarium* such as *F. avenaceum* (Fr.) Sacc., *F. poae* (Peck) Wollenw. and *F. sporotrichioides* Sherb., with *F. graminearum* Schwabe *s. l.*, *F. culmorum* (W.G. Sm.) Sacc. and *F. cerealis* (Cooke) Sacc. (= *F. crookwellense* L.W. Burgess, P.E. Nelson & Toussoun) the most prominent pathogens (Kotowicz et al., 2014). However, the past couple of years showed a major increase in dominance of *F. graminearum s.l.* (Goswami and Kistler, 2004).

On maize, *F. graminearum s.l.* causes a disease known as Gibberella ear rot (GER) (Munkfold, 2003; Sampietro et al., 2010, 2012; Boutigny et al., 2012). This is due to the occurrence of the sexual state of *F. graminearum*, namely, *G. zeae*, on the symptoms (Munkfold, 2003; Boutigny et al., 2012). The fungus colonizes the cob from the tip and rot progresses downward (Munkfold, 2003). Similarly to FHB, various mycotoxins are produced in the cob that are dangerous to those consuming the cobs or derived food.

The *F. graminearum* species complex (FGSC) consists of 15 described species contributing to FHB and GER in various parts of the world (Aoki et al., 2012). Of these, *F. graminearum s. str.* is the species most commonly associated with disease (Goswami and Kistler, 2004). Although morphologically indistinguishable, the species can be separated by twelve phylogenetic markers (Aoki et al., 2012). Some species such as *F. graminearum s. str.* occur globally, while others have only been reported from single countries such as *F. aethiopicum* O'Donnell, Aberra, Kistler & T. Aoki (Aoki et al., 2012). The various species also have certain unique chemotypes whereby they have the genetic potential to produce different combinations of mycotoxins (Aoki et al., 2012).

A number of studies have started to elucidate the distribution of members of the FGSC throughout South Africa on grain crops such as wheat, barley and maize (Lamprecht et al., 2011; Boutigny et al., 2011a, 2011b, 2012). These have shown that only six species (*F. graminearum s. str.*, *F. meridionale* T. Aoki, Kistler, Geiser & O'Donnell,

Abbreviations: 15-ADON, 15-acetyl-deoxynivalenol; FCSC, *Fusarium chlamydosporum* species complex; FGSC, *Fusarium graminearum* species complex; FHB, Fusarium head blight; FOSC, *Fusarium oxysporum* species complex; GER, Gibberella ear rot; PDA, potato dextrose agar; TEF, translation elongation factor 1-α.

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F. acaciae-mearnsii O'Donnell, T. Aoki, Kistler & Geiser, *F. brasilicum* T. Aoki, Kistler, Geiser & O'Donnell, *F. boothii* O'Donnell, T. Aoki, Kistler & Geiser and *F. cortaderiae* O'Donnell, T. Aoki, Kistler & Geiser) occur in South Africa. Rudimentary geographical patterns of these species may exist, and a level of host preference to certain crops has been observed. Of these, *F. acaciae-mearnsii* is the only species that occurs on a different type of host other than cereals, namely, wattle (*Acacia mearnsii* De Wild.) and eucalypt (*Eucalyptus grandis* W. Hill ex Maiden) trees (Aoki et al., 2012).

In the past surrounding native vegetation, or naturalized or invasive non-native plants, have been shown to play important roles in the epidemiology of diseases, where these are often alternative hosts where the pathogens can survive, increase in numbers in the absence of the known host plants or generate additional genetic diversity. Absence of such data impacts on our understanding of the current geographical distribution and possible movements of pathogens, and their potential to become genetically more diverse on these hosts. This could negatively affect disease management programmes and mycotoxin risk assessments.

Northwestern areas of South Africa range from arid to semi-arid. These areas are planted with agricultural crops such as maize, wheat, sunflowers, potatoes and groundnut. Few tree crops are grown in these areas due to lack of water, with pecan nuts [*Carya illinoinensis* (Wangenh.) K. Koch] being one of the few. Pecan is rapidly becoming a large industry in South Africa (Erasmus, 2011). Not many serious diseases are as yet published from this crop. Vegetation surrounding crops in the North-western areas of South Africa comprise of grassland and predominantly *Vachellia* and *Senegalia* tree species, which was known as *Acacia* in the past (Kyalangalilwa et al., 2013). Of these, *Vachellia erioloba* (E. Mey.) P.J.H. Hurter (camel thorn), previously named *Acacia erioloba* E. Mey. (Kyalangalilwa et al., 2013), is the dominant form.

A pilot survey was done in the Hoopstad area of South Africa (Free State Province) to investigate the co-infection of fungal endophytes and latent pathogens between non-native pecan trees and native *V. erioloba*. Endophytes are organisms living asymptomatically inside tree tissues, while latent pathogens are plant pathogens that have an endophytic phase (Slippers and Wingfield, 2007). The presence of pathogens known to infect the crops in the area was also investigated in these unrelated trees. A number of *Fusarium* isolates were obtained from these trees during the survey. *Fusarium* are well-known fungi that include devastating plant pathogens of various crops, mycotoxin producers and also pathogens of humans (Leslie and Summerell, 2006). What was of special interest was a number of isolates morphologically resembling species in the *F. graminearum* species complex (FGSC). The aim of this study was to determine which species in the complex these isolates represent and if they include pathogens known from South Africa. Possible disease reactions on pecan leaves were also assessed in bioassays.

2. Material and methods

2.1. Collection of samples

Ten trees of *V. erioloba* and pecan, respectively, were sampled at two sites about 500 m apart in the Hoopstad area, Free State. Ten branches were randomly selected from each tree, and ten leaves were cut from each branch. Ten pieces per leaf and per branch (c. 4 mm diam.) were plated onto 2% potato dextrose agar (Biolab, Merck Millipore, South Africa) after surface sterilization (1 min wash with sterile water, 5 min submersion in 3% sodium hypochloride followed by 5 min in 70% ethanol, final rinse in sterile water). Resultant colonies were purified onto PDA plates and identified morphologically. Isolates resembling *Fusarium* spp. were single spored and maintained on PDA. Representative isolates were deposited in the National Collection of Fungi, Agricultural Research Council, Pretoria, South Africa.

2.2. Identification with DNA sequence comparisons

DNA was extracted from scraped mycelium of six representative and morphologically distinct seven-day-old cultures (Fig. 1) using the method developed by Möller et al. (1992). PCR amplicons and sequences of the translation elongation factor 1-α and β-tubulin genes were obtained following the protocols of O'Donnell et al. (2000, 2004) using the Robust PCR kit (KAPA Biosystems). Amplification products were visualized on 1% agarose gels (Cleaver Scientific, AEC-Amersham, South Africa) containing Gelred DNA stain (Biotium, Anatech, South Africa) under UV illumination using a Geldoc XR + imaging system (Bio-Rad, South Africa).

Up to 20 ng/μl of PCR amplicons, purified using the EXO/SAP Amplicon Purification system (Werle et al., 1994), were used for sequencing reactions with the BigDye Terminator v3.1 cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified with EDTA/ethanol precipitation and run on an ABI 3130XL genetic analyzer (Applied Biosystems). Chromatograms were compiled into contigs with Geneious v. 7.0.6 (Biomatters, New Zealand) and the DNA sequences were submitted to Genbank (TEF 1-α: KU325471, KU325473–KU325474, KU325476, KU325479, KU325482; β-tubulin genes: KU325458, KU325461–KU325462, KU325465, KU325467, KU325469). Generated sequences were added to datasets of the TEF1-α and β-tubulin genes for the FGSC in Mega 6.06 (Tamura et al., 2013) obtained from Dr. Kerry O'Donnell (United States Department of Agriculture). Maximum likelihood analyses with 1000 bootstrap replicates were done on the datasets with MEGA 6.06 using appropriate models also obtained with MEGA. Initial analyses were done separately on the TEF1-α and β-tubulin datasets, but the datasets were combined since both gave congruent phylogenetic trees and were proven previously to be combinable (O'Donnell et al., 2000, 2004).

2.3. Bioassays

Two repeats of fresh leaf bioassays (Keith and Zee, 2010) with five isolates (Fig. 2) in the FGSC were done on fresh, mature pecan leaves to test if the isolates had an ability to cause lesions on this host. Similar assays on *V. erioloba* leaves proved too difficult due to the small size of the leaf pinnules of the compound leaves. Agar blocks (4 mm diam) with mycelium were placed on 10 surface sterilized leaves for each isolate at wounds created by a sterile needle. Clean agar blocks were used for negative controls. Leaves were kept in moist chambers at room temperature and leaf lesions were scored after three weeks (1 = no lesion, 2 = small lesion, 3 = intermediate lesion, 4 = lesion covered leaf). The average scale for each isolate and the negative control were calculated and represented as a graph.

3. Results

3.1. Collection of samples

The number of *Fusarium* isolates (33) was small relative to the c. 2000 cultures of fungi isolated from the trees. Of the 18 representatives sequenced, 12 represented the FGSC, while the others were shown to be in the *F. chlamydosporum* species complex (FCSC) and *F. oxysporum* species complex (FOSC) (data not shown). The FGSC isolates originated from both pecan and *V. erioloba* (one isolate from *V. erioloba* and 11 from pecan for the FGSC), while two isolates from *V. erioloba* and one from pecan grouped in the FCSC and three from *V. erioloba* in the FOSC.

3.2. Identification with DNA sequence comparisons

NThe combined dataset including TEF1-α and β-tubulin DNA sequences consisted of 71 taxa and 991 characters. In the FGSC, the isolates grouped with *F. boothii* (bootstrap support 84%) based on TEF1-α