New Disease Reports

First report of *Colletotrichum gloeosporioides* species complex causing anthracnose on leaves of cutnut, *Barringtonia edulis*, in Papua New Guinea

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Cutnut, *Barringtonia edulis*, is one of six indigenous edible nut tree species found in Papua New Guinea (PNG), Solomon Islands and Vanuatu, where it has market potential both for domestic consumption and as an export commodity (Wah, 1996). Here we report an anthracnose-like foliar disease observed in January 2015 on 90% (19 out of 21) cutnut trees planted for landscaping at the PNG University of Technology, Lae. Foliar symptoms gradually progressed from an angular, asymmetrical yellow discoloration to an ulcer-like necrosis causing death to the whole leaf (Fig. 1).

Portions of necrotic leaf tissues (1 cm^2) were excised and surface-sterilised with 5g/l NaOCl for five minutes, rinsed three times with sterilised water and plated onto fresh potato dextrose agar (PDA) then incubated at 25°C in the dark. A pure culture was obtained using a modified monoconidial technique by slightly agitating seven-day-old cultures over fresh PDA to release the spores under laminar airflow conditions; the resulting spore-inoculated cultures were then incubated in the dark at 25°C. After 24-48 hours, germinating spores were identified under a dissecting light microscope and transferred onto PDA using a sterile glass needle. Cultural and morphological descriptions were done after 14 days incubation at 25°C in the dark. This methodology is as described by Phoulivong *et al.* (2012). Colony pigmentations observed on PDA ranged from grey to white with slightly raised aerial mycelium to a dense cottony mycelium. Cultures exhibited salmon to bright orange acervuli arranged in concentric rings (Fig. 2), with aging the acervuli become evenly distributed towards the edges of the culture (Fig. 3). The conidial shape was straight and cylindrical with the average conidia length and width ranging from 12-15.7 and 4-5.3 µm respectively, fitting published descriptions (Photita *et al*., 2005; Weir *et al*., 2012) (Fig. 4). DNA-based PCR amplification using the forward species-specific primer, 5′-GGGGAAGCCTCTCGCGG-3′ (Mills *et al*., 1992), and universal ITS4 (reverse) primer targeting the ribosomal DNA of *Colletotrichum gloeosporioides* produced a 450 bp amplicon. A BLAST search found the amplicon sequence (GenBank Accession No. KT601169) to have 99% identity to several *C. gloeosporioides* accessions (Table 1).

Koch's postulates were fulfilled using 10 detached fifth-largest, fully-opened *B. edulis* leaves from healthy plants raised in a greenhouse which were inoculated with 0.5 ml of a 10^5 spores/ml spore suspension and incubated at 25°C in the dark. The fungus was re-isolated from anthracnose-like lesions formed on these leaves, and the identity as *C. gloeosporioides* species complex was confirmed by PCR using the same primers.

Anthracnose is mainly caused by the *C. gloeosporioides* species complex (Weir *et al*., 2012). It is reported to affect a wide range of commercially important crops (e.g. Than *et al*., 2008). To our best knowledge, this is the first report of the *C. gloeosporioides* species complex causing foliar anthracnose on cutnut, *B. edulis*, in PNG and in the world. As such, it is imperative to identify the causal pathogen(s) of the complex to assess the potential for yield loss in commercial nut production, and to evaluate the implications for the management of the disease in other crop species.

Acknowledgements

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Figure 1

Figure 2

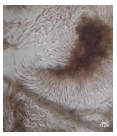

Figure 3

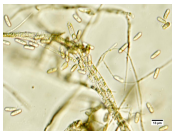

Figure 4

Table 1. Similar sequences obtained from the GenBank database (accessed in November 2015) using a BLAST search with *Colletotrichum gloeosporioides* isolated from *Barringtonia edulis*.

Species, strain/isolate	Host	Country	GenBank Accession No.	Similarity (%)
C. gloeosporioides, strain BWH1	*Hylocereus undatus*	China	KF051997	99
C. gloeosporioides, strain HCGPL2	*Jatropha curcas*	Mexico	AB859758	99
C. gloeosporioides, strain RP262	*Rhodomyrtus tomentosa*	Thailand	JF441203	99
C. gloeosporioides, isolate Cm 88	Unspecified	India	EF025941	99
C. gloeosporioides, strain TAX-1	*Eriobotrya japonica*	Pakistan	KR075160	99
C. gloeosporioides, isolate KPP15	Rubber	Sri Lanka	KT582188	99

Figure 5

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