CSIRO PUBLISHING

www.publish.csiro.au/journals/apdn *Australasian Plant Disease Notes*, 2010, **5**, 48–50

First record of *Neoscytalidium dimidiatum* and *N. novaehollandiae* on *Mangifera indica* and *N. dimidiatum* on *Ficus carica* in Australia

J. D. Ray[A,D], *T. Burgess*[B] *and V. M. Lanoiselet*[C]

[A]Australian Quarantine and Inspection Service, NAQS/OSP, 1 Pederson Road, Marrara, NT 0812, Australia.
[B]School of Biological Sciences and Biotechnology, Murdoch University, Murdoch, WA 6150, Australia.
[C]Department of Agriculture and Food, Baron-Hay Court, South Perth, WA 6151, Australia.
[D]Corresponding author. Email: jane.ray@aqis.gov.au

Abstract. *Neoscytalidium dimidiatum* is reported for the first time in Australia associated with dieback of mango and common fig. *Neoscytalidium novaehollandiae* is reported for the first time associated with dieback of mango.

Neoscytalidium dimidiatum has a wide geographical and host range. For example, it has been reported on *Albizia lebbeck*, *Delonix regia*, *Ficus carica*, *Ficus* spp., *Peltophorum petrocarpum* and *Thespesia populena* in Oman (Elshafie and Ba-Omar 2001); on *Arbutus*, *Castanea*, *Citrus*, *Ficus*, *Juglans*, *Musa*, *Populus*, *Prunus*, *Rhus*, *Sequoiadendron* in the USA (Farr *et al*. 1989); and on *Mangifera indica* in Niger (Reckhaus 1987). Stress factors such as water stress enhance the severity of disease caused by this fungus and symptoms include branch wilt, dieback, canker, gummosis and tree death (Punithalingam and Waterson 1970; Reckhaus 1987; Elshafie and Ba-Omar 2001). *Neoscytalidium novaehollandiae* was recently described from north-western Australia as an endophyte of *Adansonia gibbosa*, *Acacia synchronica*, *Crotalaria medicaginea* and *Grevillia agrifolia* (Pavlic *et al*. 2008).

A joint Plant Health Survey was carried out by the Australian Quarantine and Inspection Service (AQIS) and the Department of Agriculture and Food Western Australia (DAFWA) in the Ord River Irrigation Area (ORIA) of Western Australia (WA) during August 2008. During this survey a trial planting of >50 common fig shrubs (*Ficus carica*) and several blocks of commercial mango trees (*Mangifera indica*) attracted the attention of the plant pathologist. The fig plants were showing symptoms of dieback, root rot, canker and tree decline, whereas the mango trees were showing symptoms of dieback and canker only (Fig. 1). Several fungi were subsequently isolated from a representative symptomatic stem from each of nine different mango trees and the root and stem of a single representative symptomatic fig shrub. *Neoscytalidium* spp. were isolated from 78% (7/9) of mango trees exhibiting dieback symptoms during this survey. *Neoscytalidium* spp. were not detected in three asymptomatic mango stems. *Neoscytalidium* spp. was also isolated from the roots and stems of a single representative fig shrub exhibiting symptoms of dieback.

The *Neoscytalidium* spp. isolates were further identified as *Neoscytalidium dimidiatum* (Syn: *Fusicoccum dimidiatum*, *Torula dimidiata*, *Scytalidium dimidiatum*, *Hendersonula toruloidea*) (Crous *et al*. 2006) and *N. novaehollandiae* by sequencing of part of the internal transcribed spacer (ITS) region of the rDNA operon and part of the elongation factor 1α gene (*EF-1α*) as described by Burgess *et al*. (2005) followed by a phylogenetic analysis where sequences obtained in this study were compared with those of known isolates (Fig. 2). *Neoscytalidium dimidiatum* was isolated from fig and mango stems while *N. novaehollandiae* was only isolated from mango stems.

Fig. 1. Branch dieback symptoms observed on a *Mangifera indica* tree in the Ord River Irrigation Area, WA, Australia.

During subsequent AQIS Plant Health Surveys, *N. dimidiatum* and *N. novaehollandiae* were again isolated from symptomatic mango branches sampled at Derby, WA in September 2008.

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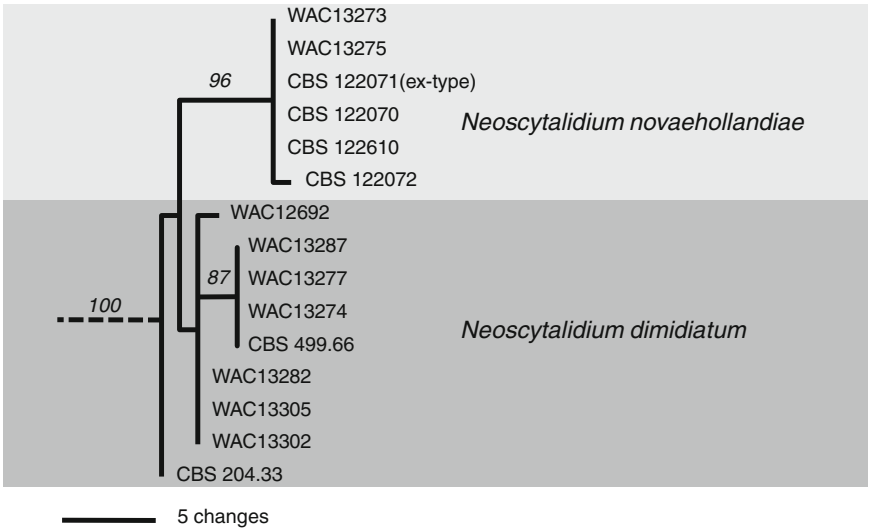

Fig. 2. One of two most parsimonious trees of 111 steps (CI = 0.98, RI = 0.99) obtained from analysis of the combined internal transcribed spacer (ITS) – elongation factor 1α gene (*EF-1α*) dataset. The tree was rooted to *Botryosphaeria dothidea* (not shown). The values above the line denote bootstrap support for the node. Isolates of *Neoscytalidium novaehollandiae* reside in a highly supported terminal clade separate from isolates of *N. dimidiatum.*

Neoscytalidium dimidiatum was also isolated from symptomatic mango branches sampled at Broome in September 2008 and Bathurst Island, Northern Territory in October 2008. All cultures have been deposited at the West Australian Culture Collection (Table 1).

Excised mango stems were inoculated to prove isolate pathogenicity and fulfil Koch's postulates. Field-collected mango stems were ~25 cm long, the ends were dipped in wax and assigned randomly to replicates. Eleven replicates of representative isolates of *N. novaehollandiae* and *N. dimidiatum* were under-bark inoculated. Under-bark inoculation was performed by using a sterile scalpel to cut a flap into the bark layer, an agar plug colonised by mycelia was placed under the flap; mycelium faced toward the stem. The control with 16 replicates was non-colonised agar. The inoculation site was wrapped with parafilm and replicates of inoculated stems were placed in plastic bags and incubated at ~22°C. After 14 days, lesions were measured. Both *N. novaehollandiae* caused lesions and *N. dimidiatum* caused lesions (27.4 ± 3.5 mm and 26.2 ± 5.2 mm, respectively) on the excised mango stems in comparison to the control (5.8 ± 3.2 mm). Koch's postulates were tested by re-isolation from the inoculated material.

Re-examination of isolates collected during previous surveys of the ORIA in 2005 from symptomatic mango trees revealed that *N. dimidiatum* has been present in the region for some time. These isolates were previously identified as *Fusicoccum dimidiatum*. Similarly, records from the Australian Plant Pest Database (APPD 2010) suggest that the pathogen may have been present in other parts of Australia for a long time. Indeed, the APPD records include a report of *Torula dimidiata* found on *Citrus aurantium* in 1914 and several reports of *Hendersonula* sp. from various hosts including *Mangifera indica* and *Citrus limon*. Molecular diagnostics are recommended to confirm the identity of these isolates from Queensland and other parts of Australia to conclusively determine if the pathogen is widespread in Australia.

The extreme climate of the north-west regions of WA and site stress factors at Bathurst Island, NT, may contribute to the susceptibility of common fig and mango to the dieback disease associated with *Neoscytalidium*. This is the first report of *N. novaehollandiae* associated with mango dieback. This is

Table 1. Isolates lodged in the Department of Agriculture Western Australia Plant Pathogen Collection, South Perth (WAC)

Culture no.	Host	Location	Collection date	Plant part	Identity	GeneBank accession
WAC13282	*Ficus carica*	Kununurra	08/2008	Stem	*Neoscytalidium dimidiatum*	ITS; GU172387 EF-1α; GU172419
WAC13302	*F. carica*	Kununurra	08/2008	Root	*N. dimidiatum*	ITS; GU172386 EF-1α; GU172418
WAC13287	*Mangifera indica*	Kununurra	08/2008	Stem	*N. dimidiatum*	ITS; GU172385 EF-1α; GU172417
WAC12692	*M. indica*	Kununurra	09/2005	Stem	*N. dimidiatum* (*F. dimidiatum*)	ITS; EF585538 EF-1α; EF585576
WAC13273	*M. indica*	Kununurra	08/2008	Stem	*N. novaehollandiae*	ITS; GU172397 EF-1α; GU172429
WAC13305	*M. indica*	Broome	09/2008	Stem	*N. dimidiatum*	ITS; GU172390 EF-1α; GU172422
WAC13277	*M. indica*	Derby	09/2008	Stem	*N. dimidiatum*	ITS; GU172388 EF-1α; GU172420
WAC13275	*M. indica*	Derby	09/2008	Stem	*N. novaehollandiae*	ITS; GU172400 EF-1α; GU172434
WAC13274	*M. indica*	Bathurst Island	10/2008	Stem	*N. dimidiatum*	ITS; GU172391 EF-1α; GU172423

also the first report of *N. dimidiatum* associated with dieback of mango and common fig in Australia.

Acknowledgements

We would like to thank Monique Sakalidis for assistance with pathogenicity testing at Murdoch University.

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Manuscript received 12 January 2010, accepted 10 May 2010