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Neofusicoccum luteum associated with leaf necrosis and fruit rot of olives in New South Wales, Australia

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Summary. *Neofusicoccum luteum* is reported for the first time from olives (*Olea europaea*), causing fruit rot and leaf necrosis. Affected fruits initially became brown with pycnidia developing on the surface, later drying out and becoming mummified. The fungus was shown to be pathogenic on both fruits and leaves. The association of Botryosphaeriaceae with rotting olive fruits in Mediterranean regions and in New South Wales, Australia indicates that these fungi play a significant role in fruit rots of olives and deserve greater attention.

Key words: Botryosphaeriaceae, drupe rot, *Olea europaea,* pathogen.

The European olive (*Olea europaea* L.) originates from the Mediterranean region and grows well in areas of Australia with a similar climate characterized by cool, wet winters and warm, dry summers. Interest in the planting of olive groves in Australia has increased significantly in recent years. This rapid expansion in all mainland states has led to disease problems not previously encountered. Diseases are often key constraints to economic production through their effects on both yield and quality. Olives are an important crop in Australia. In 2008 the estimated Australian production of olive oil was 12,000 tonnes, up from 2500 tonnes five years earlier in 2004 (Kennedy, 2009).

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In 2003–2008 a fruit rot was recorded on olives in New South Wales, Victoria, and Western Australia. The fruits showed a black rot that covered part or all of the fruit. Pycnidia developed on the surface, the fruits dried out and became mummified (Fig. 1). Necrosis of the leaves was also seen and this was mostly confined to the tips of the leaves (Fig. 1). Based on conidial morphology and ontogeny, in all cases a *Neofusicoccum* species was isolated. In 2008, following an unusually wet summer, the disease appeared again but at a far higher frequency than in previous years with up to 12% of the fruits affected.

Infected olive fruits and leaves cv. Manzanillo were collected from an olive orchard in Nemingha in the Tamworth area of northern New South Wales, Australia in March–May 2008. The orchard was sampled before harvest (March) and at harvest (April), and fruits remaining on the trees after har-

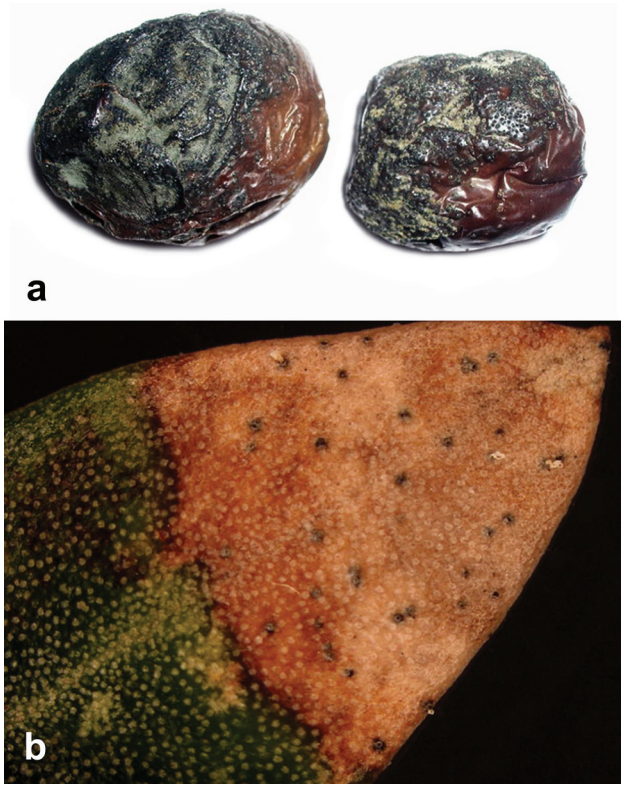

Fig. 1. Symptoms of *Neofusicoccum luteum* on olives. A, infected olive fruits covered with pycnidia. B, infected leaf with necrosis at the tip with pycnidia embedded in the leaf tissue.

vest were sampled in May. At each sampling date 50 fruits were collected from 30 trees and about 40% were infected. Thus, over the three sampling periods isolations were made from a total of 60 fruits. Isolations were made from infected fruits and leaves by transferring conidia, oozing from the pycnidia, to potato dextrose agar (PDA) and incubating overnight at 25°C. The following day single germinating conidia were transferred to fresh plates of PDA. A *Neofusicoccum* species was isolated from all the samples of leaves and fruits. Cultures on PDA were initially white, growing rapidly and forming a transient yellow pigment that diffused into the agar. Within 7–10 days the pigment became violaceous and ultimately was obscured by the mycelium, which became pale grey brown. Pycnidial conidiomata formed abundantly in cultures after 10 days, and after 14 days pycnidia were seen oozing from the ostioles or exuded in cirrhi. Conidiogenous cells (Fig. 2) were short lageniform, hyaline and thin-walled, forming a single conidium at the tip, proliferating internally to form periclinal thickenings and typical phialides (*sensu* Sutton, 1980). Conidia (Fig. 2) were hyaline, thin-walled, aseptate, ovoid, widest in the upper third, tip rounded, base truncate, (16–18–23.5(–24.5)×4.5–6(–7.5) μm. These characters placed the fungus in either *N. luteum* or *N. australe*. Cultures were deposited at the University of Western Sydney (UWS41, UWS43, UWS50).

DNA was extracted from 10 isolates following the method of Alves *et al*. (2004). Profiles generated with MSP-PCR primer GTG5 or ERIC-PCR primer (Alves *et al*., 2007) revealed identical patterns (Fig. 3) for all isolates tested. Since all isolates were thus considered to belong to the same species, three isolates were selected for amplification and sequencing of the ITS1-5.8S-ITS2 cluster following the method of Alves *et al*. (2004). Sequences (579 bp) were deposited in GenBank (FJ790237–FJ790239). A BLAST search in GenBank gave 100% similarity with *N. luteum*, including the ex-type strain of this species (PDDCC 8004 = CBS 562.92, ITS sequence AY259091). The isolates differed in 3 nucleotide positions from *N. australe*. These three differences are known to be fixed in *N. luteum* and *N. australe* (Slippers *et al*., 2004) and therefore it was concluded that the species associated with fruit rot of olives in northern New South Wales is *N. luteum*.

Pathogenicity was tested on 25 mature, symptomless fruits of olive cv. Manzanillo. The fruits were washed with sterile water containing Tween 20 (20 μL L^{-1}) and then surface-sterilized with 20% sodium hypochlorite for 2 min. The fruits were wounded by pricking with a sterile, 0.5 mm diam. needle and then inoculated by immersing in a suspension of conidia in water (2×10^5 conidia mL^{-1}). A further 25 fruits, wounded and treated with sterile water served as controls. All fruits were incubated at 25°C and 100% relative humidity. After 14 days of incubation, all the inoculated fruits showed a brown rotting with pycnidia developing on the surface, similar to the symptoms observed in the field. *Neofusicoccum luteum* was re-isolated from all inoculated fruits and identity confirmed from conidial morphology and ontogeny. No disease developed on the fruits treated with sterile water. Pathogenicity was also tested on 15 leaves of

the same olive variety. The leaves were washed and then surface-sterilized as for the fruits and inoculated by spraying with a conidial suspension (2×10^5 conidia mL^{-1}). Leaves sprayed with sterile water served as controls. After incubating at 25°C for 14 days, necrotic lesions covered with pycnidia developed at the tips of the leaves. No symptoms were seen on the control leaves. Therefore it was concluded that *N. luteum* was the cause of the fruit rot and leaf necrosis of olives in New South Wales, Australia.

Botryosphaeria dothidea is the most common Botryosphaeriaceae species associated with olive fruit rot in Italy (Lazzizera *et al*., 2008b), Greece (Phillips *et al*., 2005) and Spain (González *et al*., 2006). In the New South Wales Plant Pathology Herbarium there is a specimen of *B. dothidea* on olive fruits (DAR 61984), and another specimen (DAR 7885) labelled *B. parva* on olive leaves, also from New South Wales. However, in the absence of cultures it is not possible to verify the identities of these specimens. Other species in the Botryosphaeriaceae have been associated with olive fruit rots. Lazzizera *et al*. (2008b) reported *N. australe*, *N. vitifusiforme*, *N. parvum* and *N. mediterraneum* on olive fruits in southern Italy. Morphologically and in terms of ITS and translation elongation factor 1-α sequences, the isolates that Lazzizera *et al*. (2008b) studied were intermediate between *N. luteum* and *N. australe*. However, the isolates we studied from Australia are clearly *N. luteum*, and could be differentiated from *N. australe* and from the isolates studied by Lazzizera *et al*. (2008b) by the three fixed differences in ITS sequences. Lazzizera *et al*. (2008a) also found *Diplodia seriata* and *D. olivarum* associated with rotting olives in the same region of Italy. Although *Colletotrichum acutatum* is generally considered to be the main cause of olive fruit rot (Talinhas *et al*., 2005), the frequent occurrence of *B. dothidea* and various *Neofusicoccum* species on rotting olives in the Mediterranean region, and the present report of *N. luteum* in Australia suggests that more attention should be paid to the Botryosphaeriaceae as agents of olive fruit rot, possibly worldwide. While *Spilocaea oleagina* and *Pseudocercospora cladosporioides* are considered to be the main leaf pathogens of olives (Ávila *et al*., 2005, Spooner-Hart, 2005; Triki and Rhouma, 2008; Sergeeva *et al*., 2008), the present study shows that *N. luteum* can also cause leaf disease.

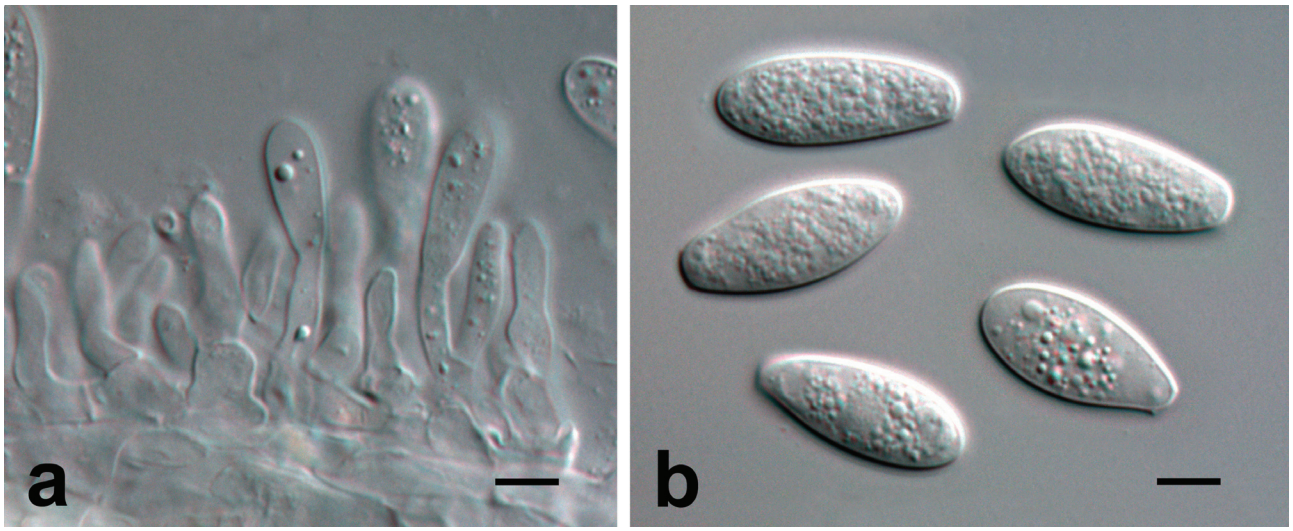

Fig. 2. *Neofusicoccum luteum* from culture. A, conidiogenous cells and developing conidia. B, ovate, hyaline conidia. Scale bars = 10 μm.

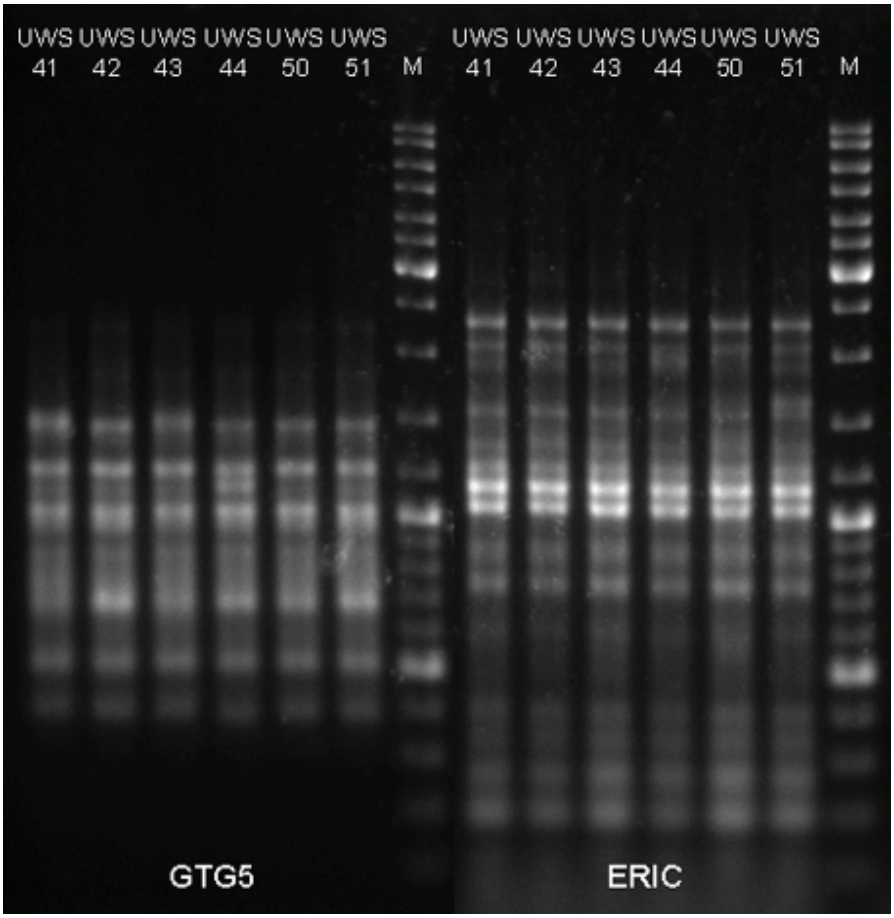

Fig. 3. MSP-PCR and ERIC-PCR fingerprints of six isolates of *Neofusicoccum luteum* isolated from infected olive fruits.

Acknowledgements

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