

# First report of *Botryosphaeria dothidea* causing shoot blight and cankers of pistachio in Australia

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**Abstract** Shoot blight and cankers are reported for the first time from pistachio in Australia from 20–26 year old trees of a plantation in the Riverina, South West NSW. Shoot blight symptoms during late summer prior to harvest included dark lesions on current-season shoots, followed by blackening and dieback of the same structures. Cankers were observed in older wood, showing wedge-shaped discolouration in cross-sections of these branches. Microscopic investigation provided evidence of black pycnidia, indicative of Botryosphaeriaceae, on the surface of old cankerous wood. Isolations from surface sterilised symptomatic young shoots and old wood yielded in Botryosphaeriaceae cultures, which were identified via conidia morphology and ITS sequencing. All isolates were identified as the pistachio pathogen *Botryosphaeria dothidea* responsible for panicle and shoot blight as well as basal cankers of pistachio in other parts of the world. Pathogenicity from six of our isolates was confirmed with in vitro pathogenicity tests. Re-inoculation of actively growing cultures from our isolates into healthy young pistachio wood resulted in dark internal lesions varying between 5 and 40 mm length, while control inoculated wood showed no lesions or internal discolouration.

**Keywords** *Pistacia vera* · ‘Bot’ canker · Panicle and shoot blight

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The pistachio nut (*Pistacia vera* L.) cultivation in Australia is a relatively new industry, with first commercial cropping in late 1980s. Currently the industry extends over 3 states along the Murray River in New South Wales (NSW), Victoria (VIC) and South Australia (SA) as well as individual orchards in Southern and central NSW, VIC and SA. The current total area of cultivation in Australia comprises 870 ha (2009 data) and has an annual production value of AU\$ 9 million (95% dry market and 5% fresh nuts). The production in Australia covers 40% of the countries consumption and currently does not supply any export markets.

The three major diseases reported from pistachio in Australia at current are pistachio anthracnose, a leaf spot and hull rot caused by *Colletotrichum acutatum* (Ash and Lanoiselet 2001a), late blight, affecting leaves and fruit caused by *Alternaria alternata* (Ash and Lanoiselet 2001b) and pistachio dieback caused by *Xanthomonas* bacterial pathogens (Facelli et al. 2002).

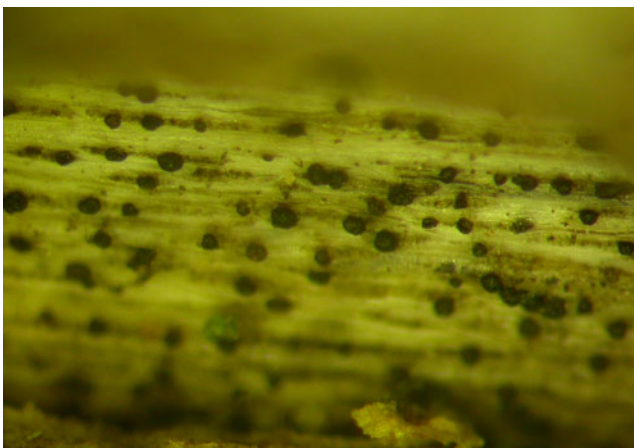
Visual observations during the growing season 2010/2011 of a decline in a pistachio orchard in the Riverina, south-western NSW, Australia, with 20–26 year old trees, showed initial dark lesions on current-season shoots, which later in the season blackened completely and died off (Fig. 1). In addition the older wood of affected trees showed cankers on stems and branches, which revealed discolouration of wood in cross-sections. Microscopic investigation of the bark on the older cankerous wood showed black pycnidia covering the wood surface (Fig. 2).

Samples of symptomatic current-season shoots and older wood were taken between harvest and pruning season and surface sterilised in 0.5% sodium hypochlorite for 2 min followed by three consecutive rinses with sterile distilled water for 1 min each, placed in potato dextrose agar (PDA; Oxoid Ltd., Basingstoke, Hampshire, England) and incubated at 25°C in the dark. Mycelium indicative of

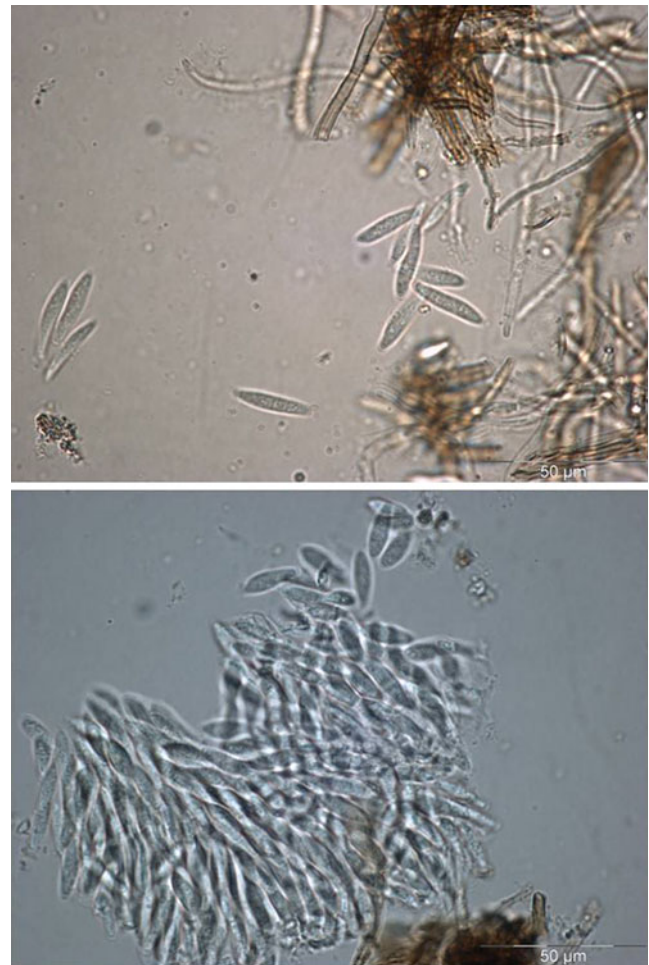


**Fig. 1** Darkening and dying of current-season twigs hosting *Botryosphaeria dothidea*

Botryosphaeriaceae growth was subcultured onto PDA after 3 days. Individual cultures were further incubated for 2 weeks and after blackening of the mycelium occurred the cultures were inspected for pycnidia inside and on the surface of the PDA. Microscopic investigation of crushed up pycnidia showed thin-walled conidia ranging from 21 to 25  $\mu\text{m}$  in length and 5–6  $\mu\text{m}$  width (Fig. 3). Conidia morphology matched the description of the anamorph *Fusicoccum aesculi*. To further confirm species and identify the holomorph, the ribosomal DNA internal transcribed spacer region of six single spore isolates was amplified and sequenced using ITS1 and ITS4 primers (White et al. 1990). Sequences were compared to sequences in GenBank using the Basic Local Alignment Search Tool. Based on the sequencing data combined with conidia morphology, the fungal cultures were identified as *Botryosphaeria dothidea*



**Fig. 2** Black pycnidia covering the bark surface of cankerous pistachio wood



**Fig. 3** Hyaline conidia from pycnidia of *Botryosphaeria dothidea* cultures

(Mough.: Fr.) Ces. & De Not.(anamorph: *Fusicoccum aesculi*).

The isolates in this study are stored at the Agricultural Scientific Collection Unit, Department of Primary Industries NSW, Orange, NSW, Australia (Herbarium code: DAR) and corresponding DNA sequences were deposited in GenBank. Accession numbers are listed in Table 1.

**Table 1** Herbarium and GenBank accession numbers for six isolates used in this study

Isolate	DAR accession number	GenBank accession number
D1	81463	JN561085
D2	81464	JN561086
D3	81465	N561087
D5	81468	JN561088
D7	81467	JN561089
D8	81466	JN561090

To confirm pathogenicity an experiment re-inoculating the six *B. dothidea* isolates (Table 1) into healthy looking ~10 mm in diameter and 80 mm long cuttings of pistachio wood, was conducted. Wood samples were surface sterilised with 70% ethanol and wounded by drilling a hole with a surface-sterilised 4 mm diameter drill bit into the pith in the middle of each piece of wood. Four mm mycelium plugs from actively growing 3 day old cultures of each of the six isolates were inserted into the holes in the pistachio wood. For each isolate there were five replicate wood pieces. All holes were covered with Parafilm and each piece of wood was placed into a Petri dish on moist filter paper. Control wood pieces were inoculated with sterile PDA plugs. Petri dishes were placed in a completely randomised design and incubated at 25°C in the dark. After 14 days the bark of each piece of wood was removed carefully with a sterile dissecting blade and lengths of visible lesions originating from the inoculation point were recorded. No lesions or discolouration in the cross sections of control inoculated wood were recorded. Lesion lengths for the wood inoculated with *B. dothidea* ranged from 5 mm to 40 mm.

To satisfy Koch's postulates small tissue samples were removed from the margin of healthy wood and lesion on each cane and plated out after surface sterilisation, as described above.

Fungi originating from these samples were identified to species level by their conidial morphology and were compliant with that of *B. dothidea*.

*B. dothidea* has been associated with pistachio panicle and shoot blight causing yield declines in California since 1991 (Michailides 1991) and this fungal species has also been identified as the cause of basal cankers on pistachio trees in South Africa (Swart and Blodgett 1998). In Australia the

association of *B. dothidea* with *P. vera* has been limited to a single isolate from a root canker, submitted to the Agricultural Scientific Collection Unit, Department of Primary Industries NSW, Orange, NSW, Australia in 1987 (DAR 59086).

To the best of our knowledge our work presents the first report of *B. dothidea* causing stem canker and shoot blight of pistachio in Australia.

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