First report of *Phoma multirostrata* in Australia

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Abstract Dark grey leaf lesions were observed on coriander (*Coriandrum sativum*) commercially grown at Wanneroo, Western Australia during November 2013. A species of *Phoma* was consistently isolated from leaf lesions. The pathogen was identified as *Phoma multirostrata* using morphological characteristics, DNA sequencing comparisons and pathogenicity testing. This is the first report of *Phoma multirostrata* causing leaf spot on coriander in Australia.

Keywords *Phoma* · Leaf lesion · *Coriandrum sativum*

The genus *Phoma* consists of a large number of endophytes, saprobes and plant pathogens, some species causing serious diseases on economically important plant species worldwide (Aveskamp et al. 2008). *Phoma multirostrata*, (P.N. Mathur, S.K. Menon & Thirum.) Dorenb & Boerema has been reported on a various hosts, including *Coriandrum sativum* (Hashmi and Ghaffar 1991), *Ajuga multiflora* (Lou and Hu 2008), *Dipteryx alata* (Anjos et al. 2009) and *Fuchsia × hybrida* (Garibaldi et al. 2010).

Coriander is usually grown for its fresh leaves; however production of the spice seed for export is also significant in Australia (Hooper and Dennis 2002; Foster and Bird 2009).

In Australia, *Pseudomonas syringae* pv. *coriandricola* and *Septoria* sp. have been reported causing leaf lesions on coriander (Hooper and Dennis 2002) but this is the first report of *P. multirostrata* on coriander.

Coriander samples presenting leaf lesions were randomly collected from a commercial property at Wanneroo, Western Australia during November 2013. The symptoms appeared as dark greyish irregular lesions that coalesced to form larger necrotic areas on the leaves (Fig. 1). The pycnidia of the fungus were black, embedded in the infected leaf tissues and developed on senescence lesions. Tissue was excised from the leading edge of lesions and surface-sterilised by immersion in a 1.25 % aqueous solution of sodium hypochlorite for 2 min, rinsed in sterile water and dried in a laminar flow cabinet. The pieces were then either (a) placed on potato dextrose agar (PDA) and incubated at 22±3 °C for 7 days, fungal colonies subsequently sub-cultured onto oatmeal agar (OA) and then single-spored to obtain pure cultures; or (b) to develop fungal morphological characteristics, placed in trays on moist filter paper and incubated at 25 °C with a 12-h dark and light cycle for two weeks. After 14 days the fungal colonies were dark-olivaceous and dark-black in reverse. Conidiomata were globose to subglobose, 165–350 µm in diam. Pycnidia were dark brown, solitary or congregated with inconspicuous neck (Fig. 2). Pycniospores were unicellular hyaline, guttulate, 4.5–7×1.5–2.5 µm (Fig. 3). Brown chlamydospores formed after three weeks on PDA. All cultural and morphological characteristics of the isolates were similar to those described for *P. multirostrata* (Boerema et al. 2004).

A culture of *P. multirostrata* was deposited in the Western Australia Plant Pathogen Collection (WAC13683).

Two representative isolates morphologically identified as *P. multirostrata* were grown on PDA for two weeks at 25 °C. DNA was extracted from fungal mycelium with the DNeasy Plant mini Kit (Qiagen, Melbourne, Vic., Australia) according to the manufacturer's instructions. The rDNA internal transcribed spacer (ITS) region was amplified with primers ITS1 and ITS4 (White et al. 1990), Actin gene (ACT) amplified with primers ACT-512 F and ACT-783R and the translation elongation factor 1-α (EF-1α) was amplified with primers

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Fig. 1 Leaf lesions on coriander (*Coriandrum sativum*) caused by *Phoma multirostrata*. Bars=5 mm

EF1-728 F and EF1-986R (Carbone and Kohn 1999). The relevant sequence information for a representative isolate was submitted to GenBank as Accession Numbers KM659039, KM891729 and KM891728 for the ITS, Actin and EF-1 α regions, respectively. BLAST results for the ITS, ACT, EF-1 α sequence indicated 100 and 99 % identity with nucleotide sequences for *P. multirostrata* (Accession No KJ767077, KJ426920, KJ427146, respectively).

A pathogenicity test was carried out on three weeks old coriander seedlings in a randomised trial with four replications inside a glasshouse. The two representative *P. multirostrata* isolates (21-d-old cultures) were used for inoculation and plants were separately sprayed to run-off with a conidial suspension (10^6 spores/mL) of each isolate. The control plants were sprayed with sterile distilled water. Inoculated plants were placed in a humidifier for 48 h then transferred to a growth-room at 22 °C and a 12-h dark and light cycle. Two weeks post inoculation disease symptoms were identical to those on naturally infected plants. Control plants remained

Fig. 2 *Phoma multirostrata*, pycnidia . Bars=40 μ m

Fig. 3 *Phoma multirostrata*, pycniospores. Bars=10 μ m

asymptomatic. Koch's postulates were fulfilled by re-isolating the pathogen from the symptomatic tissue. The identity of the pathogen was confirmed as *P. multirostrata* using morphological and DNA sequence analysis. . To the best of our knowledge, this is the first report of *P. multirostrata* causing leaf spot on coriander in Australia.

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