Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Phaeoacremonium amygdalinum* Associated with Almond Dieback and Wood Disease in Italy

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Plant Dis. 105:4166, 2021; published online as <https://doi.org/10.1094/PDIS-03-21-0565-PDN>. Accepted for publication 4 June 2021.

In the last 10 years, almond (*Prunus dulcis* [Mill.] Webb) cultivation areas in the region of Apulia (southern Italy) have increased. A recent survey in young almond orchards recorded characteristic disease symptoms including rapid collapse of branches, chlorosis of leaves (with sudden wilting and death) in June and July, and bud and shoot dieback in December to March. Internal wood symptoms on stems consisted of brown vascular streaking that led to death of young apical shoots within a few weeks. Wood samples were collected and taken to the laboratory, where they underwent disinfection according to the method of Fisher et al. (1992). Fungal isolation techniques were applied, with small pieces of wood tissue (0.5 to 1.0 cm) placed on potato dextrose agar (PDA; Oxoid Ltd.) supplemented with 300 mg/liter of streptomycin sulfate and incubated at $23 \pm 2^\circ\text{C}$ in the dark. After 10 days, based on morphological features carried out by microscopic studies, 85% ($n = 55$) of the resulting fungal colonies ($n = 65$) were preliminarily attributed to known fungal pathogens of wood, of which 12.4% ($n = 8$) belonged to the *Phaeoacremonium* genus. These last were purified by transferring single germinating conidia onto fresh PDA plates. In particular, the conidiophores were mostly short, usually unbranched, erect to flexuous, up to five-septate, brown to pale brown, verruculose on the lower part, (12 to) 15.7 to 41 (to 55) (average 29) μm long and 1.6 to 3.2 (average 2.1) μm wide ($n = 30$). Conidia hyaline, oblong ellipsoidal-obovoid or sub-cylindrical (3 to) 4 to 5 μm long and 1.5 to 3 (average = 4.4×2.1) μm wide ($n = 30$). All *Phaeoacremonium* isolates were successfully subjected to genomic DNA extraction, according to the method of Carlucci et al. (2013). Partial actin and β -tubulin genes were amplified with the

ACT-513F/ACT-783R (Carbone and Kohn 1999) and T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995) primers, respectively. Consensus sequences were compared with reference strains (ex-type) in the GenBank database, using the Basic Local Alignment Search Tool (BLAST). In particular, all isolates showed 99 to 100% similarity with reference strains of *Phaeoacremonium amygdalinum* (JN191301; JN191305). Actin and β -tubulin sequences of *P. amygdalinum* strain Pm10 were deposited in GenBank (MW716265; MW714562). In spring 2020, artificial inoculations with two isolates of *P. amygdalinum* (Pm10; Pm15) were carried out on 10 healthy 2-year-old almond seedlings (cv. 'Filippo Cea'). Agar plugs (diameter, 0.3 to 0.5 cm) taken from 10-day-old cultures grown on PDA at $23 \pm 2^\circ\text{C}$ were inserted into small wounds under the bark of the stems (length, 0.4 to 1.0 cm) made with a sterile scalpel. After inoculation, the wounds were wrapped with wet sterile cotton wool and sealed with Parafilm. Ten almond seedlings were used as controls, being inoculated with sterile agar plugs. The experiment was replicated three times. The inoculated young almond plants were grown in pots in a greenhouse without temperature control. After 150 days, the inoculated plants showed decline symptoms and internal longitudinal brown wood streaking (average length, 2.7 to 6.4 cm). *P. amygdalinum* was reisolated from symptomatic wood of 95% of the experimental almond seedlings, thus fulfilling Koch's postulates. No symptoms were observed on almond seedlings used as controls. *P. amygdalinum* was first described as a fungal pathogen of almond in Spain (Gramaje et al. 2012). To the best of our knowledge, this is the first report of *P. amygdalinum* associated with almond dieback disease in Italy.

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The author(s) declare no conflict of interest.

Keywords: almond, *Phaeoacremonium* species, dieback

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