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Page 687
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DISEASE NOTES

First Report of Damping off Caused by *Pythium aphanidermatum* on Bean (*Phaseolus vulgaris*) in Italy

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During the summer 2014, symptoms of crown and root rot were observed on bean (*Phaseolus vulgaris* L.) cultivar Billò grown in a commercial field near Cuneo (northern Italy). Forty-day-old plants were stunted with leaf chlorosis and developed symptoms of root rot and necrotic streaks on the crown area. About 20 to 25% of plants out of 30,000 suddenly collapsed at temperatures ranging from 22 to 28°C. Fifty tissue fragments were excised from roots and basal stems of 20 plants, dipped in a solution containing 1% sodium hypochlorite, rinsed in sterile water, and plated on potato dextrose agar and on a semiselective medium for oomycetes (Masago et al. 1977). Plates were incubated under constant fluorescent light at 22 ± 1°C for 3 days. Ten out of the 40 colonies with abundant aerial mycelium obtained from both media were recovered and then plated on V8 medium. Under light microscope, aseptate hyphae, 3.57 to 7.8 µm (mean 5.9 µm) wide were observed. Oogonia were globose, smooth, and measured 12.6 to 28.0 µm (mean 22.1 µm). Antheridia were barrel-shaped (8.2 to10.3 µm), and oospores were 14.8 to 22.1 µm (mean 19.3 µm) in diameter. These morphological characters identified the microorganism as a *Pythium* sp. (Spencer 2005). The internal transcribed spacer (ITS) region of rDNA of a single isolate (Py 13/14) was amplified using the primers ITS1/ITS4 and sequenced. BLAST analysis of the 777-bp segment showed a 100% similarity with the sequence of *Pythium aphanidermatum* (GenBank accession KY095191). The nucleotide sequence was deposited in GenBank under accession number MF040822. Pathogenicity tests were performed twice on bean cultivar Billò. Pots, containing 2 liters of steam-disinfested organic peat substrate, were infested with wheat and hemp kernels colonized with Py 13/14 strain of *P. aphanidermatum* at a rate of 2 g/liter. Five seeds/pot were sown 48 h after inoculation in six pots filled with the infested medium, and the same number of seeds was sown in uninfested substrate. Plants were kept in a greenhouse at 22 to 27°C under 12 h of photoperiod. Preemergence damping-off was observed in 37 to 43% of plants 10 days after the artificial inoculation. After 20 days, 47 to 60% of plants were infected, showing the same symptoms as previously described. Control plants remained healthy. *P. aphanidermatum* was consistently reisolated with 80% frequency from the lesions of the root

and crown of the plants. To our knowledge, this is the first report of the presence of *P. aphanidermatum* on bean in Italy. The same pathogen has also been reported on bean in Spain and in Oman (Al-Mahmooli et al. 2015; Serrano et al. 2008). Due to the economic importance of beans in Piedmont (Italy) in the Cuneo province, where about 5,000 ha are grown in open field generally in monoculture, the spread of the disease could cause serious damage in this area as well as elsewhere.

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