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DISEASE NOTES



Collar Rot and Crown Wilting by *Phytophthora pachypleura* on *Aucuba japonica* in Italian Nurseries

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A *Phytophthora* species was consistently associated with symptomatic collar tissue of *Aucuba japonica* during surveys carried out in Tuscan nurseries from April 2012 to the end of 2014. Plants from three different nurseries showed similar wilting and browning of crown portions and necroses at the collar level. Tissue samples were taken from active lesions on the cambium and plated in petri dishes on the selective PARPNH-V8-agar medium. Four *Phytophthora* isolates were obtained from five symptomatic individuals after two days of incubation at 23°C in the dark and monohyphal derivatives were transferred onto V8A (isolate SB131). Isolates had optimum growth at 25°C (8.2 mm/d), whereas growth ceased at 35°C. All isolates were homothallic with paragynous antheridia, smooth-walled spherical oogonia ($30.1 \pm 2.7 \mu\text{m}$; overall range 25.2 to 34.9 μm) and mostly aplerotic (86%) oospores ($26.5 \pm 1.9 \mu\text{m}$; overall range 23.1 to 30.6 μm) characterized by a very thick wall ($2.81 \pm 0.36 \mu\text{m}$). In non-sterile, filtered pond water, isolates produced semipapillate, non-caducous sporangia averaging $60.8 \pm 7 \times 33.1 \pm 3.9 \mu\text{m}$ (length:width ratio 1.86 ± 0.27). Most sporangia were ovoid in shape, but obpyriform, limoniform, ellipsoid, or distorted sporangia were also observed. Colony appearance, surface topography and texture, and micromorphological traits like thickness of the oospore wall, high presence of aplerotic oospores, and general measurements of reproductive structures suggested that all isolates belong to a single taxon in the *P. citricola* complex. To confirm this assignment, DNA was

extracted from a pure culture of the isolate SB131 using the Plant Genomic DNA Miniprep Kit (Sigma-Aldrich), and the Internal Transcribed Spacer (ITS) region of the rDNA was amplified using primers ITS6-ITS4 and sequenced (GenBank Accession No. KJ417664). A BLAST search in the GenBank database revealed complete homology (100% identity) with a sequence of *Phytophthora pachypleura* (ID KC855321) isolated from the same host *A. japonica* (Henricot et al. 2014). Molecular-based identification thus strongly supported conventional identification. To test the pathogenicity, 12 leaves of *A. japonica* and 12 twigs each of *A. japonica* and *Fagus sylvatica* were inoculated with all isolates. Similar-sized rounded lesions developed on the leaves, averaging $3.9 \pm 1.4 \times 3.6 \pm 1.3$ cm at 5 days of incubation in the dark. The oomycete also produced lesions of 11.2 ± 1.9 cm after 11 d on *A. japonica* twigs and of 10.3 ± 1.3 cm on *F. sylvatica* twigs after 3 weeks of incubation in the dark (*data not shown*). Reisolations on PARPNH-V8 agar confirmed *P. pachypleura* to be the etiologic agent. *P. pachypleura* has been recently reported from *A. japonica* in the UK. Isolate SB131 was found in a nursery in Tuscany, in the Pistoia district, from a batch of the same host species imported from France. These close events confirm that the international trade in plant material poses a serious biosecurity threat. In the globalization era, the nursery trade has a main responsibility in the spread of pathogenic microorganisms over the territory. The Pistoia district is one of the most important nursery districts of Europe, with plants that are sold all over the world. Plant pathologists have a primary role in the early identification of pathogens and preventing their transnational spread.

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