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First Report of Phytophthora acerina, P. plurivora, and P. pseudocryptogea Associated with Declining Common Alder Trees in Italy

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Since the early 1990s, common alder (*Alnus glutinosa*) trees showing a variety of symptoms such as a partial or complete canopy dieback, reddening of foliage, and bleeding cankers at the collar and lower stem were observed in several torrential mountain streams in Sardinia (Italy). In order to clarify the etiology of the symptoms observed, three riparian alder stands along three streams in northern Sardinia were surveyed in the spring of 2017. In each stand a 100-m-long transect was established, and 10 symptomatic alder trees per transect were sampled for *Phytophthora* species. *Phytophthora* isolations from 30 rhizosphere samples (300 g of soil and roots) collected around the selected alder trees and 30 inner bark samples taken from the margin of active lesions at the collar region of the same trees were performed as described by Linaldeddu et al. (2020). Disease incidence among transects ranged from 81 to 97%. Based on colony growth patterns on carrot agar (CA), morphological features of sporangia and sequence analysis of the internal transcribed spacer region (ITS1-5.8S-ITS2) of rDNA, the 40 *Phytophthora* isolates obtained were identified as *Phytophthora acerina* (seven isolates with ovoid sporangia measuring 48.9 × 33.1 µm), *P. plurivora* (24 isolates with ovoid sporangia measuring 50.1 × 32.9 µm), and *P. pseudocryptogea* (nine isolates with ellipsoid sporangia measuring 44.8 × 26.9 µm). For all *Phytophthora* species, BLAST searches in GenBank showed 100% identity with reference sequences of representative isolates including

those of ex-type cultures (JX951285, FJ665225, and KP288376). The ITS sequence of a representative isolate of each species was deposited in GenBank (*P. acerina* MN589653, *P. plurivora* MN589655, and *P. pseudocryptogea* MN589656). The representative isolates were stored at 10°C under water at the Culture Collection of the University of Padova. The pathogenicity of the representative isolate of each *Phytophthora* species was evaluated by inoculating five 3-year-old common alder seedlings per isolate. For each seedling a plastic beaker was positioned and sealed at the base of the stem, filled with 130 ml of pond water, and inoculated with 10 agar-mycelium plugs (10 mm) cut from the margin of a 5-day-old CA colony. The water of five control plants was inoculated with sterile CA plugs. Plants were kept in a laboratory at 25°C and watered regularly for 4 weeks. At the end of the experiment, all inoculated plants were symptomatic and displayed reddened to browned leaves and dark brown lesions on the inner bark. The three *Phytophthora* species were successfully reisolated from symptomatic inner bark tissues of the stem of all plants, fulfilling Koch's postulates. No disease symptoms were detected on control seedlings, and no *Phytophthora* species were isolated. The pathogenicity test was conducted twice. *P. plurivora* was the dominant species and the only species obtained from stem cankers and rhizosphere samples in all sites. This species is regarded as native to Europe and known as an aggressive pathogen of *A. glutinosa* (Aday Kaya et al. 2018; Haque et al. 2014). *P. acerina* and *P. pseudocryptogea* were obtained from both bleeding cankers and rhizosphere samples in two sites. All three *Phytophthora* species are reported for the first time to be associated with declining common alder trees in Italy, and for the first time the pathogenicity of *P. acerina* and *P. pseudocryptogea* has been demonstrated on *A. glutinosa*. The widespread occurrence and virulence of these *Phytophthora* species represents a serious threat to riparian alder ecosystems in Sardinia.

The author(s) declare no conflict of interest.

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