Diseases Caused by Oomycetes

First Report of *Phytophthora chlamydospora* Causing Postharvest Fruit Rot on Apples and Pears in the Netherlands

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Apple (*Malus domestica*) and pear (*Pyrus communis*) are important fruit crops in the Netherlands, with total production of 269,000 and 402,000 tons in 2018, respectively. In 2018 and 2019, postharvest fruit rots were observed on the apple variety Elstar (one observation) and pear varieties Conference and Doyenné du Comice (multiple observations). The symptoms were found after storage in controlled-atmosphere storage facilities on fruits from different orchards across the Netherlands. Disease incidences up to 50% of the stored fruit were observed. The diseased fruits showed circular brown to black spots with irregular and diffuse margins that enlarged rapidly to form distinctive rings, typical of *Phytophthora* infection. Several *Phytophthora* species are currently known to cause fruit rot of pome fruit (Sanchez et al. 2019). To isolate the causal agent, small portions of fruit flesh from decayed fruit were excised from the lesion margin and placed on potato dextrose agar (PDA). The plates were incubated at 20°C in the dark, and pure cultures were obtained by transferring hyphal tips on PDA. The colonies were white with petaloid and rosette-shaped patterns. The isolates grown on PDA formed irregularly branched hyphae, produced persistent nonpapillate sporangia, usually on unbranched sporangiophores, and chlamydospores were produced. The characteristics were similar to those described for *Phytophthora chlamydospora* Brasier and Hansen sp. nov. (Hansen et al. 2015). The identity of three representative isolates (KP00219, WURR121, and WURR119) from two different pear cultivars (Conference and Doyenné du Comice) and one

apple cultivar (Elstar), respectively, was confirmed by means of multilocus gene sequencing. Genomic DNA was extracted using the LGC Mag Plant Kit (Berlin, Germany) in combination with the Kingfisher method (Waltham, MA). Sequences of ITS region, COX, and EF were amplified and sequenced. The sequences have been deposited in GenBank (accession nos. MT125889, MT125891, and MT125890 [ITS]; MT153610, MT153612, and MT153611 [COX]; MT153613, MT153615, and MT153614 [EF]). MegaBLAST analysis revealed that our ITS, COX, and EF sequences matched with 100% identity to *P. chlamydospora* isolates in GenBank: AF541901 and AF541902 (ITS), JF771548 and JF771549 (COX), and JN936005 and JN936006 (EF). In order to perform Koch's postulates, a pathogenicity assay was performed using mycelial plugs of the cultures: KP00219 on pear cultivar Conference, and WURR119 and WURR121 on apple cultivar Elstar and pear cultivar Doyenné du Comice. Ten apples and pears per cultivar were disinfected and wounded using a sterile cork borer in the middle of the fruit surface area. A mycelial plug of a 2-week-old fungal culture was then placed onto the fruit. Placement of a PDA plug without fungal growth was used as a control. The fruits were incubated at 18°C at high relative humidity for 7 days. Symptoms appeared within 3 days on all fruits. Mock-inoculated controls remained symptomless. The fungus was reisolated and confirmed as *P. chlamydospora* by morphology and sequencing. *P. chlamydospora* is found in streams and wet soil worldwide and has only rarely been recovered as a pathogen from ornamental and woody species (Blomquist et al. 2012; Ginetti et al. 2014; Türkölmez et al. 2016). To our knowledge, this is the first report confirming *P. chlamydospora* as a causal agent of fruit rot of commercially produced apple and pear cultivars in the Netherlands.

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