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First Report of *Phytopythium vexans* causing decline syndrome of *Actinidia deliciosa* 'Hayward' in Italy

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Italy is the second worldwide producer of kiwifruit (*Actinidia deliciosa* C.F. Liang & A.R. Ferguson) with a production of approximately 571,020 tons/year and 26,650 ha of cultivated area. A new disease characterized by decline and root rot has been reported in Italy since 2012 and currently affects 12% (3,160 ha) of Italian kiwifruit production area (Sorrenti et al. 2019). During 2016 and 2018, 18 orchards were monitored in Piedmont (NW Italy), and 100% sampled trees showed typical symptoms of the disease with diffuse root rot, reduction of plant vigor, leaf curling, and sudden decline. Experimental trials were set up in Friuli Venezia Giulia (NE Italy), to reproduce the disease in controlled environment applying waterlogging conditions to kiwifruit plants grown on soil (sterilized and unsterilized) collected from diseased orchards. Rotting and decline appeared in 90% of the plants when flooding conditions on unsterilized soil were used, whereas decline was not observed on plants grown on flooded sterilized soil (Savian et al. submitted for publication).

Isolations were carried out by cutting pieces of symptomatic roots. Tissue fragments were surface-disinfected with 1% sodium hypochlorite for 30 s and rinsed in sterile water. Five fragments of each root were cut and plated onto corn meal agar (CMA) supplemented with pimaricin, ampicillin, rifampicin and pentachloronitrobenzene. Representative isolates were transferred onto V8 agar and morphological observations were performed according to de Cock et al. (2015). After 3 days, colonies showed typical mycelia of a *Pythium* species. Older cultures showed subglobose non-papillate sporangia (11.25 to 18.47 µm), bell-shaped antheridia, smooth oogonia and spherical zoospores typical of *Phytopythium vexans* (de Bary A.) (de Cock et al. 2015). Species identification was confirmed by sequencing rDNA internal transcribed spacer (ITS) using primers ITS1/ITS4 (White et al. 1990), the large subunit (LSU) rDNA using primers NL1/NL4 (Baten et al. 2014) and cytochrome oxidase I (COI) regions using FM85mod/OomCOILevup primers (Robideau et al. 2011). Two sequences per region were deposited in GenBank (Accession N° MN510425, MN510426, MN510427, MN510428, MN510423 and MN510424) and were BLAST-searched in GenBank, obtaining 99 to 100% homology with strains of *P. vexans* (Accession N° AY598713, HQ665090 and GU133476).

Pathogenicity was tested on 1-year-old *Actinidia deliciosa* 'Hayward' potted plants and the different isolates of *P. vexans*, grown on wheat and hemp for 7 days, were inoculated into the soil at a rate of 6 g/liter. Plants were kept in a greenhouse at 32±3°C. To mimic waterlogging conditions that were found necessary for symptom induction, three rounds of flooding and drainage were applied according to the protocol devised by Savian et al. (submitted for publication). Similar symptoms to those observed in the field occurred after 14 to 24 days in all the plants, depending on the isolate, while controls remained symptomless. To fulfil Koch's postulates, re-isolations were performed from symptomatic plants and the pathogen was molecularly identified as *P. vexans*. *P. vexans* was first described on kiwifruit by Polat et al. (2017) in Turkey. To the best of our knowledge, this is the first report of *P. vexans* causing kiwifruit decline syndrome in Italy. The identification of the causal agent

will permit to establish appropriate disease management strategies to face this emerging disease on kiwifruit.

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