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NEW DISEASE REPORT

First report of *Phytophthora vexans* and *Phytophthora litorale* associated with root rot symptoms on red raspberry (*Rubus idaeus*)

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Twenty-two root samples from raspberry plants exhibiting symptoms consistent with *Phytophthora* infection, i.e. cane wilt, chlorotic leaves, and blackened roots, were collected from two farms in Perthshire, Scotland, in November 2021 (Fig. 1). Roots were rinsed with water, cut into 20 mm sections, transferred into 70% (v/v) ethanol for 10 seconds and dried on sterile filter paper. Five root sections per sample were aseptically transferred to Petri dishes containing CMA-PARP medium (Stewart *et al.*, 2014), sealed and incubated in the dark at 18°C. The hyphal tips of growing colonies were transferred onto fresh CMA-PARP. Five isolates were obtained, with two distinct morphotypes: one produced sub-globose, non- to semi-papillate sporangia, while the second produced globose and papillate sporangia (Fig. 2). Root material (c. 1–2 g per sample) was freeze-dried in 2 mL microfuge tubes prior to DNA extraction with a PowerSoil Pro Kit (Qiagen, Germany) as per the manufacturer's instructions. PCR was performed using the *Phytophthora* genus-specific primer pair YPh1F/YPh2R (Schena *et al.*, 2008). The *Phytophthora* species *P. idaei*, *P. ilicis* and *P. plurivora* were detected in the samples but no isolates of these species were obtained from root samples. To determine the identity of the isolates, the ITS region was amplified and sequenced with ITS5 and ITS4 primers (White *et al.*, 1990). BLAST analysis revealed one isolate with a 99.49% identity

to a *Phytophthora vexans* isolate found in China (GenBank Accession No. MW800643). A fragment of 860 bp was deposited in GenBank (OQ170787). Four further isolates had a 99.54% sequence identity to a *Phytophthora litorale* isolate found in Chile (KU961896.1). A fragment of 662 bp from one isolate was deposited in GenBank (OQ449690). Metabarcoding analysis of the samples using the primers in Scibetta *et al.* (2012) detected *Pp. litorale* and *Pp. vexans* in 81% and 63% of samples, respectively.

A detached root assay, using healthy one year old raspberry cv. Tulameen, was performed. Roots were rinsed in water and submerged in 70% (v/v) ethanol for one minute, rinsed twice in sterile distilled water. Roots were placed on sterile filter paper with 500 µL of sterile water in Petri dishes, 10 replicates per isolate. The basal section of each root was suspended in 500 µL sterile water in a 1.5 mL microfuge tube sealed with plastic film to prevent drying. The apical portion was wounded with a sterile needle and 5 µL of a 1×10⁴ zoospore/mL suspension was pipetted onto the wound. Petri dishes were sealed and incubated at 20°C in the dark for 14 days (Fig. 3). Black lesions were noted on the apical zone of the roots inoculated with *Pp. vexans*, the basal root zone remained healthy. *Pp. litorale* caused blackened lesions throughout the entire root. Both species were re-isolated from the

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FIGURE 1 *Rubus idaeus* exhibiting cane wilt, leaf chlorosis and diseased roots consistent with root rot infection: a) and b) show symptoms in plants from which *Phytophthium vexans* was isolated, c) and d) show symptoms caused by infection with *Phytophthium litorale*.

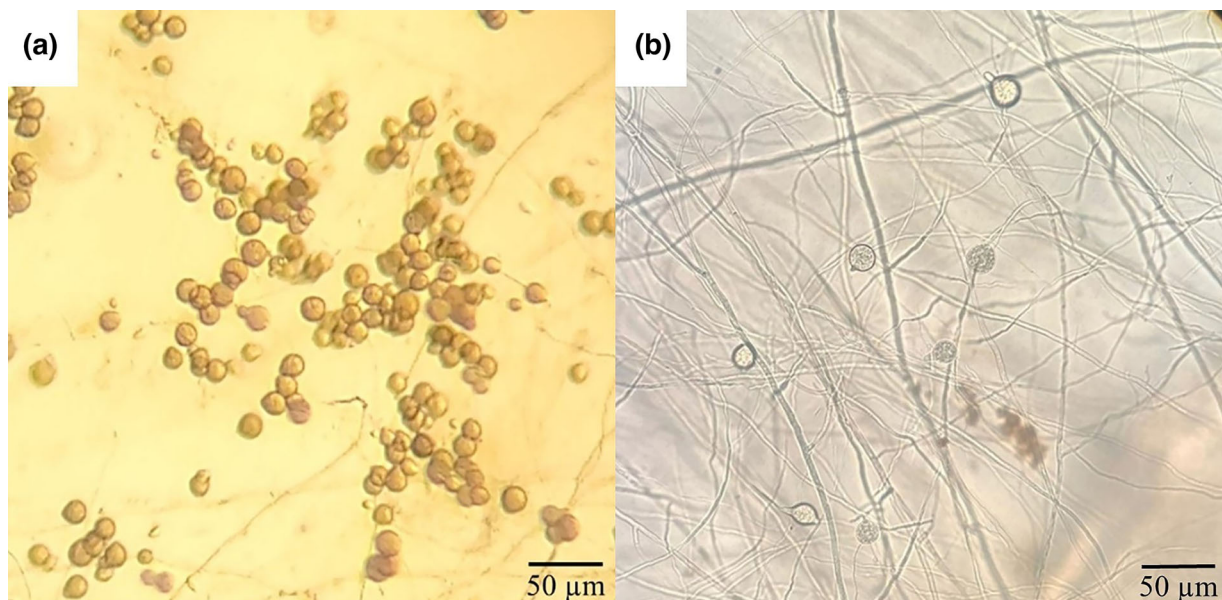


FIGURE 2 Sporangia of oomycetes isolated from diseased roots of raspberry: a) sub-globose, non-papillate sporangia at immaturity and semi-papillate prior to zoospore release, identified as *Phytophthium vexans* and b) globose and papillate sporangia, identified as *Phytophthium litorale*.

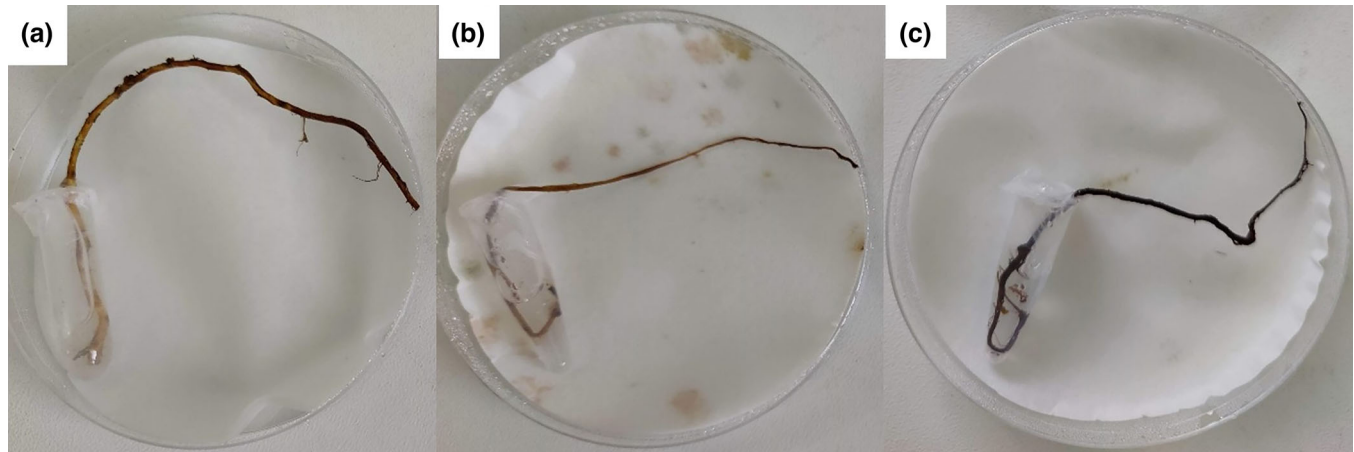


FIGURE 3 Detached raspberry roots inoculated with a) sterile water, b) *Phytophthora vexans* and c) *Phytophthora litorale* following 14 days incubation at 20°C.

diseased roots using the method above.

The co-detection of the *Phytophthora* with the *Phytophthora* species *P. idaei*, *P. ilicis* and *P. plurivora* in these samples may suggest both genera are involved in root rot on these sites. Both species have been reported on strawberry in the Czech Republic by Pánek & Střížková (2021). This is the first report of *Pp. litorale* and *Pp. vexans* exhibiting pathogenicity on red raspberry.

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