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NEW DISEASE REPORT

First report of *Phytophthora pluvialis* causing needle lesions on *Pinus pinea* in New Zealand

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During routine surveillance in October 2020, needle lesions and necrosis were observed in two 42-year-old stone pines (*Pinus pinea*) situated in an amenity planting within an infected *Pinus radiata* plantation in Nelson, New Zealand. Symptoms were widespread across the foliage of infected trees and samples were sent to Scion's Forest Health Reference Laboratory. Approximately fifteen needles displaying yellow and red bands (Fig. 1) tested positive for *Phytophthora* using a Phytophthora ImmunoStrip® (Agdia, USA), and PCR and subsequent DNA sequencing with Ypap2F/Ypap2R primers (McDougal *et al*., 2021), indicated *Phytophthora pluvialis*. A *Phytophthora* species was isolated from diseased tissue. Morphological characteristics were consistent with those described by Reeser *et al*. (2013) for *P. pluvialis*. This identification was confirmed by sequencing the internal transcribed spacer region (White *et al*., 1990). The isolate was deposited in the National Forest Culture Collection (NZFS 5280) and symptomatic needles were lodged into the National Forest Mycological Herbarium (NZFRI 5931). Foliage symptoms caused by *P. pluvialis* on other conifers, including *Pinus radiata*, *Pseudotsuga menziesii* and *Tsuga heterophylla*, typically express as distinct khaki/red lesions and black resinous bands (Dick *et al*., 2014), rather than the yellow and red bands observed on the stone pines (Fig. 2).



FIGURE 1 *Pinus pinea* needles from Nelson, New Zealand displaying yellow and red lesions



FIGURE 2 Typical foliage symptoms caused by *Phytophthora pluvialis* on other conifer species: *Pinus radiata*, *Pseudotsuga menziesii*, and *Tsuga heterophylla* (from left to right) infected with *P. pluvialis* are distinct khaki lesions which eventually senesce and turn red

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FIGURE 3 *Pinus pinea* needles artificially inoculated with sterile pond water (left) or *Phytophthora pluvialis* zoospores (right) and incubated for ten days. Olive/yellow lesions are observed on *P. pluvialis* inoculated needles only

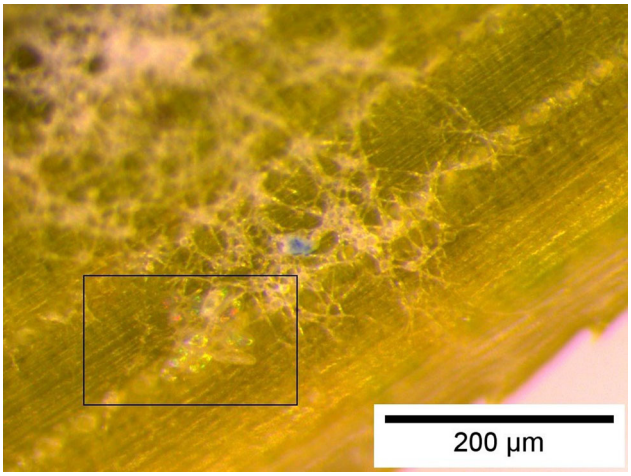


FIGURE 4 Surface mycelium and sporangia (black box) growing on *Pinus pinea* needles artificially inoculated with *Phytophthora pluvialis* zoospores

Pathogenicity testing was done by inoculating stone pine needles with *P. pluvialis* zoospores using a needle dip assay according to Dick *et al.* (2014). Stone pine needles from young healthy seedlings were placed in sterile pond water inoculated with *P. pluvialis* zoospores and incubated in darkness at 17°C for 24 hours. Control needles were placed in sterile pond water. Needles were then lain on moist paper towels in a sealed tray and incubated at 17°C at a 14-hour photoperiod. After ten days, olive/yellow lesions were observed on needles (Fig. 3). These lesions were lighter than those observed on field samples, however, this is common in detached needle assays of other species, such as *P. pluvialis* infection of *Pinus radiata* (Dick *et al.*, 2014). Sporangia were observed near the lesion margin of needles (Fig. 4). Sections of symptomatic needles were surface sterilised and plated onto a *Phytophthora*-selective media (10% carrot agar + ampicillin, pimaricin, rifampicin, nystatin and hymexazol). The resultant cultures displayed morphological features of *P. pluvialis*, fulfilling Koch's postulates (Fig. 5). Control needles remained healthy (Fig. 3).

To our knowledge, this is the first report of *P. pluvialis* causing needle disease on stone pine. Stone pine is an economically important species

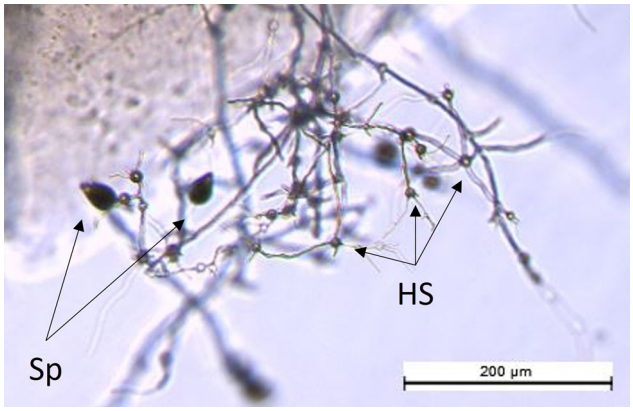


FIGURE 5 Cultures isolated from symptomatic needles, plated on *Phytophthora*-selective media then suspended in sterile pond water displaying hyphal swellings (HS) and sporangia (Sp) typical of *Phytophthora pluvialis*.

due to the production of high value pine nuts and timber. Although stone pine accounts for a small proportion (<1%) of New Zealand's forests, continued monitoring is recommended to assess the impact of disease on the growth and yield of these plantations.

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