A pre-harvest rot of persimmon fruit caused by *Phytophthora cactorum* in New Zealand

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Received: 28 October 2013 / Accepted: 18 December 2013 / Published online: 4 January 2014
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Abstract *Phytophthora cactorum* is reported causing a fruit rot of persimmon (*Diospyros kaki*) in New Zealand. The oomycete was isolated from rapidly developing brown rots of fruit that were observed pre-harvest. *P. cactorum* was identified on the basis of morphology and molecular tests, and confirmed as the causal agent by fulfilling Koch's postulates.

Keywords Persimmon · *Diospyros kaki* · *Phytophthora cactorum* · New Zealand · CARPP medium

Diospyros kaki (Japanese persimmon), a member of the Ebenaceae, was introduced into New Zealand in 1873 (Kitagawa and Glucina 1984). Persimmons are grown in the North Island of New Zealand and are of minor economic importance, with export volumes of 1,748 tonnes in 2010, 1,438 t in 2011 and 1,566 t in 2012 (NZHEA 2013).

Diseases of persimmon recorded in New Zealand include grey mould (*Botrytis cinerea*), bacterial blast (*Pseudomonas syringae* pv. *syringae*) and post-harvest rots caused by *Penicillium* spp. (Kitagawa and Glucina 1984). Other reports include *Colletotrichum acutatum* causing fruit rots (Lardner et al. 1999), *Colletotrichum horii* from anthracnose lesions on persimmon fruit and twigs (Weir and Johnston 2010), and basal root rot caused by *Cylindrocladium floridanum* (Boesewinkel 1986).

In June 2011, a small 'spray-free' orchard near Tauranga experienced pre-harvest rotting of 'Fuyu' persimmon fruit.

The orchard was planted in 1981, mainly on *D. kaki* rootstock, and had previously been a dairy farm. There was no evidence of stem cankers or collar rots on the trees. However, some of the pollinators, which had *Diospyros lotus* as the rootstock, had previously experienced tree decline, with sparse foliage and a general 'unthriftness'.

The fruit rots were patchy within the crown of the trees, and moved across the orchard as the season progressed, eventually becoming apparent in a neighbouring orchard. Approximately 30 % of the trees were affected and few fruit could be sold. The symptoms were unlike those of other common persimmon fruit-rotting pathogens in New Zealand, such as the anthracnose caused by *Colletotrichum* spp., appearing before harvest and forming circular light brown rots that progressed rapidly over the fruit, along with the distinctive smell typical of *Phytophthora*-rotted fruit. The fruit remained attached to the tree until harvest. A white mycelial web developed over the rotten areas after harvest.

Histological examination of the fruit rots revealed large numbers of typical *Phytophthora* sporangia forming on the surface of the fruit. Surface-sterilised pieces of rotted tissue were placed onto a selective medium, modified from Jeffers and Martin (1986), consisting of corn meal agar amended with 2 mg/L carbendazim, 250 mg/L ampicillin, 10 mg/L rifampicin, 5 mg/L pimaricin and 100 mg/L pentachloronitrobenzene (CARPP medium). A *Phytophthora* species was consistently isolated after 72 h growth at room temperature (c. 20 °C).

The isolated *Phytophthora* was maintained on V8 juice agar (Hine and Aragaki 1963). Morphological characteristics were studied on V8 juice agar and Difco corn meal agar (CMA) after up to 7 days growth at c. 20 °C under a 12 h/12 h light/dark regime. All isolates were homothallic, forming oogonia and sporangia readily on V8 juice agar (Fig. 1). Oogonia were parangynous, plerotic or aplerotic, 27.87–31.05 µm diameter. Sporangia were ellipsoid/ovoid (39–48 × 29–35 µm), papillate (papilla 4 µm depth, 5–7 µm width),

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caducous (pedicel 3.64 μm length). No hyphal swellings or chlamydospores were observed.

The internal transcribed spacer (*ITS*) ribosomal DNA gene region was amplified, using primers *ITS* 4 and 6 (White et al. 1990), from three isolates (ICMP 20004, 20021, 20022), producing an amplicon of approximately 900 bp. The sequences of the amplified products were deposited in the GenBank database and assigned accession numbers KF785803, KF785804 and KF785805. BLASTn analysis of the *ITS* amplicon showed that the region was 99.9 % homologous to *P. cactorum* sequences (accessions EF1932238; EF193231) in GenBank and 99 % homologous to *P. cactorum* sequences (AB217671; AF266772) in the Phytophthora-ID database version 2 (Grunwald et al. 2011). Analysis of neighbour-joining trees of the *ITS* sequences generated in Geneious Pro 5.5.6 (Biomatters, Auckland, New Zealand) placed these isolates into the same clade as *P. cactorum* (Fig. 2). Other *Phytophthora* species recorded on persimmon such as *P. cinnamomi* and *P. citrophthora* clustered into different clades. On the basis of morphology and the molecular tests, the isolates were identified as *Phytophthora cactorum*. Reference cultures have been deposited in the

Fig. 1 **a** Oogonia and **b** sporangia of *Phytophthora cactorum* growing on clarified V8 juice agar (photographs by JL Tyson)

International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand (ICMP 20004, 20021, 20022).

To fulfil Koch's postulates, a sterile scalpel was used to make skin wounds in six mature persimmon fruit. A small mycelial plug of each of three isolates of *P. cactorum* from persimmon was inserted into wounds on each of two fruit. Two persimmon fruit inoculated in the same manner with sterile V8 juice agar and two wounded un-inoculated fruit served as controls. Fruit were incubated in a humid chamber at c. 20°C for 4 days. The inoculations were carried out three times. Brown rots similar to the initial field symptoms began to form on all the inoculated fruit within one day of inoculation and the rot rapidly progressed to cover over 50 % of the fruit within 4 days (Fig. 3). No symptoms were seen on any of the control fruit. *Phytophthora cactorum* was re-isolated from all the inoculated fruit.

P. cactorum is polyphagous, "parasitising more than 200 plant species in 150 genera representing 60 plant families" (Erwin and Ribeiro 1996). It has a worldwide distribution and is best known for causing root, collar and crown rot diseases. Although fruit rots can be caused by *P. cactorum*, they are generally of minor economic importance and usually follow contact with soil particles that have been splashed upwards during heavy rain. In apples and pears, this results in dark brown lesions that can rapidly cover the entire fruit (Erwin and Ribeiro 1996).

The climatic conditions in the Tauranga region at the time disease symptoms were observed (June 2011) were slightly warmer (May 15.1 °C and June 12.6 °C) and substantially

Fig. 2 Neighbour joining tree generated from *ITS* gene sequences of *Phytophthora* species. Bootstrap values from 1,000 replicates are shown at each node

Fig. 3 Symptoms on persimmon **a** control fruit and **b** fruit 4 days after wound-inoculation with *Phytophthora cactorum* (photographs by Tim Holmes, Plant & Food Research)

wetter (May 173.2 mm and June 160.8 mm) than the 20-year average for these months. These conditions were probably ideal for *Phytophthora* spp. to be splashed from the soil onto fruit and to cause disease. There is anecdotal evidence that this type of fruit rot was also a problem in 2010. There were very few fruit rots of this type in 2012 and none observed in 2013.

Previous reports of *Phytophthora* spp. on persimmon in New Zealand consist of a brief note of *Phytophthora* sp. on *D. kaki* (Dingley 1969), and a report of *Phytophthora* sp. causing a basal rot on *D. kaki* in Hamilton in 1980 (Boesewinkel 1982). The Plant Pest Information Network database (PPIN 2013) records *P. cinnamomi* on roots, and *P. citricola* on roots and seedlings.

Worldwide, *P. cinnamomi* has been implicated in tree death of *D. pentamera* in Australia (Brown 1976); *P. capsici* has been recorded on *D. kaki* in Italy; *P. citrophthora* has been recorded on *D. kaki* in Iran; and *Phytophthora* sp. has been recorded on *Diospyros* spp. in Australia, Japan and the USA (Farr and Rossman 2013). *Phytophthora diospyri* is recorded on persimmon in the former USSR (Mkervali 1990), but no further information was found on this species.

Smith (1937) described artificial inoculations of *P. cactorum* on stems of various plants, including *D. kaki*. He found that in one of five plants inoculated, a 5-mm lesion had developed after 90 days.

To the authors' knowledge, this is the first report of *Phytophthora cactorum* affecting persimmon in New Zealand, and the first record of *P. cactorum* causing a fruit rot of persimmon worldwide. It is also likely to be the first report of natural infection of *Diospyros kaki* by *Phytophthora cactorum*.

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