Australasian Plant Dis. Notes (2015) 10:18
DOI 10.1007/s13314-015-0169-y

Pre-harvest rot of pear fruit Golden Russet Bosc caused by *Phytophthora lacustris* and *Phytophthora drechsleri* in Argentina

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Received: 26 March 2014 / Accepted: 1 April 2015

Abstract *Phytophthora lacustris* and *P. drechsleri* are reported causing a fruit rot of pear (*Pyrus communis*) Golden Russet Bosc in Argentina. The oomycetes were isolated from developing brown rots of fruit observed pre-harvest. *Phytophthora lacustris* and *P. drechsleri* were identified on the basis of morphology and molecular identification using the ITS region of ribosomal DNA and confirmed as the causal agent by fulfilling Koch's postulates. *Phytophthora drechsleri* was reported on pear in Argentina for the first time.

Keywords *Pyrus communis*- *Phytophthora*-fruit rot · Pre-harvest fruit rot

Recently, there has been an increase in the production of pears in the irrigated valleys of Rio Negro, northern Patagonia, Argentina (MinAgri 2013). 'Bosc' cultivars are the sixth most grown crop in the production area of the valleys with export volumes of nearly 30,000 tonnes (Senasa 2011).

Species of *Phytophthora* causing crown and collar rots are among the most important diseases affecting apple and pear plants as they cause significant economic losses worldwide (Jones and Aldwinckle 2002). In Argentina, the only species identified, to date, as the cause of rots in apple and pear trees is *P. cactorum* (Dobra et al. 2008; Rossini 2013).

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In recent years, in Argentina, *Phytophthora* in pear fruit was identified as the causal agent of rot during postharvest storage. *Phytophthora* sp. *salixsoil*, an undescribed species in the *P.gonapodyides -P.megasperma* Clade 6, was identified as the causal agent of rot in Bartlett pear fruit (Dobra et al. 2011). *Phytophthora lacustris* (formerly *P.* taxon *salixsoil*) caused severe damage and mortality to *Prunus persica* orchards in Italy and was isolated from the rhizosphere in India (Nechwatal et al. 2012; Das et al. 2013). *Phytophthora drechsleri* is of cosmopolitan distribution (113 genera in 40 families), affecting roots, stems, leaves, buds, flowers, fruits, tubers and bark of trunks (www.phytophthoradb.org).

Crown and collar rots caused by *Phytophthora* in apple and pear trees are widely known in Argentina; however, pre-harvest fruit rot in the orchards has not yet been studied in this country.

Fifteen days before harvest, during the first week of February 2013, a commercial orchard in the Medium Valley of Rio Negro showed rotting of "Golden Russet Bosc" pear fruit. The orchard was planted in 2004, on seedling rootstock and cultivated using the "Solaxe" system with the branches in the down position, and with the weight of the fruit located close to wet and irrigated soils (Fig. 1a–b).

The symptoms of collar rot on the tree wood were not observed. Approximately 10 % of orchard plants, planted in 7 rows (53 trees/row), showed typical symptoms in about 50 % of their fruit. The terminal inflorescence of lower branches located 15 to 30 cm from the ground's surface, showed different degrees of disease severity and premature drop of fruit to touch (Fig. 1a).

Disease symptoms on fruit showed circular brown/black spots with irregular and diffuse margins that enlarged rapidly to form distinctive rings, typical of *Phytophthora* infection (Fig. 1b–c). The rotted areas were firm at touch and the lesion diameter increased, extending from the pulp tissue to the fruit

Published online: 01 May 2015

Fig. 1 Symptoms of decay by *Phytophthora* on pear fruit "Bosc". **a** Premature drop of fruit affected by pathogen onto soil. **b**–**c** Typical symptoms on fruit. **d** Leaves on soil showing necrotic symptoms

core, eventually affecting the entire fruit. White mycelium was observed under the stereo microscope over the rotten areas of dropped fruit. Leaves sited next to fruit in the same branch showed necrotic symptoms, which initiated in the contact area with rot of fruit (Fig. 1d).

The pathogen was readily isolated from random samples of diseased fruit and not from leaves. Decayed fruit were sprayed with 70 % ethanol and air-dried. The skin of decayed fruit was peeled off with a sterile scalpel at the margin of decayed and healthy tissues. Small pieces of the fruit flesh were excised and plated on V8 juice agar (Erwin and Ribeiro 1996). Plates were incubated at 22±2 °C for 7 days and examined for culture development. Species of *Phytophthora* were consistently isolated.

Cultural (colony type) and morphological (mycelium, branching of sporangiophores and sporangia) features of isolates were studied. Asexual sporangia production was induced by flooding small tomato agar disks (0.5 mm diam) from the edge of a young culture (3 days old) in non-sterile soil-extract water (SEW) at 22±2 °C. Soil extract was prepared by flooding 50 g of soil with 1 L of distilled water for 2 days and then the solution was filtered through filter paper (Erwin and Ribeiro 1996). Disks were inspected for sporangia after 48 h. Tomato agar disks without culture were placed in SEW to confirm that they were free of *Phytophthora*.

Three morphospecies were identified on the basis of colony pattern and micromorphological features. The isolates were maintained on V8 juice agar and potato dextrose agar at 4 °C and one of each were submitted to the Oomycetes Collection of CIEFAP, Esquel, Argentina as Py342, Py343 and Py344.

The isolate Py342 had slow-growing, white, rosette-shaped colonies. Mycelium was coenocytic with globose or subglobose swellings. Sporangia were non-papillate with extended and internal proliferation. This isolate was identified as *P. lacustris* (Nechwatal et al. 2012).

The isolate Py343 showed rosette-like, slightly radiate and moderately fluffy colonies, with ovoid and ellipsoid, non-papillate sporangia, and a proliferation and formation of chlamydospores. The isolate Py344 had a similar colony to Py343 with non-papillate sporangia, but did not produce chlamydospores. Morphological characteristics (size and form of sporangia) of both isolates corresponded to *P. drechsleri*.

The identification of species was confirmed by direct sequencing of the internal transcribed spacer (ITS) ribosomal gene, amplified using primers DC6 and ITS4, according to Greslebin et al. (2007).

The BLASTN analysis of the ITS rDNA amplicon showed that the region corresponding to isolate Py342 was 100 % homologous to *P. lacustris*. The isolates Py343 and Py344 were 99 and 100 % homologous to *P. drechsleri*. The sequences of the amplified products were deposited in the GenBank database and assigned accession numbers KJ507655, KJ507656 and KJ507657, respectively.

To fulfill Koch's postulates, tests were carried out in pear fruit 'Bosc'. Three wounds (3x3 mm) per fruit were made and a small disk of mycelia was inserted into the wound on each of five fruit. Five pears were inoculated in the same manner with sterile V8 juice agar. The other five were wounded, uninoculated and served as controls. Fruit inoculated was placed in a humid chamber at 22±2 °C for 7 days. Brown rots similar to initial field symptoms began to form within 2 days of inoculation and the rot rapidly progressed in the fruit. The mean diameter of the lesions (less disk mycelia) was 20.6 mm for Py342, 16.2 mm for Py343, and 4 mm for Py344 after 7 days of incubation. No symptoms were seen on any of the control fruit. *Phytophthora* was re-isolated from inoculated fruit.

Several factors of the orchard were conducive to development of the disease. These included the growing system Solaxe, in which the fruit hangs close to wet soil, as well as the rainy weather conditions 2 weeks prior to harvest. Our findings contribute to the understanding that fruit postharvest rot was initiated in the field in fruit that are located at the sites of the orchard where the excess water occurs frequently and the fruit touch or are near the soil. Therefore, rain splash transmission of non-caducous sporangia of *P. lacustris* and *P. drechsleri*, from water or soil to fruit seems highly likely. This type of transmission from the soil to above-ground parts, via rain splash was reported in European beech by soilborne *Phytophthora* species (Nechwatal et al. 2011).

Monitoring the symptoms in pear fruit prior to harvest, will avoid the potential hazard that represents the entry of infected fruit at long-term storage. Our study suggests that *P. lacustris* and *P. drechsleri* can cause potential postharvest losses of

fruit. According to Nechwatal et al. (2006, 2012), *P. lacustris* does have pathogenic potential of cultivated fruit trees, and has apparently been introduced into the nursery trade. *Phytophthora drechsleri* is primarily a root pathogen but also attacks ripening fruit of various crops (Jeffers et al. 1982; Lamour et al. 2003; Hoover and Bates 2013).

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