## New Disease Reports

## First report of Phytophthora megasperma causing crown and root rot of almond in Turkey

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References

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Young almond trees (two-three years old) in commercial orchards of Adıyaman province (Southeastern Turkey) were observed showing decline symptoms in April-July 2013. Symptoms in affected trees included chlorosis, reddish-brown cankers progressing from the roots to the stem (Fig. 1), and dieback. Lateral and feeder roots were decayed. Plant losses of up to 15% occurred in the orchards. Diseased almond roots were washed in tap water and air-dried. Small sections, 3-5 mm diameter, were cut from the margin of the lesions and ten sections were plated on 1.7% corn meal agar amended with 4 mg/l pimaricin, 250 mg/l ampicillin, 10 mg/l rifampicin, and 75 mg/l pentachloronitrobenzene, without surface sterilization. Plates were incubated at 22°C in the dark. After three days, single hyphal tips from the edge of the growing colonies were cut and transferred onto carrot agar (CA) (200 ml boiled carrot juice, 800 ml distilled water and 20 g agar) to obtain pure cultures for identification.

The homothallic isolates produced oogonia abundantly in CA with a diameter of 30.6-49.5  $\mu m$  (mean 41.2  $\mu m$ ). Plerotic or aplerotic oospores with paragynous antheridia measured 26.9-43.5 µm (mean 35.4 µm) in diameter (Fig. 2). Non-papillate and non-caducous sporangia were ovoid (Fig. 3), obovoid, obpyriform or ellipsoid. They had both rounded and tapered bases. The ovoid and obpyriform sporangia were 34.6-66.8 µm long (mean 50.6 µm) and 24.6-44.7 µm wide (mean 34.1 µm), with lengthto-width ratios of about 1.48. External and internal proliferation of sporangiophores and nesting occurred. Cultures produced hyphal swellings in clumps or catenulate. Hyphal swellings sometimes occurred on sporangiophores in non-sterile soil extracts.

Based on these morphological characteristics, the isolates conformed to Phytophthora megasperma. Morphological identification was confirmed by sequencing the ITS. DNA was extracted from five isolates and the ribosomal DNA fragment was amplified with ITS1 and ITS2 primers (White et al., 1990) and sequenced. Nucleotide sequences of these isolates (Accession numbers KF633448, KF633449, KF633450, KF633451 and KF633452) had 99% homology with other P. megasperma isolates in GenBank (e.g. EU194387, GU258779, KC753539, HE805270 and EU301166).

Two isolates of P. megasperma were tested for pathogenicity on three-year-



Figure 2





Figure 3

old almond rootstocks. Inoculum was produced by growing isolates for two

weeks at 22°C in the dark on twice-autoclaved wheat grains moistened with

distilled water. Each of the rootstock plants was transplanted to a 5-litre pot

containing soil:sand mixture (1:1, v/v) mixed with inoculum at a rate of 5%

of the total soil volume. For each isolate five control plants were used.

Plants were incubated in a growth chamber for four months at  $25 \pm 1^{\circ}$ C and

were kept constantly wet. At the end of the experiment canker lesions

covered the whole roots, while no cankers developed in the roots of non-

inoculated plants (Fig. 4). The pathogen was re-isolated from symptomatic

P. megasperma causes root and crown rot of almond in Australia and the

USA (Wicks et al., 1997; Browne & Viveros, 1998). In Turkey, some

Phytophthora spp. such as P. cactorum, P. citrophthora and P. niederhauserii

have been reported previously on almonds (Kurbetli & Değirmenci 2010;

2011) but this is the first report of Phytophthora megasperma causing

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Figure 4

Figure 1

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