New Disease Reports

First report of wilt of almond caused by *Verticillium dahliae* in Tunisia

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Almond (*Prunus dulcis*) cultivation in Tunisia dates back to ancient times and has been known since the Carthaginian era (Gouta *et al*., 2011). In some orchards, intercropping with olive and vegetables is a common practice. Environmental conditions are typically Mediterranean, and many fungal diseases affect almond trees in the region. During a routine survey in June 2010, almond trees (cv. Masetto) grafted onto the rootstock 'Garnem' showed symptoms of yellowing, leaf fall and twig and branch dieback. Discoloration of vascular tissue was visible after cutting open affected stems (Fig. 1). These symptoms were observed in intensively managed orchards in the Northern part of Tunisia.

Samples were collected from branches and twigs showing the characteristic symptoms, and were kept on cold packs before bringing to the laboratory for isolations. Portions of symptom-bearing branches were surface sterilised with 2% sodium hypochlorite, then rinsed with sterile distilled water and allowed to air dry. Isolations from these tissues consistently yielded a *Verticillium* sp. on potato dextrose agar (PDA) amended with 25 mg/l of streptomycin sulphate. All isolates obtained produced dark colonies with small black microsclerotia on PDA, a defining feature that distinguishes *V. dahliae* from *V. albo-atrum* (Smith, 1965). Based on morphological characteristics (Hawksworth & Talboys, 1970) the fungus was tentatively identified as *Verticillium dahliae* Kleb. The identification was further confirmed by comparison of internal transcribed spacer (ITS) sequence data with reference isolates. The ITS region of rDNA was amplified by polymerase chain reaction (PCR) with primers ITS1F/ITS4 (Gardes & Bruns, 1993) and sequenced using primers ITS5/ITS4. Sequences from almond isolates in this study (GenBank Accession Nos. JQ902034 and JQ902035) were identical to other sequences in the GenBank, and matched the sequence of *V. dahliae* from tomato (GU060637).

A pathogenicity test was performed on ten grafted almond plants (cv. Mazetto, two years old). Trees were inoculated by root immersion in a suspension of 10^7 conidia/ml of *V. dahliae* (isolate for sequence JQ902034) while a set of control trees was similarly submerged in sterile water. The inoculated trees were maintained in a glasshouse at daily average temperatures between 25° and 28°C and relative humidity between 50 and 80%. Symptoms developed 60 days post inoculation and were typical of the original symptoms observed on diseased trees in the field. The symptoms consisted of chlorosis, wilting and necrosis of apical leaves that progressed downwards on the tree. *V. dahliae* was consistently re-isolated from infected vascular tissues of symptomatic trees as described above, completing Koch's postulates. Non-inoculated plants remained healthy.

Although *V. dahliae* has been previously reported on artichoke (Jabnoun-Khiareddine *et al*., 2008) and olive trees (Triki *et al*., 2006) in Tunisia, this is the first report of *Verticillium* wilting on almond trees in Tunisia. At this time, the economic importance of *Verticillium* wilt on almond cultivation in Tunisia is limited. However, *Verticillium* wilt might become an important economic problem for almond farmers in the future, since almond cultivation is expanding in many agricultural areas previously dedicated to tomato crops.

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Figure 1

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