DISEASE NOTE

A BLACK ROT OF BROAD BEAN LEAVES AND STEMS CAUSED BY A FLUORESCENT *PSEUDOMONAS* SPECIES IN CENTRAL ITALY

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In spring 2004, unusual symptoms on broad bean (*Vicia faba* L.) cv Reina blanca were observed in the province of Teramo (Abruzzo region, central Italy). Many blackish necrotic lesions along the stem were noticed, at the beginning of blossoming stage. Black lesions with irregular margins were also observed on the leaves. Fluorescent colonies were observed on King's medium B after 48 hours of incubation at 25-27°C. On nutrient agar the colonies appeared flat, with irregular margins and white-creamy coloured. The LOPAT tests yielded the following unusual results: levan-positive, oxidase-positive, potato-soft rot-negative, arginine dehydrolase-negative, tobacco hypersensitivity-positive. When inoculated into broad bean, the isolates induced similar symptoms as observed in the field 15 days after inoculation, whereas no apparent symptoms were noticed on inoculated tomato, pepper and zucchini plants. In a comparison using SDS-PAGE of protein extracts and repetitive PCR of broad bean isolates with some *Pseudomonas syringae* pathovars the broad bean strains were not similar in their fingerprints to these pathovars. Based on the description of symptoms, the disease observed in central Italy on broad bean is very similar to the one reported in China and Russia (Stead *et al*., 2003) on the same host. The causative agent, a fluorescent *Pseudomonas* not similar to known phytopathogenic pseudomonads, deserves further studies in order to define its true taxonomic status.

Stead D.E., Stanford H., Aspin A., Heeney J., 2003. Current status of some new and some old plant pathogenic pseudomonads. In: Iacobellis N.S., Collmer A., Hutcheson S.W., Mansfield J.W., Morris C.E., Murillo J., Schaad N.W., Stead D.E., Surico G. and Ullrich M.S. (eds.). *6th International Conference on Pseudomonas syringae pathovars and related pathogens, Maratea* 2003, 561-572.

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DISEASE NOTE

FIRST RECORD OF *VERTICILLIUM DAHLIAE* ON OLIVE IN MALTA

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Wilting of three-year old olive trees (*Olea europaea* L.) was observed in a grove in Għaxaq (Malta), in April 2004. *Verticillium dahliae* Kleb. (Hawksworth and Talboys, 1970) was isolated on tap-water agar (TWA) from wood explants of wilted twigs. All the isolates abundantly produced microsclerotia on TWA and on potato dextrose agar (PDA). On May 2004, colonies of *V. dahliae* grown on PDA were suspended by a blender in sterile tap water to a concentration of $5 \cdot 10^5$ CFU ml^{-1}. Five one-year-old olive seedlings were inoculated by root immersion in the inoculum suspension and transplanted in 16 cm diameter plastic pots containing a mixture (1:1:1 w/w) of soil, sand and peat. Five controls seedlings were treated in the same way with sterile tap water. All the plants were kept under climatised glasshouse conditions (20±4ºC) and watered to field capacity when needed. Epinasty and moderate defoliation were first observed on the inoculated plants one month after inoculation. In the following months smaller twigs started to wilt, then the wilting extended to larger branches. *V. dahliae* was re-isolated in December from all the inoculated plants. No symptoms were observed on control plants and all attempts to isolate the pathogen from them failed. This is the first record of *V. dahliae* on olive in Malta.

Hawksworth D.L., Talboys P.W., 1970. Descriptions of Pathogenic Fungi and Bacteria No. 256. Commonwealth Mycological Institute (C.M.I.), Kew, Surrey, U.K.

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FIRST RECORD OF *FUSARIUM OXYSPORUM* F.SP. *RADICIS LYCOPERSICI* IN MALTA

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Crown and root rot symptoms were observed on tomato (*Lycopersicon esculentum* L.) F1 hybrid Thomas (S. & G., Novartis Seeds B.V., Enkhuizen, Holland) in a greenhouse at Wardija, Malta, in November 2004. Symptoms included stem cankers starting mostly at the soil level and extending for 5-20 cm above it. Most severely affected plants wilted and died. *Fusarium oxysporum* was constantly isolated from rotten roots and crowns and from stem cankers. Colonies of a crown isolate, grown on potato dextrose agar (PDA), were suspended in sterile tap water by a blender. Ten tomato seedlings ('Thomas', growth stage: 6-8 leaves) were inoculated by root immersion in the inoculum suspension (concentration: $2.5 \cdot 10^6$ CFU ml^{-1} on PDA) for 5 min and were successively transplanted in 16 cm diameter plastic pots containing a mixture (1:1:1 w/w) of soil, sand and peat. Ten seedlings (controls) were treated in the same way with sterile tap water. All the plants were kept under glasshouse conditions (15±5ºC), regularly watered, and examined after one month. The inoculated plants showed typical symptoms of crown and root rot, including 1-3 cm cankers, and *F. oxysporum* was re-isolated from all of them. The controls were healthy, and the attempts to isolate the pathogen from them failed. The morphology of the fungus and the symptoms, observed both on the crop and after artificial inoculation, coincided with those described for *F. oxysporum* Schlecht. f.sp. *radicis lycopersici* Jarvis and Shoemaker (FORL) (Jarvis and Shoemaker, 1978; Brayford, 1996). Therefore we conclude that FORL was the causal agent of the disease reported above. This is the first record of FORL in Malta.

Jarvis W.R., Shoemaker R.A., 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. *Phytopathology* **68**: 1679-80.

Brayford D., 1996. *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Mycopathologia* **133**: 61-63.

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FIRST REPORT OF FRUIT TREE PHYTOPLASMAS AND THEIR PSYLLID VECTORS IN BOSNIA AND HERZEGOVINA

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During autumn 2004, orchards were surveyed in seven districts of north-western, central and southern Bosnia and Herzegovina (BiH) for the presence of phytoplasma diseases. Symptoms of apple proliferation (AP), pear decline (PD) and European stone fruit yellows (ESFY) were observed in several districts. Samples were collected for laboratory analyses from a number of symptomatic and symptomless fruit trees. In the same orchards, during spring 2005, the vectors of AP, *Cacopsylla costalis* and *Cacopsylla melanoneura*, of PD, *Cacopsylla pyri*, and of ESFY, *Cacopsylla pruni*, were collected and analysed for the presence of the respective phytoplasmas. After nucleic acid extraction from plants and insects, nested PCR assay was done using the phytoplasma universal primers P1/P7 followed by R16F2n/R2. The R16F2n/R2 amplicons, when digested with *Mse*I, showed the restriction profile typical of the 16SrX phytoplasma group. The identities of the detected agents was confirmed by a second nested PCR using 16SrX phytoplasma group specific primer pair f01/r01 (Lorenz *et al*., 1995), followed by RFLP with *Ssp*I and *Bsa*AI. Laboratory analyses showed the presence of phytoplasmas belonging to: i) 16SrX group, subgroup A (*Candidatus* Phytoplasma mali) in apples, in *C. costalis* and in *C. melanoneura*; ii) 16SrX group, subgroup C (*Candidatus* Phytoplasma pyri) in pears and in *C. pyri*; (iii) 16SrX group, subgroup B (*Candidatus* Phytoplasma prunorum) in apricots and peaches, and in *C. pruni* (Anonymous, 2004). Following the report of pear decline in BiH (Duduk *et al*., 2005), we have demonstrated, for the first time, the presence of AP, ESFY and the respective psyllid vectors in the country. The presence of both typically epidemic diseases and infected vectors represents a serious threat for fruit-growing in Bosnia and Herzegovina.

Anonymous, 2004. '*Candidatus* Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. *International Journal of Systematic and Evolutionary Microbiology* **54**: 1243-1255.

Duduk B., Botti S., Trkulja V., Ivanovic M., Stojcic J., Bertaccini A., 2005. Occurrence of pear decline in Bosnia and Herzegovina. *Journal of Plant Pathology* **87**: 75.

Lorenz K.H., Schneider B., Ahrens U., Seemüller E., 1995. Detection of apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. *Phytopathology* **85**: 771-776.

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