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A severe outbreak of crown and root rot of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Malta

Angelo Porta-Puglia[1], Robert Tanti[2] and David Mifsud[1]

[1]Ministry for Rural Affairs and the Environment, Department of Plant Health, Agricultural Research & Development Centre, Ghammieri, Marsa CMR 01, Malta
[2]Ministry for Rural Affairs and the Environment, Department of Plant Health, Plant Biotechnology Centre, Annibale Preca Street, Lija BZN 04, Malta

Summary. A severe outbreak of crown and root rot of tomato was observed in greenhouses in Malta in eight locations during the period November 2004 – February 2005. Besides root and crown symptoms, several plants showed cankers at the basal part of the stem. *Fusarium oxysporum* was constantly isolated from these plants. One isolate from each location was tested for pathogenicity on tomato seedlings. All these isolates caused severe necrotic lesions of the crown and roots, and stem cankers. It was concluded that *F. oxysporum* f. sp. *radicis-lycopersici* (*FORL*) was the cause of the outbreak. Incidence ranged from 10 to 50%. Cold weather conditions occurring in Malta during the late fall and winter of 2004–2005 probably favoured the disease. The use of tomato cultivars or hybrids resistant to *FORL* is suggested for winter tomato crops in south Mediterranean areas.

Key words: *FORL*, winter tomato, disease incidence.

Introduction

Crown and root rot is an important fungal disease of tomato (*Lycopersicon esculentum* L.). It was reported for the first time in Japan in 1969. Subsequently it was found in California and now also occurs in many parts of the EU, including several of its Mediterranean member countries, as well as Israel and Australia (Brayford, 1996). In Sicily the pathogen is considered a major limiting factor in greenhouses during winter, when it attacks tomato plants that are starting production (Cartia and Asero, 1994). In contrast to Fusarium wilt caused by FOL, crown and root rot is favoured by low (10°C to 20°C) soil temperatures (Roberts *et al*., 2001).

The causal agent was initially thought to be a new race of *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*), but was subsequently attributed to a new *forma specialis* and designated as *Fusarium oxysporum* Schlecht. f. sp. *radicis-lycopersici* (*FORL*) by Jarvis and Shoemaker (1978).

FORL was first observed in Malta in November 2004 (Porta-Puglia and Mifsud, 2005). From this time until February 2005 crown and root rot symptoms have been observed in greenhouses in seven other locations on the island. This paper reports on investigations carried out to ascertain the diffusion and incidence of *FORL* in Malta during the cold season 2004–2005.

Corresponding author: A. Porta-Puglia
Fax: +356 25904211
E-mail: angelo.porta-puglia@gov.mt

Materials and methods

Thirty tomato-growing greenhouses in Malta were surveyed from the beginning of November 2004 until 10 February 2005. The number of plants in each greenhouse varied between 600 and 5000; but in one there were 32,000 plants (which remained free of *FORL*). When symptoms attributable to *FORL* were observed, diseased plant samples were collected and taken to the laboratory. After surface disinfestation of the plant organs (10 sec in 90% ethanol followed by 5 min in a solution of sodium hypochlorite, 2% active Cl), small pieces of tissues were collected aseptically from the roots, crowns and stems affected with cankers and plated on tap-water agar (TWA). Fungal colonies were subsequently transferred to potato dextrose agar (PDA) plates for colony observation, and to PDA slants for storage.

Disease incidence was determined during the last week of February. Depending on the size of the tomato stands, 100–500 plants from randomly selected rows or portions of rows were inspected. Plants were counted as diseased when they showed irreversible wilt accompanied by symptoms at the basal part attributable to *FORL*. Incidence values were rounded up/down to 5%.

One isolate from each location was tested for pathogenicity on young tomato seedlings according to Apodaca-Sanchez *et al*. (2001). Seeds of the tomato F1 hybrid Thomas (Novartis seeds B.V., Holland) were disinfested in a solution of sodium hypochlorite (2% active Cl) for 5 min, rinsed in sterile water then placed in Petri dishes on TWA around a plug (5 mm diameter) of PDA cut with a sterile cork-borer from the active growing margin of *FORL* colonies. Two dishes containing 6 seeds each were prepared for each isolate. Two PDA plates with plugs of sterile PDA were used as controls. Developing seedlings were observed daily for two weeks to detect symptoms.

Re-isolations from inoculated and control seedlings were carried out on PDA. Fragments of roots, crown and stem tissues were surface-disinfested for 3 min in a sodium hypochlorite solution (2% active Cl), rinsed in sterile water and plated on PDA. Developing fungal colonies were observed visually and microscopically for identification.

Results and discussion

Locations in which symptoms of *FORL* were observed including the estimated incidence of the disease at the end of February are shown in Table 1. Diseased plants were mostly randomly scattered through the greenhouse, with subsequent diffusion along the rows. Symptoms included crown and root rot and in several cases stem cankers at the basal part of the stem. Cankers extended mostly for up to 25 cm above soil level but it was not uncommon to see longer cankers, especially in greenhouses where primary stems were trained horizontally (a common practice in Malta). Conidiophores producing abundant microconidia and several macroconidia of *F. oxysporum* were frequently observed on the cankers.

Fusarium oxysporum was consistently isolated from symptomatic roots, crowns and stems at all locations.

All the isolates induced crown and root rot symp-

Table 1. Locations in which *FORL* was observed from November 2004 to February 2005 in Malta.

Location	Disease first observed on	Variety	Incidence (%) at end of Feb 05
Wardija	05 Nov. 04[a]	Thomas [b]	30
Burmarrad	16 Nov. 04	Jeffrey [c]	10
Mġarr	27 Dec. 04	Jeffrey	50
Żabbar	29 Dec. 04	Thomas	10
Bidnija (Mosta)	31 Dec. 04	Jeffrey	45
Attard	03 Jan. 05	Jeffrey	20
Dingli	11 Jan. 05	Jeffrey	10
St. Paul's Bay	08 Feb. 05	Thomas	35

[a] Planting was started between mid September and the beginning of October in all the greenhouses under study.
[b] Hybrid (F 5576, F1), S&G, Novartis Seeds.
[c] Hybrid (DRW 3414, F1), De Ruiter Sementi.

toms on the plantlets. Initial symptoms were observed 2 to 4 days after the growing mycelium came in contact with the seedlings. The severity of crown and root symptoms increased in subsequent days, with 1–4 mm-long cankers appearing on some stems. All inoculated seedlings were severely affected. The different isolates did not show visible differences in virulence. *F. oxysporum* was re-isolated from all diseased seedlings.

The present study showed that in all the eight Maltese locations *FORL* was the cause of the rather severe damage caused. It is not easy to explain why the disease appeared at more or less the same time in all locations. One relevant factor may be that temperatures were lower than usual for much of the 2004/2005 growing season (E. Mifsud, personal communication).

Since the greenhouses, which consisted mostly of metallic frames covered with polyethylene film, were unheated, temperatures would have been favourable for *FORL* during most of the November to February period.

The origin of the inoculum can only be hypothesised. On most of the tomato plantations studied the soil was disinfested with methyl bromide either shortly before planting or in the year before that. The substantial absence of weeds and of other major soil-borne diseases confirmed the efficacy of the soil treatments given. Although re-colonisation from fungal propagules located deep in the soil or surviving in the greenhouse structure cannot be excluded, it seems more likely that the primary infection started from infested transplants or from aerial inoculum coming from outside.

The disease progress suggested a monocyclic phase followed by a polycyclic phase, as reported by Rekah *et al*. (2001). The polycyclic phase could account for the rapid increase of inoculum when climatic conditions were conducive to epidemics. Once the primary infection foci were established, the disease could progress to neighbouring plants by root-to-root dissemination, as shown by Rekah *et al*. (1999).

In view of the fact that average temperatures like those recorded during the period under study are not rare in southern Mediterranean areas, and that methyl bromide is now banned, the use of cultivars or hybrids resistant to *FORL* (gene Fr-1) should be considered for Malta and other tomato-growing areas of the region.

When *FORL* appears, sanitation measures should be applied immediately. In our experience, growers who act early, remove diseased plants and follow other measures suggested (disinfesting the soil around the uprooted plants, and adjusting irrigation to minimise dissemination of inoculum) suffer lower yield losses.

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