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FUSARIUM OXYSPORUM F. SP. *RADICIS-LYCOPERSICI* – THE CAUSE OF FUSARIUM CROWN AND ROOT ROT IN TOMATO CULTIVATION

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Abstract: *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) leading to fusarium crown and root rot is one of the most destructive soilborne diseases of tomatoes occurring in greenhouse and field crops. Physiological races of FORL were not defined but nine vegetative compatibility groups (VGCs) were identified. Infection followed by wounds and natural holes and infection is not systemic. The optimum soil temperature for pathogen development is 18°C. Infection may cause plants to wilt and die completely or infection may lower fruit quality. *Fusarium oxysporum* f. sp. *radicis-lycopersici* has the ability to produce a specific enzyme, tomatinase, which breaks down α-tomatine and protects the pathogen. In contrast tomato also has a defence system which consists of the enzymes chitinase and β-1, 3-glucanase. Tomato resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* is determined by a single dominant gene *Frl,* localized on the long arm of chromosome 9. It was introduced to cultivars from *Licopersicum peruvianum* (L.) Mill.

Key words: disease control, epidemiology, FORL, Fusarium crown and root rot, tomato

INTRODUCTION

Fusarium oxysporum f. sp. *radicis-lycopersici* (FORL) is a saprophytic fungus occurring in the rhizosphere of many plant species. The pathogen has a broad range of host species but host specialization of isolates is more circumscribed. Isolates in the same host species are assigned to a *forma specialis* (Kim *et al*. 2001). More than seventy *forma specialis* (f. sp.) were described by Armstrong and Armstrong (1981). In tomato there occur two *forma specialis* named *Fusarium oxysporum* f. sp. *lycopersici* (FOL), and *F. oxysporum* f. sp. *radicis-lycopersici* (Armstrong and Armstrong 1981; Steinkellner *et al*. 2005). The first reports on FORL came from Japan (1969) and California (1971), (Benhamou *et al*. 1989; Fazio *et al*. 1999). Fusarium crown and root rot (FCRR) caused by *F. oxysporum* f. sp. *radicis-lycopersici* is one of the most destructive diseases of tomatoes. It is widespread and leads to substantial yield losses in both greenhouse and soil production systems. Katan *et al*. (1991), Katan and Katan (1999) did not report the physiological races of FORL but identified nine VCGs (Vegetative Compatibility Groups) which are indicators of a high level of genetic variation within *F. oxysporum* f. sp. *radicis-lycopersici*. These nine groups were identified in isolates obtained from Western Europe, North America, and the Mediterranean region (Balmas *et al*. 2005).

Development of disease

F. oxysporum f. sp. *radicis-lycopersici* has a greater host range than *F. oxysporum* f. sp. *lycopersici* and occurs on *Lycopersicon* spp. *Capsicum frutescens* L. *Solanum melongena* L., *Arachis hypogeal* L., *Astragalus glycyphyllos* L., *Glycine mas* L. Merr., *Phaseolus vulgaris* L., *Pisum sativum* L., *Trifolium* spp., *Vicia faba* L., *Cucumis* spp., *Beta vulgaris* L. and *Spinacia oleracea* L. (Jarvis and Shoemaker 1978). The disease caused by *F. oxysporum* f. sp. *radicis-lycopersici* is characterized by a long period of incubation. When infection occurs immediately after planting, external symptoms appear immediately before harvest. If however infection occurs during the production of seedlings the disease may manifest itself at the time of flowering (Ślusarski 2000). According to Brayford (1996) the fungus can be isolated near the lesions and does not spread systemically. Infection occurs through the wounds and natural holes created by the newly formed root (Steinkellner *et al*. 2005). In the case of soilless growing, the sources of primary infection are microconidia transferred from air (Ślusarski 2000). The disease develops rapidly in cool soil (18°C), (Sato and Araki 1974; Yamamoto *et al*. 1974; Jarvis and Thorpe 1976; Sonoda 1976; Kim *et al*. 2001). At higher substrate temperatures, the disease is asymptomatic, although it is the cause of tail tissue browning (Ślusarski 2000). The pathogen may by introduced into a new area of tomato cultivation through contaminated seeds, infested soil or compost (Di Primo *et al*. 2001). Infected plants may wilt and die or remain in a state of weakness. A weakened plant will produce lower quality fruits (Jarvis and Shoemaker 1978; Steinkellner *et al*. 2005). An example can been seen in figure 1 where outside the shoot, just above the soil level,

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Fig 1. Fusarium crown and root rot symptoms on tomato rot (Author J. Sobolewski)

a necrotic injury appears involving the neck of the root and the stem base. The pink raid of the fungus occurs on the dead tissues. The pathogen grows rapidly in arid soils whereas in soils inhabited by various saprophytic organisms the pathogen poses practically no risk (Ślusarski 2000). Infected plants release a honeysuckle smell. Damage roots can be colonized by secondary pathogens. The disease affects both greenhouse and field crops (Jones *et al*. 1991; Kamilova *et al*. 2006).

Epidemiology in soilless culture, and disease control in tomato

Infected plants growing in the field produce many conidia that may be sources of airborne propagules (Rekah *et al*. 2000). Plant invasion by the FORL is enhanced by a wound in the tomato foliage. Symptoms of FCRR have not been observed in the field earlier than 63 days after planting. If symptoms are not observed, plants that were aerially infected may still be colonized by FORL and may infect neighbouring tomatoes by root to root contact and by increased inoculum in the soil for the next season (Rekah *et al*. 1999). The authors suggested that aerially disseminated propagules play a significant role in the epidemic development of the pathogen. Rowe and Farley (1981) confirmed that airborne spores may reinfest the soil after steaming. They suggested three approaches to the control of soil reinfestation: 1) eliminating spores of FORL by soil steaming and formaldehyde disinfestation, 2) using post-steaming soil treatments with captafol, and 3) developing resistant tomato cultivars.

Tomato production in greenhouses in the USA has begun to shift from ground culture to hydroponic rock wool and stonewool (Mihuta-Grimm *et al*. 1990). The advantages of the use of these substrates are higher crop yield, better control of growth, and independence from soil quality problems (van Os 1999). The studies by Mihuta-Grimm *et al*. (1990) showed that *F. oxysporum* f. sp. *radicis lycopersici* colonized sterile rock wool substrates with or without plant nutrients and confirmed that this system may be less vulnerable to the rapid spread of FCRR. The researchers reported that production of healthy transplants is very important in disease control and the use of a benomyl in a rock wool system reduced growth and colonization by FORL and slowed disease development.

In the field, methyl bromide/chloropicrin and captafol were used to reduce disease development (Datnoff *et al*. 1995). Biological controls such as fungi or bacteria are alternatives to the use of fungicides (Cook and Baker 1983). *Trichoderma harzianum* Rifi and *Glomus intraradices* Schenck and Smith (VAM – vesicular-arbuscular mycorrhizal fungi) have been effective as biological control agents for FORL (Caron *et al*. 1986). The use of both agents

together is more effective than when they are used alone (Datnoff *et al*. 1995). Sivan and Chet (1993) used *T. harzianum* combined with a sub-lethal dose of methyl bromide or with soil solarization. These combinations positively controlled FORL development in tomato cultivation. In the research of Menzies and Ehret (1997), three fungal isolates were used: isolate rf18 of *F. culmorum* (Smith) Sacc., isolate rf34 of *Penicillium brevicompactum* Dierckx, and isolate rf41 of *P. crustosum* Thom. The researchers observed that these fungi have the ability to increase the growth and yield of tomatoes in a soilless culture. These fungi also reduced the degree of infection by *F. oxysporum* f. sp. r*adicis-lycopersici*. *F. oxysporum* and *F. solani* which are avirulent to tomato. Root rot was also reduced (Louter and Edgington 1990). *Bacillus megaterium* (c96) and *Brukholderia cepacia* (c91) may be used as biocontrol agents. The first isolate reduced disease by 75%, and the second by 88%. *B. cepacia* (c91) in combination with carbendazim reduced symptoms by 46% compared with the 20% reduction obtained with the bacterium alone, and the fungicide alone. A combination of *B. megaterium* (c96) and fungicide reduced symptoms by 84% compared to an inoculated control, and by 77% compared to carbendazim alone (Omar *et al*. 2006). *Pseudomonas fluorescens* strain CHA0 in combination with zinc and copper significantly decreased FCRR symptoms in soilless tomato culture. Zinc improved biocontol by stimulation of the biosynthesis of antibiotics such as PHL (2,4-diacetylphloroglucinol), PLT (pyoluteorin) as well as phenazine-type antibiotics. Zinc also had an effect on FA (fusaric acid) production (Duffy and Defago 1997). Benhamou *et al*. (1994) in their research observed that chitosan used in seed coating was an inducer of plant defence reactions and may be useful in disease control. In new stonewool substrates, *P. fluorescens* strain WCS365 reduced the disease caused by FORL from 96 to 7%. The positive effect of biocontrol is due to the absence of other microorganisms on a sterile surface and lack of competition between microbes (Kamilova *et al*. 2006).

Pathogen – host relation

F. oxysporum f. sp. *radicis-lycopersici* has the ability to tomatinase production while protecting from the harmful effect of α-tomatine, steroidal glycoalkaloid. α-Tomatine is combined with free 3β-hydroxyl groups of fungi membrane sterols. The complexes cause loss of fungi membrane integrity (Roddick *et al*. 1974; Roddick and Drysdale 1984; Lairini *et al*. 1996; Ito *et al*. 2005). The enzymes as β-1, 3-glucanase and chitinase were induced in infected tomato plants. Researchers have reported that chitinase accumulates around the damaged hyphae in tomato root tissues infected by FORL. Its accumulation is mediated by fungal elicitors. In contrast, β-1, 3-glucanase locates itself in uncolonized tissues of resistant plants, which may indicate a different function of this enzyme in plant responses to the pathogen (Benhamou *et al*. 1990). Mauch *et al*. (1988) suggested that the plant enzymes β-1, 3-glucanase and chitinase play a significant role in the inhibition of fungal growth in vitro, and act synergistically. *F. oxysporum* f. sp. *radicis-lycopersici* has the ability to produce polygalacturonases (PGs), induce pectin depolymerisation, and facilitate colonisation of the host tissue. Polygalacturonases have an endo or an exo mode of action. The pathogen produces some isoforms of PGs whose expression is dependent on isolates (de las Heras et al. 2003). Lagopodi *et al*. (2002) used *F. oxysporum* f. sp. *radicis-lycopersici* transformed GFP (Green Fluorescence Protein) and demonstrated that the contact between pathogen and the root is initiated at the root hair. The next observation showed that colonization sites on the root surface are the junctions along the epidermal cells. The fungus forms hyphae which grow and fill all the junctions of the epiderma. In the crown region, development of hyphae is more rapid (Lagopodi *et al*. 2002).

Genetic resistance to FORL

Resistance to FORL was introduced into *L. esculentum* from *L. peruvianum* (L.) Mill. (Fazio *et al*. 1999). Berry and Oakes (1987) reported that resistance to crown root was segregated as a single dominant gene. Studies by Vakalounakis (1988) confirmed the dominant inheritance of resistance to *F. oxysporum* f. sp. *radicis-lycopersici*, and he designated this gene as *Frl*. The *Frl* gene is closely linked with the *Tm-2* gene responsible for resistance to tobacco mosaic virus (TMV) (Elkind *et al*. 1988). The genetic distance between *Frl* and *Tm-2* is approximately 5,1 cM ,and *Frl* is near the centromere on the long arm of chromosome 9 (Vakalounakis *et al*. 1997; Fazio *et al*. 1999).

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