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

Wojciech Szczechura, Mirosława Staniaszek, Hanna Habdas*

Department of Genetics, Breeding and Biotechnology of Vegetable Plants
Research Institute of Horticulture
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

Received: January 22, 2013
Accepted: April 4, 2013

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,

*Corresponding address:
wojciech.szczechura@inhort.pl

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).

This work was performed in the frame of the Multiannual Programme "Development of sustainable methods of horticultural production to ensure the horticultural products' high biological and nutritional quality, and to preserve the biodiversity of the environment while protecting its resources" financed by the Polish Ministry of Agriculture and Rural Development; Task 6.6.

REFERENCES

Armstrong G.M., Armstrong J.K. 1981. Formae speciales and races of *Fusarium oxysporum* causing wilt disease. p. 392–399. In: "Fusarium: Disease, Biology and Taxonomy" (P.E. Nelson, T.A. Toussoun, R.J. Cook, eds.). University Park: The Pennsylvania State University, 457 pp.

Balmas V., Scherm B., Di Primo P., Rau D., Marcello A., Migheli Q. 2005. Molecular characterization of vegetative compatibility group in *Fusarium oxysporum* f. sp. *radicis-lycopersici* and f. sp. *lycopersici* by random amplification of polymorphic DNA and microsatellite-primed PCR. Eur. J. Plant Pathol. 111 (1): 1–8.

Benhamou N., Charest P.M., Jarvis W.R. 1989. Biology and host parasite relations of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. p. 95–105. In: "Vascular Wilt Disease of Plants: Basic Studies and Control" (E.C. Tjamos, C.H. Beckman, eds.). NATO ASI Ser Ser H Cell Biol Vol H28 Berlin, 604 pp.

Benhamou N., Joosten M.H.A.J., De Wit P.J.G.M. 1990. Subcellular localization of chitinase and of its potential substrate in tomato root tissues infected by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Plant Physiol. 92 (4): 1108–1120.

Benhamou N., Lafontaine P.J., Nicole M. 1994. Induction of systemic resistance to fusarium crown and root rot in tomato

plants by seed treatment with chitosan. Phytopathology 84: 1432–1444.

Berry S.Z., Oakes G.L. 1987. Inheritance of resistance to fusarium crown and root rot in tomato. HortScience 22 (1): 110–111.

Brayford D. 1996. IMI descriptions of fungi and bacteria set 127. Mycopathologia 133 (1): 61–63.

Caron M., Fortin J.A., Richard C. 1986. Effect of phosphorus concentration and *Glomus intraradices* on fusarium crown and root rot of tomato. Phytopathology 76: 942–946.

Cook R.J., Baker K.F. 1983. The Nature and Practice of Biological Control and Plant Pathogens. APS Press, St. Paul, MN, 539 pp.

Datnoff L.E., Nemec S., Pernezny K. 1995. Biological control of fusarium crown and root rot of tomato in Florida Rusing *Trichoderma harzianum* and *Glomus intraradices*. Biol. Control 5 (3): 427–431.

de las Heras A. Patino B., Posada M.L., Martines M.J., Vazques C., Gonzales Jaen M.T. 2003. Characterization and *in vitro* expression patterns of an exopolygalacturonase encoding gene from *Fusarium oxysporum* f.sp. *radicis lycopersici*. J. Appl. Microbiol. 94 (5): 856–864.

Di Primo P., Cartia G., Katan T. 2001. Vegetative compatibility and heterokaryon stability in *Fusarium oxysporum* f. sp. *radicis-lycopersici* from Italy. Plant Pathol. 50 (3): 371–382.

Duffy B.K., Defago G. 1997. Zinc improves biocontrol of fusarium crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogene metabolites inhibitory to bacterial antibiotic biosynthesis. Phytopathology 87: 1250–1257.

Elkind Y., Kedar N., Katan Y., Couteaudier Y., Laterrot H. 1988. Linkage between Tm-2 and *Fusarium oxysporum* f. sp. *radicis-lycopersici* resistance (FORL). Rep. Tomato Genet. Coop. 38, p. 22.

Fazio G., Stevens M.R., Scott J.W. 1999. Identification of RAPD markers linked to fusarium crown and root rot resistance (Frl) in tomato. Euphytica 105 (3): 205–210.

Hartman J.R., Fletcher J.T. 1991. Fusarium crown and root rot of tomatoes in the UK. Plant Pathol. 40 (1): 85–92.

Ito S., Nagata A., Kai T., Takahara H., Tanaka S. 2005. Symptomless infection of tomato plants by tomatinase producing *Fusarium oxysporum* formae speciales nonpathogenic on tomato plants. Physiol. Mol. Plant Pathol. 66 (5): 183–191.

Jarvis W.R., Thorpe H.J. 1976. Susceptibility of Lycopersicon species and hybrid to the foot and root rot pathogen *Fusarium oxysporum*. Plant Dis. Rep. 60 (12): 1076–1031.

Jarvis W.R., Shoemaker R.A. 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68: 1679–1680.

Jones J.B, Jones J.P., Stall R.E., Zitter T.A. 1991. Compendium of Tomato Diseases. APS, St. Paul, MN, USA, 100 pp.

Kamilova F., Kravchenko L.V., Shaposhinkova A.I., Makarova N., Lugtenberg B. 2006. Effect of tomato pathogen *Fusarium oxysporum* f.sp. *radicis-lycopersici* and of the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the composition of organic acids and sugars in tomato root exudates. Mol. Plant Microbe Interact. 19 (10): 1121–1126.

Katan T., Zamir D., Sarfatti M., Katan J. 1991. Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Phytopathology 81: 255–262.

Katan T., Katan J. 1999. Vegetative compatibility in *Fusarium oxysporum* f.sp. *radicis-lycopersici* from the UK, the Netherlands, Belgium and France. Plant Pathol. 48 (4): 541–9.

Kim J.T., Park I.H, Oung I.H., Yu S.H. 2001. Crown and root rot of greenhouse tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Korea. Plant Pathol. J. 17 (5): 290–294.

Lagopodi A.L., Ram A.F.J., Lamers G.E.M., Punt P.J., Van den Hondel C.A.M.J.J., Lugtenberg B.J.J, Bloemberg G.V. 2002. Novel aspects if tomato root colonization and infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. Mol. Plant Microbe Interact. 15 (2): 172–179.

Lairini K., Perez-Espinosa A., Pineda M., Ruiz-Rubio M. 1996. Purification and characterization of tomatinase from *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Appl. Environ. Microbiol. 62 (5): 1604–1609.

Louter J.H., Edgington L.V. 1990. Indications of cross-protection against fusarium crown and root rot of tomato. Can. J. Plant Pathol. 12 (3): 283–288.

Mauch F., Mauch-Mani B., Boller T. 1988. Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and β-1, 3-glucanase. Plant Physiol. 88 (3): 936–942.

Menzies J.G., Ehret D.L. 1997. Root fungi increase the growth and yield and decrease the severity of fusarium crown and root rot of tomato plants grown in soilless culture (refered). International Symposium Growing Media and Plant Nutrition in Horticulture. ActaHort. 450: 457–466.

Mihuta-Grimm L., Erb W.A., Rowe R.C. 1990. Fusarium crown and root rot of tomato in greenhouse rock wool systems: sources of inoculum and disease management with benomyl. Plant Dis. 74 (12): 996–1002.

Omar I., O'Neill T.M., Rossall S. 2006. Biological control of fusarium crown and root rot of tomato with antagonistic bacteria and integrated control when combined within the fungicide carbendazim. Plant Pathol. 55 (1): 92–99.

Rekah, Y., Shtienberg, D., Katan, J. 1999. Spatial distribution and temporal development of fusarium crown and root-rot of tomato and pathogen dissemination in field soil. Phytopathology 89: 831–839.

Rekah Y., Shtienberg D., Katan J. 2000. Disease development following infection of tomato and basil foliage by airborne conidia of the soilborne pathogens *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *basilica*. Phytopathology 90: 1322–1329.

Roddick J.G. 1974. The steroidal glycoalkaloid α-tomatine. Phytochemistry 13 (1): 9–25.

Roddick J.G., Drysdale R.B. 1984. Destabilization of liposomy membranes by the steroidal glycoalkaloid α-tomatine. Phytochemistry 23: 543–547.

Rowe R.C., Farley J.D. 1981. Strategies for controlling fusarium crown and root rot in greenhouse tomatoes. Plant Dis. 65 (2): 107–112.

Sato R., Araki T. 1974. On the tomato root-rot disease occurring under vinyl-house conditions in southern Hokkaido. Ann. Rep. Soc. Plant Prot. North Jap. 25: 5–13.

Sivan A., Chet I. 1993. Integrated control of fusarium crown and root rot of tomato with *Trichoderma harzianum* in combination with methyl bromide or soil solarization. Crop Prot. 12 (5): 380–386.

Sonoda R.M. 1976. The occurrence of fusarium root rot of tomatoes in Florida. Plant Dis. Rep. 60: 271–274.

Steinkellner S., Mammerler R., Vierheilig H. 2005. Microconidia germination of the tomato pathogen *Fusarium oxysporum* in the presence of root exudates. J. Plant Interac. 1 (1): 23–30.

Ślusarski Cz. 2000. Choroby odglebowe pomidorów (cz. II). W bezglebowej uprawie pod oslonami. Haslo Ogrodnicze 2: 46–49.

van Os E.A. 1999. Closed soilless growing system: a sustainable solution for Dutch greenhouse horticulture. Water Sci. Technol. 39 (5): 105–112.

Vakalounakis D.J. 1988. The genetic analysis of resistance to fusarium crown and root rot of tomato. Plant Pathol. 37 (1): 71–73.

Vakalounakis D.J., Laterrot H., Moretti A., Ligoxigakis E.K., Smardas K. 1997. Linkage between Frl (*Fusarium oxysporum* f. sp. *radicis-lycopersici* resistance) and Tm-2 (tobacco mosaic virus resistance-2) loci in tomato (*Lycopersicon esculentum*). Ann. Appl. Biol. 130 (2): 319–323.

Yamamoto I., Konad H., Kuniyasu M., Saito M., Ezuka A. 1974. A new race of *Fusarium oxysporum* f. sp. *lycopersici* including root rot of tomato. Proc. Kansai Plant Proc. Soc. 16: 17–29.