Potential of Non-Pathogenic *Fusarium oxysporum* Isolates for Control of Fusarium Wilt of Tomato

Juliano C. da Silva[1] & Wagner Bettiol[2]

[1]UNESP, Departamento de Produção Vegetal, Cx. Postal 237, CEP 18603-970, Botucatu, SP; [2]Embrapa Meio Ambiente, Cx. Postal 69, CEP 13820-000, Jaguariúna, SP, Fax: (019) 38678740, e-mail: bettiol@cnpma.embrapa.br, Bolsista do CNPq

(Aceito para publicação em 02/03/2005)

Autor para correspondência: Wagner Bettiol

SILVA, J.C. & BETTIOL, W. Potential of non-pathogenic *Fusarium oxysporum* isolates for control of Fusarium wilt of tomato. Fitopatologia Brasileira 30:409-412. 2005.

ABSTRACT

This study was done to evaluate the efficiency of non-pathogenic *Fusarium oxysporum* isolates (141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5, and 257) in controlling vascular wilt caused by *F. oxysporum* f. sp. *lycopersici*, race 2 (isolates C-21A, TO11, and TO245) in tomato (*Lycopersicon esculentum*) cv. Viradoro seedlings. In order to determine the effect of non-pathogenic *F. oxysporum* isolates in tomato plants, the root system of 30-day-old seedlings was immersed in conidial suspensions (10^6 ml^{-1}) of each isolate and the seedlings were transplanted to a cultivation substrate. Thirty-five days after transplanting it was observed that the non-pathogenic *F. oxysporum* isolates were not pathogenic to the cv. Viradoro nor did they affect seedling development. The efficiency of the non-pathogenic *F. oxysporum* isolates in controlling Fusarium wilt was determined by immersing the tomato seedling roots in the conidial suspension (10^6 ml^{-1}) of each isolate and then transplanting them into substrates previously infested with isolates of *F. oxysporum* f.sp. *lycopersici*, race 2 (10^5 conidia ml^{-1} of substrate). Evaluations were performed 35 days after transplanting, for severity in scale with 1=healthy plant to 6=dead plant or plant showing vessel browning and wilted leaves up to the leader shoot and seedling height. The non-pathogenic *F. oxysporum* isolates were efficient in reducing the severity of the disease and maintaining normal plant development. These results provide evidence of the antagonistic activity of non-pathogenic *F. oxysporum* isolates in controlling vascular wilt caused by *F. oxysporum* f. sp. *lycopersici* race 2 in tomato.

Additional keywords: biological control, *Fusarium oxysporum* f.sp. *lycopersici* race 2, nonpathogenic *F. oxysporum.*

RESUMO

Potencial de isolados de *Fusarium oxysporum* não patogênico no controle da murcha de Fusarium do tomateiro

O trabalho avaliou a eficiência dos isolados (141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5 e 257) de *Fusarium oxysporum* não patogênico ao tomateiro (*Lycopersicon esculentum*), no controle da murcha vascular causada por *Fusarium oxysporum* f. sp. *lycopersici*, raça 2 em plântulas de tomateiro cv. Viradoro. Para verificar o efeito dos isolados de *F. oxysporum* não patogênicos, o sistema radicular de plântulas de tomateiro, com 30 dias de idade, foi imerso na suspensão de conídios (10^6 ml^{-1}) e as mudas transplantadas para substrato de cultivo. Após 35 dias do transplante foi verificado que esses isolados não foram patogênicos às plantas de tomateiro, nem afetaram o desenvolvimento das mudas. A eficiência dos isolados de *Fusarium oxysporum* não patogênicos no controle da murcha foi determinada imergindo-se as raízes de mudas de tomateiro em suspensão de conídios (10^6 conídios ml^{-1}) e transplantando-as em substratos previamente infestados com os isolados de *F. oxysporum* f.sp. *lycopersici*, raça 2 (10^5 conídios ml^{-1} de substrato). Transcorridos 35 dias do transplante, foram realizadas as avaliações da severidade na escala de 1=planta sadia a 6=planta morta ou com vasos coloridos e folhas murchas até o ponteiro e altura das mudas. Os isolados de *F. oxysporum* não patogênicos foram eficientes em reduzir a severidade da doença e em manter normal o seu desenvolvimento. Esses resultados evidenciam a atividade antagônica dos isolados de *F. oxysporum* não patogênico no controle da murcha vascular do tomateiro, causada por *Fusarium oxysporum* f. sp. *lycopersici* raça 2.

Palavras-chave adicionais: controle biológico, *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* não patogênico.

The tomato (*Lycopersicon esculentum* Mill.) is one of the world's most cultivated vegetable crops, and Brazil is one of the major producers. Tomato plants are affected by several diseases, including Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen, which can cause serious economic losses. Methods used to control vascular wilt are either not very efficient or are difficult to apply. The best way to control the disease is by selecting resistant varieties of tomatoes. Although commercial varieties of tomato resistant to *F. oxysporum* f. sp. *lycopersici* races 1 and 2 are available, both additional pathogenic strains, and other races of the pathogen have been

reported in several countries. For this reason, alternative methods of controlling the disease have been studied, with emphasis on biological control. Soils naturally suppressive to Fusarium wilt (Garibaldi *et al*., 1990; Alabouvette, 1999) have been reported in different regions of the world. Although several antagonistic microorganisms have been evaluated to control Fusarium wilt, the most promising are non-pathogenic *F. oxysporum* isolates (Rouxel *et al*., 1979; Garibaldi *et al*., 1987; Minuto *et al*., 1995ab). Saprophytic species of *Fusarium* have been found to be effective in reducing *F. oxysporum* in cyclamen (*Cyclamen persicum* Mill.), gerbera (*Gerbera jamesonii* Hook.), basil (*Ocimum basilicum* L.), asparagus (*Asparagus officinalis* L.), eggplant (*Solanum melongena* L.), carnation (*Dianthus canyophyllus* L.), watermelon [*Citrullus lanatus* (Thumb.) Matsumi & Nakai], tomato, chick pea (*Cicer arietinum* L.) and cucumber (*Cucumis sativus* L.) (Mandeel & Baker, 1991; Postma & Rattink, 1992; Yamagushi *et al*., 1992; Hervás *et al.*, 1995; Minuto *et al.*, 1995ab; Larkin & Fravel, 1999; He *et al*., 2002; Reid *et al.*, 2002).

The objective of this work was to evaluate the efficiency of non-pathogenic *F. oxysporum* isolates for biological control of tomato wilt caused by *F. oxysporum* f. sp. *lycopersici* race 2.

Tomato cv. Viradoro, resistant to race 1 but susceptible to race 2 of *F. oxysporum* f. sp. *lycopersici*, was used in all assays. The tomato seedlings were produced for transplanting on Multihort® planting substrate in a styrofoam tray (35 mm × 35 mm) in a greenhouse.

The *F. oxysporum* f. sp. *lycopersici* race 2 isolates were supplied by Dr. Sami J. Michereff, Universidade Federal Rural de Pernambuco (isolate C-21A) and by Dr. Rômulo Fujito Kobori, Sakata Seed Sudamérica (isolates TO11 and TO245). The non-pathogenic *F. oxysporum* isolates 141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5, and 257, isolated from carnation plants grown in suppressive soils in Italy, were supplied by Dr. Angelo Garibaldi, from Università degli Studi di Torino, Italy. The antagonistic isolates were introduced into Brazil through Laboratório de Quarentena Costa Lima (Brazil's official quarantine facility), of Embrapa Meio Ambiente (MA Proceeding no. 21052.011767/99-04). The inocula of all isolates were produced in potato-dextrose broth in shake culture (150 rpm), for ten days, at 25±2 °C. The medium in flasks was seeded with 5mm diameter discs of PDA culture of the respective *Fusarium* sp. isolates. The culture was filtered through a double layer of sterilized gauze.

Pathogenicity test

The plant growth substrate consisted of a soil and cattle manure (3:1 v/v) mixture. The soil (Yellow Latosol) showed the following chemical composition: P=5 mg dm^{-3}; K=1.5, Ca=7, H+AL=95, BS=9.5, CEC=104.5 mmolc dm^{3}; and V=9%. Each kilogram of the substrate was enriched with 0.2 g potassium chloride, 0.5 g single superphosphate and 6g dolomitic lime. The substrate was infested with respective isolates of *F. oxysporum* f. sp. *lycopersici* at concentrations of 10^3, 10^4, 10^5 and 10^6 conidia ml^{-1} of substrate, ten days prior to transplanting the 30-day-old seedlings. The plants were raised in the greenhouse for 35 days at which time disease severity and plant height were evaluated. The severity rating was done using the scale proposed by Tokeshi & Galli (1966), modified as follows: 1=healthy plant; 2=plant with brown vessels in the first internode region, without other visible symptoms; 3=plant with brown vessels up to the height of the first leaf, with yellowing of at least one leaflet; 4=plant showing vessel browning up to half of the stem length, with yellowing of two or more leaves; 5=plant showing vessel browning nearly to the leader shoot, with most leaves wilted, except the leader shoot; 6=dead plant or plant showing vessel browning and wilted leaves up to the leader shoot.

Effect of Fnp isolates on tomato

The root systems of tomato seedlings were washed in tap water, then immersed in a conidial suspension (10^6 ml^{-1}) of respective non-pathogenic *F. oxysporum* isolates 141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5, and 257, for 5 min. The seedlings were then transplanted to 500 ml pots containing the substrate. In addition to the non-pathogenic *F. oxysporum* isolates, the assay included a non-inoculated control and a control treated with the autoclaved PD culture medium. The plants were grown in the greenhouse and evaluations for disease severity and plant height were performed 35 days after transplanting, as previously described.

Effect of Fnp isolates on the control of Fusarium wilt in tomato

The tomato seedling root system was immersed in a conidial suspension (10^6 ml^{-1}) of non-pathogenic *F. oxysporum* isolates 141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5, and 257, for 5 min, after which the seedlings were transplanted to a substrate previously infested with a *F. oxysporum* f. sp. *lycopersici* isolates C-21A, TO11 and TO245 (10^5 ml^{-1} of substrates). The plants were grown in the greenhouse and evaluations for disease severity and plant height were performed 35 days after transplanting, as previously described.

Statistical analysis

A completely randomized experimental design with ten replicates was adopted for all assay. For the statistical analysis the data were transformed to sqrt (x + 0.5) and compared by the Tukey test at 5% probability, using the SAS System Software Package, version 8.

Race 2 of *F. oxysporum* f. sp. *lycopersici* isolates C-21A, TO11 and TO245 were found to be pathogenic to the cultivar Viradoro at all inoculum concentrations tested (Table 1), causing a drastic reduction of plant height. The isolate TO245 was the most virulent, causing the maximum diseases severity in plants grown in substrate infested with 10^6 and 10^5 conidia ml^{-1} of substrate. These results agree with those

of Andrade & Micherref (2000), who demonstrated that tomato plants of different cultivars, inoculated with 10^6 conidia ml^{-1} of isolates C-1, C-7, C-21A, and F-23 of *F. oxysporum* f. sp. *lycopersici* race 2, showed a 50% disease incidence. He *et al.* (2002) also showed that 10^6 CFU g^{-1} soil of *F. oxysporum* f. sp. *asparagi* caused the death of asparagus plants.

Tomato seedlings whose root systems were immersed in the conidial suspension of non-pathogenic *F. oxysporum* isolates 141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5, and 257, did not show symptoms of vascular diseases and developed normally. The non-pathogenic *F. oxysporum* isolates were obtained from carnation rhizospheres (Garibaldi *et al*., 1985), so were not pathogenic to the tomato plants. This is important because the same non-pathogenic *F. oxysporum* isolates can be useful for other hosts, as demonstrated by Minuto *et al.* (1995ab) for cyclamen and basil and by Garibaldi *et al.* (1990) for melon (*Cucumis melo* L.) and radish (*Raphanus sativus* L.).

When the tomato seedling root systems were immersed in inocula of non-pathogenic *F. oxysporum* isolates and the plants were grown in substract previously infested with race 2 of *F. oxysporum* f. sp. *lycopersici* isolates C-21A, TO11 and TO245, all non-pathogenic *F. oxysporum* isolates were efficient in controlling the disease; plants showed lower disease severity and greater height (Table 2), with no significant degree of difference between the non-pathogenic *F. oxysporum* isolates. These results agree with Garibaldi *et al*. (1987), Postma & Rattink (1992), and Minuto *et al*. (1995ab), who reported that non-pathogenic *Fusarium* spp. isolates, introduced by root immersion before transplanting, were efficient in colonizing the rhizosphere and in controlling Fusarium wilt. There are reports of non-pathogenic *F. oxysporum* that show they act by competing for infection sites and for nutrients, and by induction of resistance (Mandeel & Baker, 1991; Alabouvette & Couteaudier, 1992; Larkin & Fravel, 1999; Benhamou et al., 2001). In order to control vascular wilt caused by *F. oxysporum* f. sp. *lycopersici*, with non-pathogenic *F. oxysporum* it is necessary to study the best method for applying the non-pathogenic *F. oxysporum*, i.e., by treating the root systems by deepening or by applying the non-pathogenic *F. oxysporum* in soil/substrate in which the tomato is grown.

TABLE 1 - Severity of Fusarium wilt and plant height (cm) of tomato (*Lycopersicon esculentum*) cv Viradoro grown in substrate infested with race 2 *Fusarium oxysporum* f.sp. *lycopersici* isolates

Inoculum concentration (conidia ml^{-1} of substrate)	Isolates of *F. oxyxporum* f.sp. *lycopersici*					
	C-21A		TO11		TO245	
	Severity*	Height	Severity*	Height	Severity*	Height
0	1.00c	51.36a	1.00d	46.23a	1.00d	46.23a
10^3	3.16b	38.11b	2.33c	35.98b	3.16c	36.41b
10^4	3.33b	35.71bc	2.66c	34.48cb	3.83b	36.50b
10^5	3.66ab	33.83dc	3.66b	30.71c	6.00a	0d
10^6	4.16a	30.66d	4.83a	30.21c	5.00ab	29.58c

*Disease severity – ratings: 1=healthy plant to 6=dead plant or plant showing vessel browning and wilted leaves up to the leader shoot. Means followed by the same letter do not differ (Tukey p>0.05)

TABLE 2 - Severity of Fusarium wilt and plant height (cm) of tomato (*Lycopersicon esculentum*)cv Viradoro treated with non-pathogenic *Fusarium oxysporum* isolates and grown in substrates infested (10^5 conidia ml^{-1} of substrate) with race 2 of *Fusarium oxysporum* f.sp. *lycopersici*

Isolate of non pathogenic *Fusarium oxysporum*	Isolate of *F. oxysporum* f.sp. *lycopersici*					
	C-21A		TO11		TO245	
	Severity*	Height	Severity*	Height	Severity*	Height
Control	1.00b	52.33a	1.00c	52.63a	1.00c	53.06a
Fol	3.66a	41.36c	4.16a	30.43e	4.83a	30.21c
233	2.33ab	49.88ab	3.16ab	43.48cd	2.50b	49.80a
233/1	2.16ab	50.18a	3.83ab	50.80ab	2.00b	50.85a
141/3	2.16ab	51.46a	2.83b	49.25abc	2.16b	51.93a
251	2.33ab	52.61a	3.00ab	48.35abc	2.33b	43.15b
251/2	2.16ab	44.66b	2.66ab	39.68d	3.00b	39.21b
245	2.16ab	47.11ab	3.00ab	48.55abc	2.50b	43.51b
245/1	2.00b	48.08ab	2.83b	50.38ab	2.50b	50.10a
257	1.83b	49.25ab	3.33ab	44.81bcd	2.50b	42.08b

*Disease severity - ratings: 1=healthy plant to 6=dead plant or plant showing vessel browning and wilted leaves up to the leader shoot. Means followed by the same letter do not differ (Tukey p.0.05).

ACKNOWLEDGEMENTS

To Prof. Dr. Angelo Garibaldi, of Università degli Studi di Torino, for donating the non-pathogenic *Fusarium oxysporum* isolates; to Prof. Dr. Sami Jorge Michereff, of Universidade Federal Rural de Pernambuco and to Dr. Rômulo Fujito Kobori, of Sakata Seed Sudamérica, for donating the strain 2 *Fusarium oxysporum* f. sp. l*ycopersici* isolates; and to Embrapa Hortaliças, for supplying the cv. Viradoro tomato seeds.

LITERATURE CITED

ALABOUVETTE, C. Fusarium wilt suppressive soils: an example of disease suppressive soils. Australasian Journal of Plant Pathology 28:57-64. 1999.

ALABOUVETTE, C. & COUTEAUDIER, Y. Biological control of Fusarium wilts with nonpathogenic Fusaria. In: Tjamos, E.S. (Ed.). Biological control of plant diseases. New York. Plenum Press, 1992. pp.415-426.

ANDRADE, D.E.G.T. & MICHEREFF, S. Arranjo espacial da murcha de fusário do tomateiro no Agreste de Pernambuco. Summa Phytopathologica 26:316-319. 2000.

BENHAMOU, N. & GARAND, C. Cytological analysis of defense-related mechanisms induced in pea root tissues in response to colonization by the non-pathogenic *Fusarium oxysporum*, strain Fo47. Phytopathology 91:730-740. 2001.

GARIBALDI, A., BRUNATTI, F. & ALLOCHIO, A. Terreni repressivi verso *Fusarium oxysporum* f.sp. *dianthi*: isolamento di microrganismi e loro ativitá antagonistica in vaso. La difesa delle piante 2:101-106.1985.

GARIBALDI, A.,BRUNATTI, F. & GULLINO, M.L. Evaluation of several antagonistics and different methods of applications against Fusarium wilt of carnation. EPPO Bulletin 17:625-629. 1987.

GARIBALDI, A., GUGLIELMONE, L. & GULLINO, M. L. Rhizosphere competence of antagonistic Fusaria isolated from suppressive soils. Symbiosis 9:401-404. 1990.

HE, C.Y., HSING, T. & WOLIN, D.J. Induction of systemic disease resistence and pathogen defense response in *Asparagus officinalis* inoculated with nonpathogenic strains of *Fusarium oxysporum*. Plant Pathology 51:225-230. 2002.

HERVÁS, A., TRAPERO-CASAS, J.L. & JIMENEZ-DIAZ, R.M. Induced resistence against Fusarium wilt of chickpea by nonpathogenic races of *Fusarium oxysporum* f. sp. *ciceris* and nonpathogenic isolates of *F. oxysporum*. Plant Disease 79:1110-1116. 1995.

LARKIN, R.P. & FRAVEL, D.R. Mechanisms of action and dose-response relationships governing biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 89:1152-1161. 1999.

MANDEEL, Q. & BAKER, R. Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. Phytopathology 81:462-469. 1991.

MINUTO, A., MIGHELI, Q. & GARIBALDI, A. Evaluation of antagonistic strains of *Fusarium* spp. in the biological and integrated control Fusarium wilt of cyclamen. Crop Protection 14:221-226. 1995a.

MINUTO, A., MOCIONI, M. & GARIBALDI, A. Preliminary trials on biological control of Fusarium wilt of basil. Acta Horticulturae 382:173-177. 1995b.

POSTMA, J. & RATTINK, H. Biological control of Fusarium wilt of carnation with a non-pathogenic isolate of *Fusarium oxysporum*. Canadian Journal of Botany 70:1199-1205. 1992.

ROUXEL, F., ALABOUVETTE, C. & LOUVET, J. Recherches sur la resistence des sols aux maladies. IV. Mise envidence du role des *Fusarium* autochtones dans la resistence d´un solè la fusariose vascularie du melon. Annales de Phytopathologie 11:199-207. 1979.

TOKESHI, H. & GALLI, F. Variabilidade de *Fusarium* f. *lycopersici* Sny & Hans em São Paulo. Anais da Escola Superior de Agricultura "Luiz de Queiroz" 23:217-227. 1966.

YAMAGUSHI, K., SANO, T., ARITA, M. & TAKAHASHI, M. Biocontrol of Fusarium wilt of tomato and Verticilium wilt of eggplant by non-pathogenic *Fusarium oxysporum* MT0062. Annals of the Phytopathological Society of Japan 58:188-194. 1992.

04033