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FUNGI COLONIZING ROOTS OF ZUCCHINI (*Cucurbita pepo* L. var. *giromontina*) PLANTS AND PATHOGENICITY OF *Fusarium* spp. TO ZUCCHINI SEEDLINGS

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Abstract

Zucchini is a very valuable vegetable, easy to grow both in the field and under covers but it is often attacked by soil-borne fungi. The investigations were carried out in a farm near Lublin where three zucchini cultivars: Astra, Atena and Soraya, were grown in a tunnel and in the field in 2008-2009. Plants with stem and root rot were collected for laboratory tests. Mycological analysis showed that the predominant fungi were *F. culmorum*, *F. equiseti* and *F. oxysporum*. More *Fusarium* colonies were isolated from the roots of zucchini grown in the tunnel. In the pathogenicity test, all tested isolates of F. culmorum, *F. equiseti* and *F. oxysporum* proved to be pathogenic to zucchini seedlings, causing stunting, stem and root rot. The highest disease index was noticed in the combination with *F. culmorum* isolate fck61.

Key words: zucchini, root and stem base rot, *Fusarium* spp., pathogenicity

INTRODUCTION

Zucchini (*Cucurbita pepo* L. var. *giromontina*) is a summer squash cultivated in many countries on all continents. The fruits are low in calories and contain useful amounts of vitamins B, C and PP, provitamin A, carotene, magnesium, potassium as well as flavonoid poly-phenolic antioxidants which help to scavenge harmful oxygen-derived free radicals and reactive oxygen species that play a role in aging and cancer development (Muntean et al. 2006).

Zucchini grown under greenhouse and field conditions is usually infected by pathogens specific for Cucurbitaceae but also by microorganisms pathogenic to other crops (Kimati et al. 1997; Sumner et al. 1983, 1995). The most important among them are *Fusarium* spp. surviving in the soil environment as saprotrophic mycelium and chlamydospores (Nelson et al. 1983, Jamiołkowska, 2009). *F. avenaceum* and *F. culmorum* (Smith) Sacc. are known to be pathogens of zucchini and other vegetables causing plant decay due to the colonization of their underground organs (Nelson et al. 1983). The most important pathogens seem to be *Fusarium oxysporum* Schl. responsible for plant wilting and *F. solani* (Mart.) Sacc. causing stem base and root rot (Kwaśna et al. 1991; Wagner, 2004).

The aim of the study was to investigate the occurrence and diversity of fungi colonizing zucchini plants grown in a plastic tunnel and in the field as well as to evaluate the pathogenicity of some *Fusarium* spp. to zucchini seedlings.

MATERIALS AND METHODS

Mycological analysis. The experiments were conducted in 2008 and 2009 at a horticultural farm in Łuszczów (15 km north-east from Lublin). The objects of the study were three cultivars of zucchini (*Cucurbita pepo* L. var. *giromontina*): Astra, Atena and Soraya. The seedlings were produced at the Felin Experimental Farm in Lublin. They were planted in a plastic tunnel (1st decade of May) and in the field (2nd decade of May) with a spacing of 100 cm in the row and 80 cm between rows. For each cultivar, 40 seedlings in 4 rows were planted. After soil analysis before the experiment, fertilizers were applied according to the recommendation for Cucurbitaceae. Pesticides were not applied and weeds were removed manually. The observations of plant health status were conducted at the beginning of fructification (2nd decade of July) when four plants from each combination were collected for mycological analysis. Roots were precleaned, rinsed with tap water for 20 minutes and then surface

disinfected with 50% ethanol and 0.1 % sublimate for 1 minute. The disinfected plant material was rinsed 3 times in sterile distilled water. Next, 3mm fragments were placed on mineral medium in Petri dishes as described by Jamiołkowska (2007). For each experimental treatment 10 dishes with plant material, 10 plant fragments per dish, were prepared and incubated at 20-22°C for 7 days in darkness. The obtained colonies of fungi were transferred to potato-dextrose slants (PDA-Difco) and identified to species with the available monographs.

Pathogenicity test. To estimate the harmfulness of *Fusarium* spp. to zucchini, the pathogenicity test was conducted using Mańka's method described by Wagner (1997). The seeds of cv. Astra (obtained from Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN sp. z o. o. Kraków) were precleaned, rinsed in tap water for 20 minutes and disinfected with 0.1% sodium hypochlorite for 1 minute. The disinfected seeds were rinsed 3 times in sterilized distilled water, placed into sterilized soil and covered with plastic foil to germinate. The inoculation was carried out in 0.5 l pots filled with sterilized substrate up to 3 cm below the edge. Slices of PDA overgrown with mycelium of single spore cultures of the tested fungi were put on the surface. In the control combination, slices of medium without fungus were used. When cotyledons appeared, healthy looking seeds were placed in pots on medium with or without inoculum and covered by the substrate. Zucchini seedlings were grown in a growth chamber at 24–25°C and 85% air humidity with 14 hrs photoperiod (from 6 a.m. to 8 p.m.).

In the experiment, the following isolates were tested:

– *Fusarium culmorum*: fck15, fck53, fck61,
– *Fusarium equiseti*: fek16 fek26, fek59,
– *Fusarium oxysporum*: fok1, fok19, fok35.

The isolates were selected on the basis of macro- and microscopic properties of fungi isolated in 2009 from the roots of zucchini growing in a tunnel and in the field. For each combination, 15 seedlings were tested in 5 replications.

After four weeks of plant growth in the pots, the seedlings were counted and disease severity was evaluated, using a 4-degree scale: 0 – no symptoms, 1 – small necrotic spots on all lateral roots, no symptoms on leaves or stem, 2 – necrosis on tap-root and stem base, 3 – completely rotten roots and stem base, stunting of aboveground parts of the plant. Then, the number of seedlings in each degree was computed. The disease index was calculated for each replication using Mc Kinney's formula described by Wagner (1997). Afterwards, the mean disease index was computed for each combination. To check if the symptoms were caused by the pathogen, the disinfected fragments of the affected stems and roots were placed on PDA. After seven days the growing colonies were identified.

The data were analyzed with Tukey's test at a 5% significance level using the SAS statistical system (SAS Version 9.1, SAS Inst., Cary, N.C., U.S.A.).

RESULTS AND DISCUSSION

Mycological analysis. During field observations, stem base and root rot were noticed (Fig. 1). Mycological analysis resulted in 974 isolates of fungi belonging to 20 species. From the underground parts of zucchini, *Fusarium* spp. were isolated most frequently. These fungi are important in the pathogenesis of zucchini and other vegetable crops grown under covers and in the field (Jamiołkowska, 2009; Jamiołkowska and Buczkowska, 2009). More *Fusarium* isolates were obtained from the plants grown in the tunnel than from those grown in the field. The conditions in the tunnel are conducive to *Fusarium* spp. development that depends on such abiotic factors as humidity, temperature and pH (Kacprzak and Mańka, 2000). The frequency of *Fusarium* spp. was higher in the roots of cvs. Atena and Astra than in those of cv. Soraya. The roots were colonized mostly by *F. culmorum*, *F. equiseti*, and *F. oxysporum*. The predominant species in the fungal communities from the roots proved to be *F. oxysporum* (33.2% of total number of colonies) (Table 1). Ito et al. (2005) report that the infection by *F. oxysporum* causes wilting and dying of plants due to the colonization of underground organs and xylem. Wagner et al. (2001) also proved the harmfulness of *F. oxysporum* to tomato seedlings indicating the decrease of plant fluorescence resulting from the pathogen presence. The negative impact of *F. oxysporum* might be the effect of the activity of its toxins. T-2 toxin, HT-2 toxin and diacetoxyscirpenol might have been involved in the pathogenesis (Mirocha et al. 1989).

F. equiseti was numerous in the underground parts in 2009, especially in Atena cultivated in the field (Table 1). Several authors (Zalewska and Machowicz-Stefaniak, 2004; Jamiołkowska and Wagner, 2005) reported strong pathogenic properties towards some vegetable and spice plants. Jamiołkowska (2009) proved the strong pathogenicity of *F. equiseti* to hot pepper seedlings. The fungus caused root rot and decay of plants. *F. equiseti*, as other species of the genus, produces toxins and antibiotics, especially trichothecenes and equisetin (Wheeler et al. 1999; Hestbjerg et al. 2002). *F. culmorum*, frequently isolated from roots (12% of all colonies) (Table 1), also produces mycotoxins – culmorin and trichothecenes involved in the pathogenesis (Langseth et al. 2001).

Fig. 1 Root rot of zucchini stem base (photo by A. Jamiołkowska)

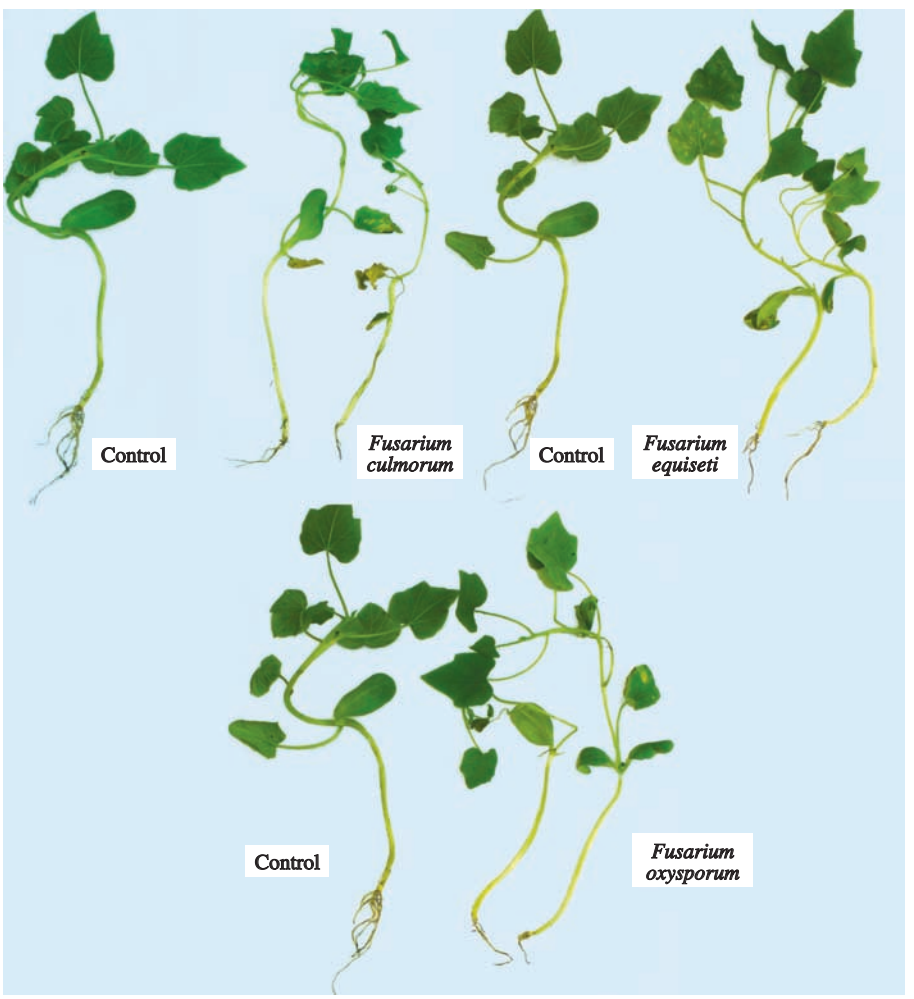

Fig. 2. Root rot and stunting zucchini seedlings inoculated with the investigated fungi (photo by A. Jamiołkowska)

Table1.
Fungi isolated from roots of zucchini (*C. pepo* var. *giromontina*) in 2008-2009

Species of fungi	Number of isolates														Total number (%)
	2008							2009							
	Tunnel			Field				Tunnel			Field				
	I	II	III	I	II	III	Total	I	II	III	I	II	III	Total	
Alternaria alternata (Fr.) Keiss		2		29		7	38								38 (4.0)
Aureobasidium pullulans (de Bary) Arnoud									1					1	1 (0.1)
Chaetomium funicolum Cooke									1					1	1 (0.1)
C*ladosporium fulvum* Cooke									6	5	10		11	32	32 (3.2)
Epicoccum purpurascens Ehr. ex Schl.									6			4		10	10 (1.0)
Fusarium avenaceum (Fr.) Sacc.		1		7	4	12	24								24 (2.5)
Fusarium culmorum (Smith) Sacc.		3		13	6	1	23	24	5	34	8	12	9	92	115 (12.0)
Fusarium equiseti (Corda) Sacc.		4		3		6	13	26	14	5	31	65	10	151	164 (16.8)
Fusarium oxysporum Schl.	74	49	53	7	35	16	234	6	37	21	9	2	14	89	323 (33.2)
Fusarium solani (Mart.) Sacc.	6	17		3	23		49								49 (5.0)
Gliocladium catenulatum Gilman et Abbott										4				4	4 (0.4)
Mucor hiemalis Wehm.			1		6	4	11	23	3	3	35	6		70	81 (8.3)
Papulaspora irregularis Hotson												4	16	20	20 (2.1)
Penicillum expansum Link ex S.F. Gray								2	1	6			3	12	12 (1.2)
Penicillium janczewskii Zaleski										1			3	4	4 (0.4)
Penicillium purpurogenum Stoll									1					1	1 (0.1)
Rhizoctonia solani Kühn.		1					1			2				2	3 (0.3)
Trichoderma hamatum (Bonord) Bain.					3	6	9		6	8			28	42	51 (5.2)
Trichoderma harzianum Rifai.						1	1	15	6	1	1	3	2	28	29 (3.0)
Trichoderma koningii Oud.		3	3	6			12								12 (1.2)
Total	80	80	57	68	77	53	415	96	87	90	94	96	96	559	974 (100)

I- cultivar Astra, II- cultivar Atena, III – cultivar Soraya

Table 2.
Disease index for zucchini seedlings inoculated with the investigated isolates of *Fusarium* spp.

Pathogen species	Isolate	Disease index
Fusarium culmorum	fck61	84.44 a
Fusarium oxysporum	fok35	77.77 b
Fusarium culmorum	fck53	75.55 c
Fusarium equiseti	fek16	68.88 d
Fusarium equiseti	fek59	66.66 e
Fusarium oxysporum	fok1	55.55 f
Fusarium oxysporum	fok19	51.11 g
Fusarium culmorum	fck15	44.44 h
Fusarium equiseti	fek26	42.22 i
–	control	0.0 j
LSD 0.05		4.73451

Values followed by the same letters do not significantly differ at 5% error (Tukey's test).

Saprotrophic fungi were also isolated: *Trichoderma* spp., *Penicillium* spp., *Papulaspora irregularis*, *Mucor hiemalis*, *Epicoccum purpurascens* and *Cladosporium fulvum* (Table 1). According to some authors (Ahmed et al. 2000; Suarez-Estrella et al. 2007), the presence of these fungi is always a positive phenomenon.

Pathogenicity test. The results confirmed the pathogenic abilities of the tested isolates of *F. culmorum*, *F. equiseti* and *F. oxysporum* (Fig. 2). The affected plants were stunted, with yellow leaves. Necrosis was noticed on the roots and some seedlings were decayed. (Fig. 2). The highest disease index was noticed for *F. culmorum* isolates fck61 and fck53. In these combinations, all seedlings were affected, showing growth stunting and root rot. Their disease index amounted to 84.44% and 75.55%, respectively, and differed significantly from the control (Table 2). The harmfulness of *F. culmorum* to many vegetables results very often in damping-off (Burgieł, 2005) and is dangerous due to polyphagous abilities of this fungus (Nelson et al. 1983). Root rot was also noticed in the combination with *F. oxysporum* isolate fok35. The disease index in this combination was 77.77%, differing significantly from the control (Table 2). For other isolates of *F. oxysporum*, the disease index was lower (51.11% and 55.55%) but significantly different from the control. In the combinations with *F. equiseti*, necrotic spots were observed on the roots as well as other symptoms. The disease index ranged from 42.22% (fe26) to 66.66% (fek16) and it was significantly higher than in the control (Table 2). *F. equiseti* is regarded as the cause of damping-off, root and fruit rot not only in Cucurbitaceae (Adams et al. 1987; Vigliola, 1994) but also in pepper and other vegetable plants (Jamiołkowska and Wagner, 2005; Jamiołkowska, 2008). The results of the tests depended not only on the species but also on individual isolates. This can be explained by the biodiversity that occurs in every population of organisms (Wagner, 1997).

CONCLUSIONS

1. *Fusarium oxysporum* and *F. equiseti* predominated among the fungi isolated from zucchini roots.
2. *F. oxysporum* was the most frequent on the roots of zucchini grown in the tunnel, while *F. equiseti* – on the roots of zucchini grown in the field.
3. The pathogenicity tests proved the ability of *F. culmorum*, *F. equiseti* and *F. oxysporum* to inhibit the growth of zucchini seedlings.

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Grzyby zasiedlające korzenie roślin cukinii (*Cucurbita pepo* L. var. *giromontina*) oraz patogeniczność grzybów rodzaju *Fusarium* dla siewek cukinii

Streszczenie

Cukinia jest bardzo wartościowym warzywem, łatwym do uprawy zarówno w polu, jak i pod osłonami, ale często jest porażana przez grzyby zasiedlające glebę. Badania prowadzono w gospodarstwie w okolicy Lublina, w którym trzy odmiany cukinii: Astra, Atena i Soraya były uprawiane w tunelu foliowym i w polu w latach 2008-2009. Rośliny z objawami nekrozy postawy pędu i korzeni pobierano do badań laboratoryjnych. Analiza mikologiczna wykazała, że dominującymi gatunkami były: *Fusarium culmorum*, *F. equiseti* and *F. oxysporum*. Więcej kolonii grzybów rodzaju *Fusarium* uzyskano z korzeni cukinii uprawianej w tunelu. W teście patogeniczności wszystkie badane izolaty *F. culmorum*, *F. equiseti* i *F. oxysporum* okazały się patogeniczne dla siewek cukinii, powodując zahamowanie wzrostu i zgniliznę korzeni i podstawy pędu. Najwyższy wskaźnik chorobowy zanotowano w kombinacji z izolatem fck61 *F. culmorum*.