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Interaction of *Fusarium oxysporum* (Schltdl.) W. C. Snyder et H. N. Hansen with other root-associated fungi

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Fusarium oxysporum is one of the most widespread root-associated fungi. It was detected in the roots of rye, wheat, barley, as well as of ten species of concomitant wild-growing plants. The distribution frequency of this fungus reached 7.1%. The interaction *in vitro* between *F. oxysporum* and nine most widespread root-associated fungi were diverse and strongly determined by the species and isolate of a fungus. Fungistatic trophic and mutual antagonisms dominated among four ascertained interaction forms of microorganisms. The most evident antagonism was revealed between the tested isolates of *F. oxysporum* with *Aspergillus ochraceus, Bipolaris sorokiniana* and *Gliocladium catenulatum.* All the fungi studied (except *B. sorokiniana*) suppressed the growth of *F. oxysporum* mycelium, more or less overgrew and even destroyed it. The suppression of *Rhizoctonia* spp. was particularly strong. The growth of *F. oxysporum* is effected by other root-associated fungi. *F. oxysporum* can be ousted by most of them. The interaction of *F. oxysporum* with other fungi occurring in the injured roots can influence the pathological process and the damage caused by it. The knowledge of fungal interactions is essential for a long-term success of biocontrol.

Key words: *Fusarium oxysporum*, root-associated fungi, interaction, cereal, wild-growing plants

INTRODUCTION

F. oxysporum (Schltdl.) W. C. Snyder et H. N. Hansen is the most economically important fungus of the genus *Fusarium* Link: Fr. It occurs chiefly as a soil saprotrophe, being distributed worldwide and extremely common in a wide range of soils. Numerous strains of this species are the causal agents of vascular wilts, damping-off and rots on hundreds of different host plants. More than a hundred formae speciales and races exist within *F. oxysporum* which are more or less host-specific on woody plants, field crops, vegetables, ornamentals [1–4]. Among them there are also strains responsible for severe lesions of cereals.

F. oxysporum, as most soil pathogens, occurs in association with other fungi and is observed on lesions on the same plant. These fungi, participating in the process of plant injury, can influence the growth and distribution of each other. The establishment of interactions among them is of particular significance for the control of pathogens' harmfulness and disease suppression.

The present work was undertaken to ascertain the distribution of *F. oxysporum* in roots of cereals and concomitant wild-growing plants and to study its interactions with other most widespread root-associated fungi.

MATERIALS AND METHODS

The investigation was carried out in 2000. The injured plants were collected in 26 localities of Akmenė, Mažeikiai, Raseiniai, Šilalė and Vilnius regions. Root-associated fungi were isolated from the roots of cereals (*Secale cereale* L., *Triticum aestivum* L., *Hordeum distichon* L., *Avena sativa* L.) and 26 concomitant wild-growing plant species (*Agropyron repens* (L.) P. B., *Arthemisia vulgaris* L., *Centaurea cyanus* L., *Cirsium arvense* Scop., *Dactylis glomerata* L., *Chenopodium album* L., *Ch. hybridum* L., *Erigeron annuus* (L.) Pers., *Euphorbia helioscopia* L., *Medicago lupulina* L., *Melilotus albus* Med., *Mentha arvensis* L., *Myosotis arvensis* (L.) Hill, *Plantago major* L., *Phleum pratense* L., *Rumex acetosella* L., *Taraxacum officinale* Weber, *Sonchus arvensis* L., *Trifolium arvense* L., *T. hybridum* L., *T. pratense* L., *T. repens* L., *Tripleurospermum perforatum* (Mérat) M. Laínz, *Tussilago farfara* L., *Vicia angustifolia* Grufb., *V. sativa* L.). In every locality five plants of each species were collected before cereal harvest time.

Pure cultures of fungi were isolated employing the generally applied methods [2, 5, 6]. The fungal species were identified on the basis of their cultural and morphological characteristics according to [2, 3, 7–9]. The distribution frequency (DF) of separate fungi species and the percentage they made up of the total number of isolates were calculated [10]. Single spore cultures of the selected isolates were transferred onto malt extract agar medium (MEA) in tubes for preservation and are deposited in the fungi collection of the BILAS.

Fifty four isolates of nine micromycete species (*Fusarium avenaceum* (Fr.) Sacc. 10 isolates, *F. culmorum* (Wm. G. Sm.) Sacc. 6, *F. sambucinum* Fuckel var. *minus* Wollenw. 7, *Bipolaris sorokiniana* (Sacc.) Shoemaker 6, *Rhizoctonia* spp. 5, *Aspergillus ochraceus* K. Wilh. 3, *Chaetomium globosum* Kunce: Fr. 2, *Gliocladium catenulatum* J. C. Gilman et E. V. Abbott 9, *Talaromyces flavus* (Klöcker) Stolk et Somson 6), widespread in roots of the plants tested, were selected to investigate their interaction with thirteen isolates of *F. oxysporum*. The research on the interaction of micromycetes was carried out *in vitro* in a dual-plate assay on the MEA, evaluating them after 5, 10, 15 and 20 days of growth. For evaluation of the interaction between micromycetes, forms of microorganism interaction proposed by I. Babushkina [11] were applied.

RESULTS AND DISCUSSION

Our investigation confirmed the data of other researchers [12–16] that the whole complex of fungi is associated with the roots of injured plants. Forty-eight taxa of root-associated micromycetes, belonging to 26 genera (*Acremoniella* Sacc., *Acremonium* Link: Fr., *Alternaria* Nees, *Apiosordaria* Arx et W. Gams, *Arthrinium* Kunze: Fr. in Kunze et J. C., *Aspergillus* P. Michel ex Link: Fr., *Bipolaris* Shoemaker, *Cylindrocarpon* Wollenw., *Chaetomium* Kunze: Fr., *Cladosporium* Link: Fr., *Fusarium* Link: Fr., *Gliocladium* Corda, *Nigrospora* Zimm., Papulaspora Preuss, *Penicillium* Link: Fr., *Periconia* Tode:Fr., *Phoma* Sacc., *Rhizoctonia* DC, *Sepedonium* Link: Fr., *Stemphyllium* Wallr., *Ulocladium* Preuss, *Talaromyces* C. R. Benj., *Thielaviopsis* Went, *Trichoderma* Pers.: Fr., *Zygodesmus* Corda, *Zygorrhynchus* Vuill.) were ascertained in roots of cereals and concomitant wild-growing plants [17]. There were pathogens actively participating in the process of root injury, as well as saprotrophes, which are involved in root destruction not being the cause of injury. Fungi of the genera *Fusarium* (DF 64.7%), *Phoma* (DF 13.4%), *Rhizoctonia* (DF 10.2%), *Talaromyces* (DF 10.0%), *Alternaria* (DF 6.1%) and *Gliocladium* (DF 5.8%) were most prevalent and made up 74.2% of the total amount of the isolates detected in roots of the plants studied. The richest species composition (15 species and 2 varieties) was determined in the genus *Fusarium*. *F. sambucinum* var. *minus*, *F. culmorum*, *F. avenaceum* and *F. oxysporum* predominated and amounted to 69.2% of all *Fusarium* isolates [17]. *F. oxysporum* was one of the most frequently distributed micromycetes, making up 10.9% of the total number of *Fusarium* and 4.8% of all isolates. It was identified in roots of *Secale cereale*, *Triticum aestivum*, *Hordeum distichon* as well as of ten concomitant wild-growing plant species (*Agropyron repens, Chenopodium album, Medicago lupulina, Melilotus alba, Phleum pratense, Trifolium arvense, T. pratense, Tussilago farfara, Vicia angustilolia, V. sativa*). The distribution frequency of this fungus in the roots of cereals and wild-growing plants was similar and reached on the average 7.9% and 6.7%, respectively. Most frequently in the cereal crops it was revealed in the roots of barley (DF 13.5%), more rarely – in wheat roots (DF 10.0%) and particularly rarely in rye roots (DF 2.9%). The highest frequency (80.0%) of *F. oxysporum* distribution was established in *Trifolium arvense* roots. The distribution frequency of this fungus in the roots of *Medicago lupulina* and *Vicia sativa* amounted to 20.0% and in the roots of other wild-growing plants to 4.0–15.0%.

Analysis of the interaction between *F. oxysporum* and nine most widespread root-associated micromycetes demonstrated the diversity and strong dependence of these interactions upon the species and the isolate of fungus. Four forms of micromycete interaction were revealed:

no impact – the fungus overgrows the pathogen, but the growth of the latter does not stop; both of them grow on;

fungistatic trophic antagonism – the fungus overgrows the pathogen, the growth of the latter stops;

territorial antagonism – the fungus overgrows the pathogen; a zone where pathogen does not grow forms;

mutual antagonism – both fungi negatively influence the growth of each other; the zone could form where neither of the fungi studied grows (Table).

The forms of fungistatic trophic and mutual antagonisms prevailed, amounting to 57.4% and 38.0%, respectively. Territorial antagonism was revealed in 1.9% and no impact between the fungi in 2.7% of the cases.

The fungistatic trophic antagonism dominated in the interaction between the tested isolates of *F. oxysporum* with *T. flavus* (93.1%), as well as with *A. ochraceus* (83.3%), *F. avenaceum* (70.0%), *F. culmorum* (55.1%) and *Rhizoctonia* spp. (50.0%). This form of interaction was also frequent between *F. oxysporum* and *G. catenulatum, C. globosum, B. so-*

Table. **Forms of interaction between separate isolates of *Fusarium oxysporum* and other root-associated fungi**

Micromycetes	Isolates	*Fusarium oxysporum* isolates												
		10039	10281	10499	10226	10348	10575	10510	10536	10196	10193	10081	10103	10098
F. avenaceum	10395	–	II	–	–	II	II	II	–	II	–	–	II	–
	10141	–	II	–	–	II	II	II	–	II	–	–	IV	–
	10038	–	IV	–	–	IV	IV	II	–	II	–	–	II	–
	10201	–	II	–	–	IV	II	II	–	II	–	–	IV	–
	10169	–	IV	–	–	II	II	II	–	IV	–	–	II	–
	10189	–	I	–	–	I	II	II	–	II	–	–	II	–
	10231	–	II	–	–	IV	II	II	–	II	–	–	IV	–
	10244	–	II	–	–	IV	II	II	–	II	–	–	IV	–
	10093	–	II	–	–	IV	II	II	–	II	–	–	II	–
	10053	–	II	–	–	IV	IV	II	–	II	–	–	IV	–
F. culmorum	10230	II	II	IV	IV	IV	II	II	IV	II	IV	II	IV	II
	10580	II	II	II	IV	IV	IV	II	II	II	IV	IV	II	IV
	10398	IV	IV	IV	II	IV	II	IV	IV	IV	II	I	IV	IV
	10302	II	II	IV	IV	IV	II	II	IV	I	II	II	II	II
	10316	IV	II	IV	II	IV	II	II	IV	I	II	II	II	II
	10326	IV	II	IV	II	IV	II	II	IV	I	II	II	II	II
F. sambucinum	10034	–	IV	–	–	II	II	II	–	II	–	–	IV	–
var. *minus*	10215	–	IV	–	–	II	II	II	–	II	–	–	IV	–
	10444	–	IV	–	–	IV	IV	IV	–	II	–	–	IV	–
	10418	–	IV	–	–	II	IV	IV	–	IV	–	–	IV	–
	10271	–	IV	–	–	IV	IV	IV	–	IV	–	–	II	–
	10311	–	IV	–	–	IV	IV	IV	–	IV	–	–	II	–
	10185	–	IV	–	–	IV	II	II	–	II	–	–	II	–
Rhizoctonia spp.	10051	II	II	II	II	IV	II	–	II	II	IV	–	IV	–
	10633	IV	III	II	IV	II	II	IV	IV	IV	I	I	II	IV
	10478	IV	II	II	IV	II	II	II	II	I	II	II	IV	II
	10110	IV	II	II	II	II	II	II	II	IV	IV	IV	IV	II
	10252	II	IV	I	IV	II	IV	IV	IV	IV	IV	II	IV	IV
Bipolaris	10289	III	II	II	IV	IV	II	IV	II	II	II	Ii	II	–
sorokiniana	10364	IV	IV	IV	IV	IV	IV	IV	IV	IV	II	IV	IV	–
	10409	IV	II	IV	IV	IV	IV	IV	IV	II	II	IV	IV	–
	10413	II	IV	IV	II	IV	IV	IV	IV	IV	II	II	II	–
	10232	IV	II	IV	II	II	IV	IV	IV	IV	II	II	II	–
	10276	II	II	II	IV	II	IV	II	IV	II	II	IV	IV	–
Gliocladium	10050	–	III	–	–	IV	II	II	–	II	–	–	IV	–
catenulatum	10363	–	IV	–	–	IV	II	II	–	IV	–	–	IV	–
	10337	–	III	–	–	IV	II	II	–	II	–	–	III	–
	10323	–	IV	–	–	IV	IV	IV	–	IV	–	–	IV	–
	10275	–	III	–	–	IV	II	II	–	II	–	–	IV	–
	10452	–	II	–	–	IV	II	II	–	II	–	–	IV	–
	10235	–	III	–	–	IV	II	II	–	II	–	–	IV	–
	10044	–	III	–	–	IV	II	II	–	II	–	–	IV	–
	10250	–	IV	–	–	IV	II	II	–	II	–	–	III	–
Talaromyces	10360	II	II	II	II	II	II	II	II	II	II	II	II	–
flavus	10628	II	II	II	II	II	II	II	II	II	II	II	II	–
	10504	II	II	II	IV	II	II	II	II	I	II	II	II	–
	10392	II	II	II	II	II	II	II	II	II	II	II	II	–
	10458	IV	II	II	II	II	II	II	II	II	II	IV	II	IV
	10467	II	II	II	II	II	II	II	II	II	II	II	II	II
Aspergillus	10352	–	II	–	–	II	II	II	–	II	–	–	IV	–
ochraceus	10290	–	II	–	–	II	II	II	–	II	–	–	IV	–
	10433	–	II	–	–	II	II	II	–	II	–	–	IV	–
Chaetomium	10623	IV	IV	IV	IV	IV	II	IV	IV	II	IV	IV	II	II
globosum	10604	II	II	II	II	IV	II	II	I	II	I	IV	IV	IV

I – no impact; II –- fungistatic trophic; III – territorial; IV – mutual antagonisms.
"–" – the interactions were not investigated.

rokiniana, *F. sambucinum* var. *minus* and made up more than 40%. Mutual antagonism prevailed in the interaction between *F. oxysporum* and *F. sambucinum* var. *minus, B. sorokiniana* and *C. globosum* (61.9%, 56.9% and 50.0% of all cases, respectively), while with other fungi it amounted to 5.6–26.7%. Territorial antagonism was detected in the interaction of *F. oxysporum* with *G. catenulatum* (13.0%), *Rhizoctonia* spp. (1.6%) and *B. sorokiniana* (1.4%) only. No impact was ascertained in the interaction between separate isolates of *F. oxysporum* and *C. globosum*, *Rhizoctonia* spp., *F. culmorum* and *F. avenaceum* (7.7%, 6.5%, 5.1% and 3.3% of the cases, respectively).

Most of the fungi tested were found to suppress the growth of *F. oxysporum*.

It was ascertained that the growth of *F. oxysporum* was inhibited by all *F. avenaceum* isolates. The *F. oxysporum* isolates 10348 and 10103 were most resistant to the impact of *F. avenaceum.* Mutual antagonism was revealed between these *F. oxysporum* isolates and the isolates of *F. avenaceum*; they inhibited the growth of each other, still the mycelium was not overgrown and a distinct border of the colonies' contiguity remained. The resistance of all other *F. oxysporum* isolates was significantly weaker: their growth was stopped and the colonies were overgrown. The *F. oxysporum* isolate 10281 was most sensitive to *F. avenaceum.* Fungistatic trophic antagonism prevailed among the isolates of *F. oxysporum* and *F. avenaceum* studied (Figure).

The interaction of *F. oxysporum* and *F. culmorum* depended upon their isolates, although the majority of *F. culmorum* isolates grew more intensively than *F. oxysporum.* The *F. culmorum* isolate 10302 abundantly overgrew all *F. oxysporum* isolates. The *F. oxysporum* isolates 10226, 10575, 10536, 10196, 10103, 10098 were most resistant, while the isolates 10039 and 10281 were most sensitive to *F. culmorum.* The fungistatic trophic antagonism prevailed in the interactions between *F. oxysporum* and *F. culmorum* as well.

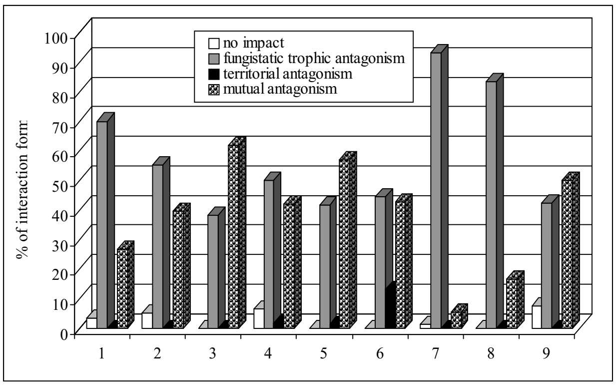

Figure. Forms of interaction of *Fusarium oxysporum* with other root-associated fungi (%) (1 – *Fusarium avenaceum*, 2 – *F. Culmorum*, 3 – *F. sambucinum* var. *minus*, 4 – *Rhizoctonia* spp., 5 – *Bipolaris sorokiniana*, 6 – *Gliocladium catenulatum*, 7 – *Talaromyces Flavus*, 8 – *Aspergillus ochraceus*, 9 – *Chaetomium globosum*)

Two forms of interaction between *F. oxysporum* and *F. sambucinum* var. *minus* were ascertained. The mutual antagonism predominated between them, amounting up to 61.9% of the cases (Figure). It was most evident between the *F. oxysporum* isolates 10281, 10575, 10510 and 10196 with the *F. sambucinum* var. *minus* isolate 10418. The isolates of *F. sambucinum* var. *minus* suppressed the growth of *F. oxysporum*, nevertheless the suppression was isolate-dependent. The highest suppression was ascertained for the *F. sambucinum* var. *minus* isolates 10034, 10215 and 10185, while the *F. oxysporum* isolate 10103 was most resistant to their impact.

All *Rhizoctonia* spp. isolates inhibited the growth of the majority of *F. oxysporum* isolates and more or less overgrew their mycelium. The *Rhizoctonia* spp. isolates 10478 and 10110 completely destroyed the mycelium of *F. oxysporum.* The *F. oxysporum* isolates 10499, 10575 and 10536 were most sensitive and the isolates 10226, 10196, 10193, 10281, 10103 most resistant to the impact of *Rhizoctonia* spp. Four forms of interaction were detected between the isolates of these fungi (Table).

Fungistatic trophic antagonism was most prevalent in the interactions between *F. oxysporum* and *T. flavus*, amounting even to 93.1% of all cases (Figure). All *T. flavus* isolates more or less overgrew and destroyed the mycelium of *F. oxysporum,* however, the *T. flavus* isolate 10467 showed the highest suppression and abundantly overgrew and destroyed the mycelium of all *F. oxysporum* isolates. The *F. oxysporum* isolates 10226 and 10348 were most resistant to the impact of *T. flavus.*

Fungistatic trophic antagonism prevailed in the interactions between *F. oxysporum* and *A. ochraceus* as well. It amounted up to 83.3% of all cases (Figure). All *A. ochraceus* isolates stopped the growth of *F. oxysporum,* abundantly overgrowing its mycelium. The *F. oxysporum* isolates 10348 and 10103 demonstrated the highest resistance to the impact of *A. ochraceus.* The antagonism was evident in the interactions between these *F. oxysporum* isolates and *A. ochraceus*.

The form of interaction between *F. oxysporum* and *C. globosum* mostly depended upon the *C. globosum* isolate. Mutual antagonism dominated in the interactions between the isolates of *F. oxysporum* with the *C. globosum* isolate 10623, while the fungistatic trophic antagonism with *C. globosum* isolate 10604 (Table). The *C. globosum* isolate 10623 inhibited the growth of the majority of *F. oxysporum* isolates, abundantly overgrowing them. The *F. oxysporum* isolates 10226, 10348 and10081 were most resistant to the impact of *C. globosum* and even suppressed the growth of the *C. globosum* isolate 10604.

The interaction between *F. oxysporum* and *G. catenulatum* depended upon the isolates as well. *F oxysporum* was more or less overgrown by *G. catenulatum* in most cases. The *G. catenulatum* isolate 10452 abundantly overgrew all *F. oxysporum* isolates. The *F. oxysporum* isolate 10348 was most resistant and the isolates 10575, 10510 and 10196 were most sensitive to the impact of *G. catenulatum.* An evident mutual antagonism was ascertained between all *F. oxysporum* isolates and the *G. catenulatum* isolate 10323.

Most of the *F. oxysporum* and *B. sorokiniana* isolates inhibited the growth of each other, however, *F. oxysporum* grew more intensively. The *F. oxysporum* isolates 10039, 10226 and 10103 showed the most evident suppression on the growth of *B. sorokiniana* and abundantly overgrew all its isolates at the edge of their colonies. The *B. sorokiniana* isolates 10232 and 10276 were most sensitive to the impact of *F. oxysporum* and were overgrown by all *F. oxysporum* isolates. The most evident antagonism was revealed between the *F. oxysporum* isolate 10348 and all *B. sorokiniana* isolates.

The results of this investigation indicated that the interaction of *F. oxysporum* with other root-associated fungi is diverse and depends upon the species and particularly on the micromycete isolate. The majority of fungi suppressed the growth of *F. oxysporum.* They stopped it, more or less overgrew or even destroyed the mycelium of *F. oxysporum* isolates. Only the growth of *B. sorokiniana* was suppressed by *F. oxysporum.* The antagonism was most evident between some *F. oxysporum* isolates and the tested isolates of *A. ochraceus, B. sorokiniana, G. catenulatum* and *F. sambucinum* var. *minus* isolate 10418. The *F. oxysporum* isolates 10226 and 10348 were most resistant while the isolates 10039 and 10281 most sensitive to the impact of the root-associated fungi. The highest suppression of the growth of *F. oxysporum* was shown by *Rhizoctonia* spp.: their isolates 10478 and 10110 destroyed the mycelium of *F. oxysporum* and demonstrated a fungistatic or even a fungicidal impact.

The growth intensity of *F. oxysporum* is affected by other root-associated fungi. *F. oxysporum* can be ousted by most of them. The interaction between *F. oxysporum* with other fungi occurring in the injured roots can influence the pathological process and the damage caused by it. The knowledge of fungal interactions is essential for a long-term success of biocontrol.

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***Fusarium oxysporum* (SCHLTDL.) W. C. SNYDER ET H. N. HANSEN IR KITŲ GRYBŲ, APTINKAMŲ AUGALŲ ŠAKNYSE, TARPUSAVIO SĄVEIKA**

Santrauka

Tyrimai parodė, kad *Fusarium oxysporum* yra vienas labiausiai pažeistose augalų šaknyse paplitusių grybų. Jis buvo nustatytas rugių, kviečių, miežių, taip pat dešimties rūšių šalia augančių augalų šaknyse. Jo paplitimo dažnis siekė 7,1%. *F. oxysporum* tarpusavio sąveika *in vitro* su devyniais dažniausiai augalų šaknyse aptinkamais grybais buvo labai įvairi ir priklausė nuo mikromiceto rūšies bei izoliato. Nustatytos keturios grybų tarpusavio sąveikos formos, tarp kurių vyravo fungistatinis trofinis ir tarpusavio antagonizmas. Ryškiausiu antagonizmu pasižymėjo *F. oxysporum* ir *Aspergillus ochraceus, Bipolaris sorokiniana* bei *Gliocladium catenulatum* izoliatai. Visi tirti grybai (išskyrus *B. sorokiniana*) stabdė *F. oxysporum* grybienos augimą, daugiau ar mažiau ją apaugo ir net suardė. Labiausiai *F. oxysporum* augimą slopino *Rhizoctonia* spp. Grybai, kartu aptinkami pažeistų augalų šaknyse, veikia *F. oxysporum* augimą. Daugelis jų gali jį nustelbti ir taip paveikti šio grybo sukeliamą patologinį procesą ir jo žalingumą. Grybų tarpusavio sąveikos tyrimai yra labai svarbūs rengiant biologinės apsaugos priemones.