

Impact of selected antagonistic fungi on *Fusarium* species – toxigenic cereal pathogens

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Fusarium-ear blight is a destructive disease in various cereal-growing regions and leads to significant yield and quality losses for farmers and to contamination of cereal grains with mycotoxins, mainly deoxynivalenol and derivatives, zearalenone and moniliformin. *Fusarium* pathogens grow well and produce significant inoculum on crop residues. Reduction of mycotoxins production and pathogen sporulation may be influenced by saprophytic fungi, exhibiting antagonistic effect.

Dual culture bioassays were used to examine the impact of 92 isolates (belonging to 29 fungal species) against three toxigenic species, i.e. *Fusarium avenaceum* (Corda) Saccardo, *F. culmorum* (W.G.Smith) Saccardo and *F. graminearum* Schwabe. Both *F.culmorum* and *F. graminearum* isolates produce trichothecene mycotoxins and mycohorhormone zearalenone and are considered to be the most important cereal pathogens worldwide. Infection with those pathogens leads to accumulation of mycotoxins: deoxynivalenol (DON) and zearalenone (ZEA) in grains. *Fusarium avenaceum* isolates are producers of moniliformin (MON) and enniatins. Isolates of *Trichoderma* sp. were found to be the most effective ones to control the growth of examined *Fusarium* species. The response of *Fusarium* isolates to antagonistic activity of *Trichoderma* isolates varied and also the isolates of *Trichoderma* differed in their antagonistic activity against *Fusarium* isolates. The production of MON by two isolates of *F. avenaceum* in dual culture on rice was reduced by 95% to 100% by *T. atroviride* isolate AN 35. The same antagonist reduced the amount of moniliformin from 100 µg/g to 6.5 µg/g when inoculated to rice culture contaminated with MON, which suggests the possible decomposition of this mycotoxin.

Key words: antagonistic fungi, *Fusarium*, moniliformin, *Trichoderma*

INTRODUCTION

Fusarium species are cosmopolitan necrotrophic pathogens of cereals, pulse crops and many other plants, important in Agricultural and forest landscape. *Fusarium* ear (head) blight (FHB=scab) has been known for more than 100 years and is one of the important diseases of wheat and other small grain cereals. In Europe it is caused mostly by *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*. Other *Fusarium* species are less important due to their lower incidence and aggressiveness (Arseniuk et al. 1999; Bai, Shaner 1994; Parry et al. 1995; Chełkowski 1998; Wakuliński, Chełkowski 1993; Bottalico, Perrone 2002). FHB leads to significant loss of grain yield and quality. Several fungal secondary metabolites, e.g. deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA) and moniliformin (MON) and also their derivatives may contaminate cereal grains (McMullen et al. 1997; Jones, Mirocha 1999; Bottalico 1998; Chełkowski 1998). The disease has been re-emerging in many cereal-growing regions worldwide (Parry et al. 1995; McMullen et al. 1997; Jones, Mirocha 1999; Bottalico, Perrone 2002).

Three chemotypes are common within *F. culmorum* and *F. graminearum* species: nivalenol (NIV), 3acetyl-deoxynivalenol (3-AcDON) and 15acetyl-deoxynivalenol (15-AcDON). These chemotypes can be identified by chemical analyses of fungal cultures or by chemotype-specific DNA markers (Quarta et al.2005).

Fusarium culmorum and *F. graminearum* survive saprophytically on plant residues after harvest and serve as a source of inoculum for the subsequent year. Both species colonize particularly frequently maize stalks and *F. graminearum* produces significant amounts of ascospores and conidia (Sutton 1982). *Fusarium avenaceum* is a cosmopolitan species and one of the most important ones in agriculture and the forest environment in the moderate climatic zone (Nelson et al. 1983; Kwaśna et al. 1991; Leslie, Summerell 2006).

Simultaneous co- occurrence of the three mycotoxins: moniliformin, deoxynivalenol and nivalenol was found in high percentage of positive samples (33% and 35%), during 1998 and 1999 epidemics of FHB in wheat in Poland (Tomczak et al. 2002).

There are few species among soil microorganisms antagonistic to *Fusarium*, able to reduce their population in soil and debris. Several fungal species were examined for the ability to reduce the inoculum potential of *Fusarium* pathogens, mainly by reduction of biomass in plant residues colonized by *Fusarium* (Łacicowa, Pięta 1985; Ligitt et al. 1997; Luongo et al. 2005; Lutz et al. 2003; Dawson et al. 2004). Several antagonists were found to reduce infection of ears and accumulation of DON in wheat grains (Ligitt et al. 1997; Dawson et al. 2004). *Clonostachys*, *Gliocladium* and *Trichoderma* species reduced the colonization of wheat and maize by pathogenic *Fusarium* species and suppressed the sporulation of the latter (Woo et al. 2005; Luongo et al. 2005). There is only scarce information on fungal antagonists of toxigenic *Fusarium* species and their ability to reduce the production of mycotoxins in solid substrates. *Trichoderma* species have been examined for more than 50 years and are known as highly effective in biological control of a wide range of plant pathogens of soil origin. They are also known to produce over 120 secondary metabolites, including antifungal metabolites (Kubicek, Harman 1998; Woo et al. 2005).

Cooney et al. (2001) showed that 6-pentyl-alpha-pyrone (6PAP), which is a metabolite of *T. harzianum* (THF2/3), can reduce the production of deoxynivalenol

by *F. graminearum* on agar medium by 66 to 81%. The authors developed an agar bioassay technique to examine *Fusarium-Trichoderma* interaction on their secondary metabolites level.

T. harzianum type 4 and named *T. aggressivum* has been found recently to be competitor of commercial mushroom *Agaricus bisporus*, known to be the cause of green mold and significant losses to mahrooms producers (Savoie, Mata 2003).

The aim of this paper was to examine the effect of 29 fungal species isolates (including *Trichoderma*) originated from soil, compost and cereals, on the growth of three toxigenic *Fusarium* species in dual cultures and a mutual interaction between the antagonists and *Fusarium* isolates in bioassays, and to examine the reduction of moniliformin production in dual cultures of *F. avenaceum* with selected effective *Trichoderma* antagonists.

MATERIAL AND METHODS

Fungal isolates. Isolates of tested fungi originated from culture collections of the Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland (KF and AN), the Institute of Science of Food Production, Bari, Italy (ITEM) and the Department of Forest Pathology, Agricultural University, Poznań, Poland (Tab. 1). Fungal isolates originating from soil, compost and cereal grains were maintained on a synthetic low nutrient agar SNA (Nirenberg 1981; Kwaśna et al. 1991). Identification and nomenclature of *Trichoderma* and *Gliocladium* isolates was followed according to Gams and Bissett 1998.

***Fusarium* isolates – species and chemotype identification.** Species were identified based on their macroconidia structure using an Olympus optical microscope at a 400-500x magnification according to Nelson et al. (1983), Kwaśna et al. (1991) and Nirenberg (1981) manuals. Species identification was also performed using DNA markers. Fungal DNA was extracted using the modified CTAB method (Chełkowski et al. 2002). PCR amplification mixture consisted of 0.5 U of Taq DNA polymerase (Finnzymes), 2.5 µl of PCR buffer, 12.5 pmol of forward/reverse primers, 2.5 mM of each dNTP and about 10 ng of fungal DNA. For the identification of *F. culmorum*, *F. graminearum* and *F. avenaceum* species, the following markers were used: Fc01 (570 bp), UBC85 (332 bp), Fg16N (280 bp), Fa (920 bp) (Nicholson et al. 1998; Schilling et al. 1996, Doohan et al. 1998) and to identify 3Ac-DON, 15Ac-DON and NIV chemotypes Tri3 (708 and 354 bp) and Tri7 (625 bp) markers were used (Quarta et al. 2005).

Dual culture bioassays. A modified bioassay of Mańka (1974) was applied to examine growth reduction of three toxigenic species isolates: *F. avenaceum*, *F. culmorum* and *F. graminearum*. A *Fusarium* isolate and a tested fungus (an antagonist) were inoculated onto 9 cm Petri dishes at a distance of 1cm on potato dextrose agar (PDA). As a control each fungus was cultured separately. Agar plates were then incubated at 25 °C at diffused daylight. Inhibition of *Fusarium* growth by the antagonist was evaluated using a modified Mańka (1974) scale –8 to +8 presented in figure 1, where 0 indicated no inhibition and +8 a total inhibition, with the *Fusarium* mycelium in >95% overgrown by the antagonist. Growth rate of each control culture was measured after 2-7 days. At least two replications were performed.

Table 1
Growth rate of 92 candidate antagonistic isolates from soil, compost and cereals
and *Fusarium* isolates on PDA medium in mm/day at 25 °C

Species group	Species of potential competitors	No. of isolates	Accession code (AN)	Mean growth rate mm/day
I	<i>Trichothecium roseum</i> (Pers; Fries) Link	1	27	10
II	<i>Trichoderma viride</i> Pers. & Fries	12	14, 15, 16, 17, 18, 45, 46, 47, 48, 51, 52, 61	11-21
III	<i>Trichoderma pseudokoningii</i> Rifai	1	60	21
IV	<i>Trichoderma polysporum</i> Link: Pers	1	55	20
V	<i>Trichoderma longibrachiatum</i> Rifai	1	22	21
VI	<i>Trichoderma koningii</i> Oudemans	4	49, 59, 65, 66	21
VII	<i>Trichoderma harzianum</i> Rifai	12	3, 4, 5, 6, 13, 53, 54, 58, 62, 63, 64, 94	21-31
VIII	<i>Trichoderma hamatum</i> (Bon) Bainier	2	21, 56	20-21
IX	<i>Trichoderma citrinoviride</i> Bissett	1	89	31
X	<i>Trichoderma aureoviride</i> Rifai	1	57	21
XI	<i>Trichoderma atroviride</i> Karsten	4	19, 35, 50, 90	21-30
XII	<i>Trichoderma asperellum</i> Samuels, Lieckf. & Nirenberg	1	93	25
XIII	<i>Pythium</i> sp.	1	72	7
XIV	<i>Paecilomyces carneus</i> (Duché & R. Heim) A.H.S. Br. & G. Sm.	1	42	1
XV	<i>Paecilomyces farinosus</i> Holmskjold	1	41	1
XVI	<i>Mortierella</i> sp.	1	71	14
XVII	<i>Melanospora fimicola</i> E.C. Hansen	3	29, 31, 44	6-21
XX	<i>Idriella bollei</i> Sprague	1	83	18
XVIII	<i>Hypocrea hunua</i> Dingley	1	20	21
XIX	<i>Gliocladium virens</i> J.H. Mill., Giddens & A.A. Foster	6	68, 69, 70, 73, 74, 75	20-21
XXI	<i>Gliocladium roseum</i> Bainier	4	24, 25, 26, 78	2-7
XXII	<i>Gliocladium catenulatum</i> Glim & Abbot	4	23, 32, 43, 88	7-9
XXIII	<i>Gilmaniella</i> sp.	4	67, 76, 77, 79	5-7
XXIV	<i>Fusarium flocciferum</i> Corda	3	84, 85, 86	7-13
XXV	<i>Fusarium equseti</i> (Corda) Sacc.	7	1, 2, 7, 9, 10, 11, 12	5-15
XXVI	<i>Epicoccum nigrum</i> Link	6	36, 37, 38, 39, 40, 87	2-12
XXVII	<i>Cladosporium cladosporioides</i> (Fr.) de Vries	2	81, 82	17-19
XXVIII	<i>Chaetomium cochlioides</i> Palliser	4	28, 30, 33, 34	12-21
XXIX	<i>Acremonium</i> sp.	1	80	5
	<i>Fusarium</i> sp.			
XXX	<i>Fusarium avenaceum</i> (Corda) Sacc.	2		15
XXXI	<i>Fusarium culmorum</i> (W.G.Smith) Sacc.	3		19
XXXII	<i>Fusarium graminearum</i> Schwabe	13		9-20

To examine the reduction of *Fusarium* toxin production in solid substrates 50 g of commercial rice were soaked with 15 ml of distilled water in a 300 ml Erlenmayer flask overnight before sterilization run for 30 min. at 121 °C. Then it was inoculated with *Fusarium* and the tested fungus (antagonist). Inoculation was made with four 4mm diameter discs of each fungus cut from the edge of 1-week-old culture on PDA and 2 ml of sterile water. Cultures were incubated at 25 °C and shaken each day to support a uniform growth of mycelium. In the control each fungus was cultured

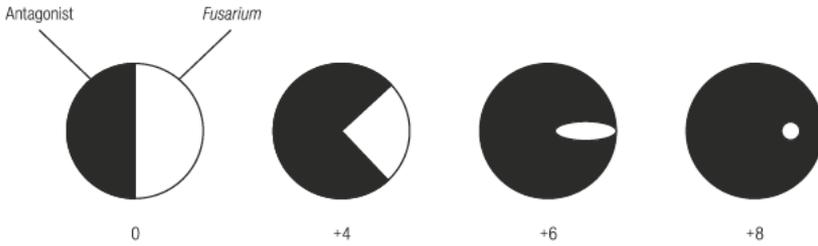


Fig. 1. Schematic presentation of scale used for evaluation of *Fusarium* isolates growth rate reduction by candidate antagonistic fungi.

separately. After 21 days rice colonized by fungi was dried in a thin layer at room temperature and mycotoxin content was analyzed. Two replications were made for each combination of paired cultures and for each control. *Fusarium avenaceum* isolate KF 2603 (ITEM 3411) rice culture, producing high amounts of moniliformin, was microwaved to kill mycelium cells and inoculated with the AN 35 *T. atroviride* isolate. After 21 days of inoculation at 25 °C the culture was dried as above and prepared for MON analyses.

Mycotoxin analyses. Moniliformin content in dry rice was analyzed by HPLC described in details by Tomczak et al. (2002) with 90% MON recovery and detection limit of 10 µg/kg.

RESULTS AND DISCUSSION

Fast growth is important character for a potential antagonist used in biological control of plant pathogens. It suggests strong competition for nutrients and space, which is very useful for the inhibition and possible elimination of pathogens. Growth rates of the examined *Fusarium* antagonists varied between species and isolates. Considering the growth rate on PDA, 92 isolates were divided into 3 groups:

1. Fast growing isolates with a growth rate >20mm/day,
2. Moderately growing isolates with a growth rate of 10 - 20mm/day,
3. Slowly growing isolates with a growth rate <10 mm/day.

The first group contained mostly *Trichoderma* species (the number of isolates is given in brackets), i.e.: *T. atroviride* (4), *T. aureoviride* (1), *T. asperellum* (1), *T. citrinoviride* (1), *T. hamatum* (1), *T. harzianum* (12), *T. koningii* (4), *T. longibrachiatum* (1), *T. pseudokoningii* (1), *T. viride* (10) and several isolates of different species: *Melanospora fimicola* (1), *Gliocladium virens*, syn. *T. virens* (5), *Chaetomium cochlioides* (1) and *Hypocrea hunua* (1). The following belonged to the second group: *Trichothecium roseum* (1), *Fusarium equiseti* (6), *Mortirella* sp. (1), *Idriella bolei* (1), *Cladosporium cladosporioides* (2), *Epicoccum nigrum* (2), *Chaetomium cochlioides* (3), *Gliocladium virens* (1) and a limited number of *Trichoderma* isolates. The following were included in the last group: *Epicoccum nigrum* (4), *Paecilomyces farinosus* (1), *Paecilomyces carneus* (1), *Gliocladium roseum* (4), *G. catenulatum* (3), *Gilmaniella* sp. (4), *Melanospora fimicola* (2), *Phytium* sp. (1), *Acremonium* sp. (1) and *Fusarium equiseti* (1).

Growth rates of *Fusarium* species, i.e. *F. avenaceum*, *F. culmorum* and *F. graminearum* isolates, were lower than growth rates of the first group of the antagonists and

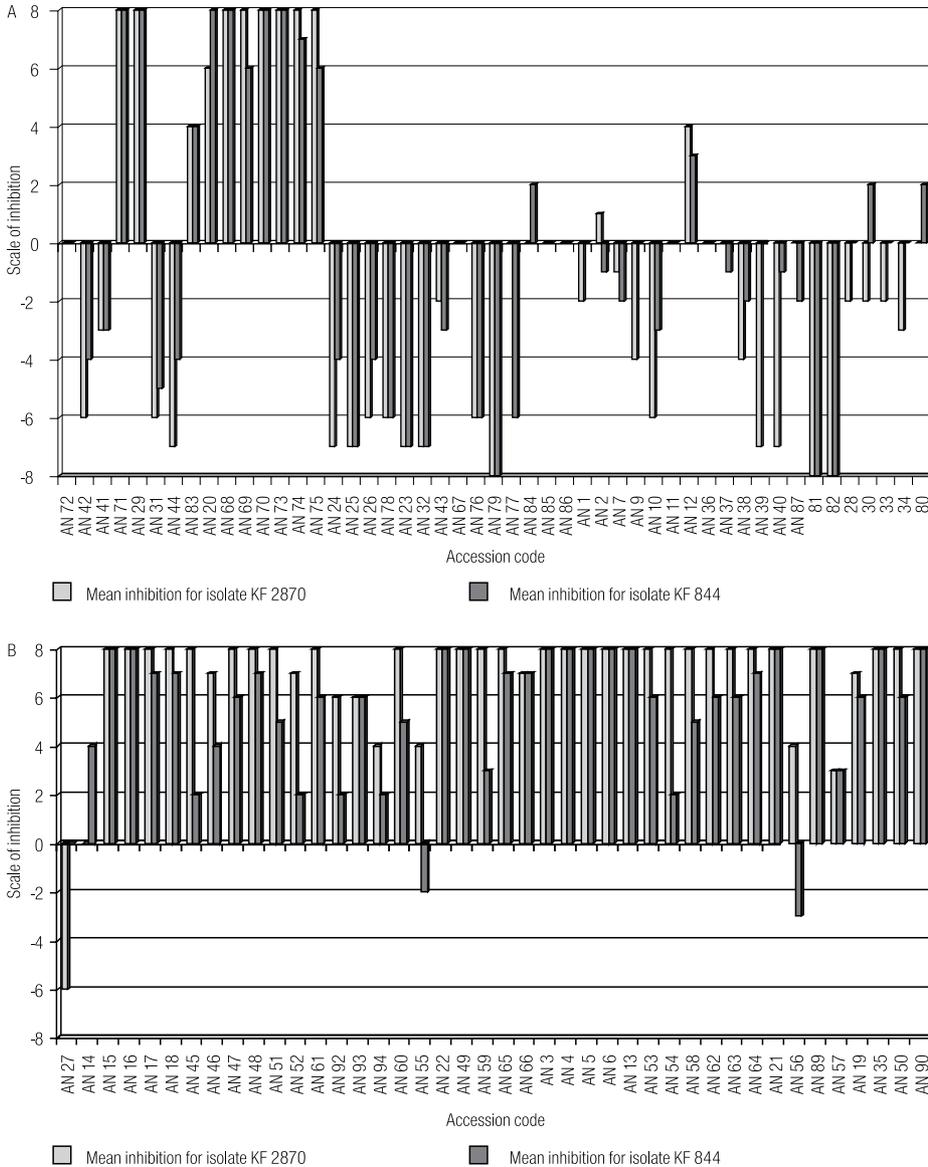


Fig. 2 A. A comparison of impact of 92 candidate antagonistic fungi isolates against two *F. graminearum* isolates KF 844 and KF 2870 in bioassay on PDA medium after 7 days at 25°C (in -8 to +8 scale of inhibition).

Fig. 2 B continued. A comparison of impact of 92 candidate antagonistic fungi isolates against two *F. graminearum* isolates KF 844 and KF 2870 in bioassay on PDA medium after 7 days at 25°C (in -8 to +8 scale of inhibition).

ranged from 9 to 20 mm/day (Tab. 1). Three chemotypes were identified using specific primers for PCR amplification of DNA among *F. culmorum* and *F. graminearum* isolates used in our experiments: 3-AcDON chemotypes (mostly *F. culmorum*

isolates), 15AcDON chemotype (most of *F. graminearum* isolates) and NIV chemotype (several isolates of both species).

Competitive abilities of candidate fungi against toxigenic *Fusarium* isolates were examined in dual culture bioassays on agar and solid substrate bioassay. *Fusarium avenaceum*, *F. culmorum* and *F. graminearum* produce aurofusarin and other carmine-red pigments on PDA medium (Vesonder, Goliński 1989). *Trichoderma* isolates produce a green pigment of polyphenolic nature. Colony pigmentation is a useful marker when studying the interaction between *Fusarium* isolates and their antagonists. *Fusarium* isolates growth in dual cultures with competitive isolates were visually reduced after 4 days. A week after inoculation growth inhibition and mycoparasitism of *Trichoderma* isolates were observed – the plate was green and overgrown with *Trichoderma* with significant production of abundant conidia in pustules over mycelium of *Fusarium* isolates, with only a small spot of the plate remaining red. *Fusarium* growth and red pigment production were strongly inhibited by isolates of *T. atroviride*, *T. harzianum*, *T. hamatum*, *T. longibrachiatum* and *T. koningii*.

Isolates of *F. graminearum* (KF 844 and KF 2870) were grown simultaneously with 92 antagonistic isolates and exhibited different susceptibility to the presence of the antagonists: isolate KF 2870 was significantly less resistant and isolate KF 844 exhibited a higher level of resistance against the same antagonistic mycoparasite (Fig. 2A, B). The antibiosis effect was not found in any of the candidate antagonists in dual culture on PDA.

The interaction between eight isolates of *Trichoderma* and six isolates of *Fusarium* is presented in tables 2 and 3. Thus, on one hand the same *Trichoderma* isolate exhibited different antagonism against various *Fusarium* isolates, and on the other hand antagonistic isolates exhibited various aggressiveness against the same isolate of *Fusarium*.

Trichoderma isolates are able to produce antibiotics – inhibitors of other fungi (such as 6PAP), and several enzymes, which hydrolyse fungal structures – conidia and mycelia – and macromolecules – chitin, cellulose, hemicellulose, beta glucan, xylem and proteins (Łacicowa, Pięta 1985; Kubicek, Harman 1998; Cooney et al. 2001). This ability allows *Trichoderma* isolates to utilise the mycelium of *Fusarium*

Table 2

Growth inhibition of four isolates of *F. graminearum* and *F. culmorum*, belonging to three chemotypes (3AcDON, 15AcDON and NIV) by eight isolates of *Trichoderma* in dual cultures on PDA medium after 7 days at 25 °C (in -8 to +8 scale)

Accession code	Antagonist species	<i>Fusarium</i> species and their chemotypes			
		KF 350 <i>F. culmorum</i> NIV	KF 846 <i>F. culmorum</i> 3Ac-DON	KF 844 <i>F. graminearum</i> 15Ac-DON	KF 2870 <i>F. graminearum</i> 15Ac-DON
AN 13	<i>T. harzianum</i>	+4	+6	+8	+8
AN 16	<i>T. atroviride</i>	+5	+6	+5	+6
AN 35	<i>T. atroviride</i>	+7	+8	+8	+8
AN 89	<i>T. citrinoviride</i>	+2	+2	+8	+8
AN 90	<i>T. atroviride</i>	+4	+6	+8	+8
AN 92	<i>T. harzianum</i>	-2	0	-2	+6
AN 93	<i>T. viride</i>	+8	+4	+6	+6
AN 94	<i>T. harzianum</i>	+2	+2	+2	+4

Table 3
Growth inhibition of two isolates of *Fusarium avenaceum* in dual culture by eight isolates *Trichoderma* (in -8 to +8 scale) on PDA medium after 7 days at 25 °C

Accession code	Antagonist species	<i>Fusarium avenaceum</i> isolate	
		KF 203 (ATCC 64451)	KF 2818
AN 13	<i>T. harzianum</i>	+4	+4
AN 16	<i>T. atroviride</i>	+5	+4
AN 35	<i>T. atroviride</i>	+6	+7
AN 89	<i>T. citrinoviride</i>	+6	+7
AN 90	<i>T. atroviride</i>	+7	+8
AN 92	<i>T. harzianum</i>	+6	+4
AN 93	<i>T. viride</i>	+2	+2
AN 94	<i>T. harzianum</i>	+6	+6

as a source of nutrients. Our biotest experiments confirmed the mycoparasitism of *Trichoderma* species over all three species and chemotypes of toxicogenic *Fusarium*.

Three types of interaction between *Trichoderma* species and plant pathogens have been recognized: antibiosis, competition for nutrients and hyperparasitism (Woo et al. 2006). No antibiosis was observed in our experiments. Results of our previous paper (Buško et al. 2008) correspond well with the finding of Cooney et al. (2001) on the impact of *T. harzianum* isolates on a DON-producing *F. graminearum* in the agar medium bioassay. The same authors proved the inhibition of DON production by a *Trichoderma* metabolite 6PAP, by as much as 80%. The mechanism of DON content reduction in *F. graminearum* cultures remains unsolved. It was shown that DON when added to an agar medium inoculated with *Trichoderma* was not metabolised by the fungus (Cooney et al. 2001).

A question arises whether isolates of *Trichoderma*, growing on the mycelium of toxicogenic *Fusarium* species, are also able to transform or degrade such mycotoxins as DON, NIV, MON, ZEA, and others – in total 19 mycotoxins identified in grain samples (Chelkowski 1998; Bottalico 2002). Until now, the ability to decompose DON has been found very rarely among microorganisms. Only one mixed culture among 1285 microbial cultures, isolated from farmland soil, cereal grains and others sources, transformed DON into two products that can be separated chromatographically (mainly 3-keto-4-deoxynivalenol; Voelkl et al. 2004).

In our previous laboratory experiments *F. culmorum* and *F. graminearum* cultures grown on rice produced five trichothecenes: fusarenone X (up to 21 mg/kg), nivalenol (up to 3.7 mg/kg), deoxynivalenol (up to 310 mg/kg), 3Ac-DON (up to 228 mg/kg) and 15Ac-DON (up to 184 mg/kg). Production of five trichothecene mycotoxins DON, 3AcDON, 15AcDON, nivalenol and fusarenone X was reduced by over 95% in dual culture bioassay by *Trichoderma* isolates AN 22 and AN 35 (Buško et al. 2008). DON concentration in the bioassay in microcosms was reduced by 45% and fungal biomass was reduced by 15% in studies of Noef et al. 2006. Consequently, mean DON production per biomass was significantly lower - 36% in dual culture with *T. atroviride* than in solitary culture of *F. graminearum* and the above mentioned authors did not find any evidence for the role of DON production in *F. graminearum* defence against *T. atroviride*.

Table 4

Reduction of moniliformin produced by two isolates *F. avenaceum* in dual culture bioassay and decomposition of MON by *Trichoderma harzianum* AN 35 isolate on rice after 21 days at 25°C

<i>F. avenaceum</i> isolate	MON produced by <i>F. avenaceum</i> ($\mu\text{g/g}$)	MON produced in dual culture <i>F. avenaceum/T. atroviride</i> ($\mu\text{g/g}$)	Reduction of MON level (%)
KF 203 (ATCC 64451)	58.5	3.4	94.2
KF 2818	296.2	ND	100
<i>F. avenaceum</i> isolate	MON ($\mu\text{g/g}$)	MON after <i>T. atroviride</i> culturing ($\mu\text{g/g}$)	Decomposition efficiency (%)
KF 2603 (ITEM 3411)	100	6.5	93.5

A significant reduction of wheat head infection by *F. graminearum* and DON accumulation in kernels was found by Dawson et al. (2004) when ears were preinoculated by fungal antagonists, such as *T. harzianum*, *Clonostachys rosea* and *F. equiseti*.

Recently three major compounds exhibiting antifungal activity were identified to be produced by T22 and T39 isolates of *T. harzianum*, that are already used as active agents in a variety of commercial biopesticides (Vinale et al. 2006). Over 50 commercial biopesticides were found effective in biological control of soil and residue borne pathogens (Woo et al. 2005).

Fusarium avenaceum isolates produced moniliformin up to 100 mg/kg (Tab. 4). The isolate *T. atroviride* AN35 was found the most effective among the examined accessions against toxigenic *Fusarium* isolates used in this study. This antagonist was able to reduce moniliformin production in dual culture bioassay on rice by 95-100%, depending on the applied *F. avenaceum* isolate. The same isolate reduced by 93% the amount of MON in a rice culture of *F. avenaceum* ITEM 3411 (KF 2603) – from 100 $\mu\text{g/g}$ to 6.5 $\mu\text{g/g}$ (Tab. 4).

The application of *Trichoderma* competitors may reduce growth of *Fusarium* species through competition in crop debris and mycotoxin production as well. However, the development of the formulation of biofungicides for practical control of diseases remains a very important task (Kubicek, Harman 1998; Woo et al. 2006).

Both in the literature and in our experiments presented in this paper significant interaction between toxigenic *Fusarium* species and *Trichoderma* competitor species was found to be of complex character, with importance of several characters such as growth rate, enzymes and secondary metabolites production (Harman 2006).

It can be concluded that competitive *Trichoderma* isolates are candidate fungi for biological control of toxigenic *Fusarium* species aggressive to cereals (such as *F. culmorum*, *F. graminearum* and *F. avenaceum*) and in reducing their inoculum, as well as preventing mycotoxin accumulation in plant tissues and crop residues in field.

It seems to be important to underline, that saprophytic species *Trichoderma harzianum* and *T. atroviride* are not antagonistic to *A. bisporus*. Both species isolates can be distinguished by DNA analyses from *T. aggressivum*, however when high amount of inoculum is present in mushroom compost both species may compete for nutrients with *A. bisporus* mycelium. Identification and nomenclature of *Trichoderma* species was recently modified by Gams and Bissett (1998). There is high similarity

of *Trichoderma* isolates morphology under laboratory conditions and there are many confusions in the literature, concerning identification of species. Actual list of over 40 species and their descriptions are available on Website: <http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm>.

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Antagonistyczne oddziaływanie wybranych grzybów na toksynotwórcze gatunki *Fusarium* patogeniczne dla zbóż

Streszczenie

Fuzarioza kłosa jest w wielu regionach produkujących zboża chorobą wyniszczającą i powoduje straty powstające na skutek obniżenia plonowania i jakości ziarna. Ziarno z roślin porażonych jest zanieczyszczone mikotoksynami, przede wszystkim deoksyniwalenolem i jego pochodnymi, zearalenonem i moniliforminą. Gatunki *Fusarium* dobrze rozwijają się na resztkach poźniwnych i obficie na nich zarodnikują. Grzyby saprotroficzne o cechach antagonistycznych wobec tych patogenów mogą przyczyniać się do zmniejszenia zarodnikowania patogenów *Fusarium* i obniżenia ilości tworzonych przez nie mikotoksyn.

Antagonistyczne oddziaływanie 92 izolatów grzybów należących do 29 gatunków testowano w bikulturach z izolatami trzech toksynotwórczych gatunków *F. avenaceum* (Corda) Saccardo, *F. culmorum* (W.G.Smith) Saccardo i *F. graminearum* Schwabe. Gatunki *F. culmorum* i *F. graminearum* tworzą mikotoksyny trichotecenowe i mikohormon zearalenon oraz należą do najistotniejszych patogenów zbóż w skali światowej. Porażenie kłosów zbóż przez te gatunki powoduje akumulację w ziarniakach deoksyniwalenolu (DON) i zearalenonu (ZEA). Izolaty *F. avenaceum* tworzą moniliforminę (MON) i enniatyny.

Izolaty gatunków *Trichoderma* okazały się najbardziej efektywnymi dla redukcji wzrostu izolatów wymienionych gatunków. Efekt antagonistyczny poszczególnych izolatów *Trichoderma* względem tych samych izolatów *Fusarium* różnił się znacząco. Również stopień redukcji wzrostu poszczególnych izolatów *Fusarium* przez te same izolaty *Trichoderma* był znacząco różny. Ilość moniliforminy produkowanej przez dwa izolaty *F. avenaceum* w bikulturach na ryżu była zredukowana o 95-100% przez izolat *T. atroviride* AN35. Ten sam grzyb antagonistyczny redukował zawartość moniliforminy z poziomu 100 µg/g do 6.5 µg/g w kulturze na ryżu, co sugeruje możliwość dekompozycji tej mikotoksyny przez ten izolat.