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Research Papers

DON on wheat crop residues: effects on mycobiota as a source of potential antagonists of *Fusarium culmorum*

Sabrina SARROCCO[1], Fabiola MATARESE[1], Antonio MORETTI[2], Miriam HAIDUKOWSKI[2] and Giovanni VANNACCI[1]

[1] Department of Tree Science, Entomology and Plant Pathology "Giovanni Scaramuzzi", Faculty of Agriculture, University of Pisa, Via del Borghetto 80, I-56124, Pisa, Italy.
[2] Institute of Sciences of Food Production, National Research Council, Via Amendola, 122/O, 70126 Bari, Italy.

Summary. *Fusarium culmorum*, a pathogenic fungal species associated with Fusarium Head Blight (FHB) of wheat, can produce the mycotoxin deoxynivalenol (DON) that is frequently found as contaminant in cereals. Wheat haulms, as decomposing plant material, are an important inoculum source of *F. culmorum* for subsequent crops. In the present work we exploited the mycobiota of haulms buried in natural soil as a source of potential antagonists of *F. culmorum* and evaluated the role played by DON in determining the composition of such mycobiota. DON was added to wheat haulm pieces that were incubated in three natural soils, namely sandy, clayey and mixed, all with a previous history of wheat cultivation. Composition of mycobiota associated with DON-treated cultural debris was evaluated and compared with mycobiota from control haulms, in order to evaluate the effect of soil type and of the presence of the mycotoxin. Our results showed that DON affected neither the number nor species profile of fungal isolates. Among fungi associated with cultural debris, thirty-nine *Pythium* spp. isolates were collected and five of them, both from treated and not treated haulms, were tested for their antagonistic ability against *F. culmorum.* The two strains showing the highest antagonistic activity were further tested against *F. oxysporum,* a species considered a major competitor of *F. culmorum* for wheat residues, and frequently recovered from haulms in our experiments. The two *Pythium* spp. isolates showed a positive antagonistic and mycoparasitic activity against *F. culmorum*, without affecting the growth of *F. oxysporum*. Mycobiota of haulms buried in soil represents an interesting source of potential antagonists and competitors for future exploitation in a multitrophic approach for biocontrol of FHB.

Key words: deoxynivalenol, straw competition, mycobiota, biological control.

Introduction

Fusarium Head Blight (FHB) represents one of the most economically devastating disease of wheat (*Triticum aestivum*) worldwide (Parry *et al*., 1995; Windels, 2000), causing significant reductions in grain yield and quality. FHB of wheat is caused by a complex of species belonging mostly to the genus *Fusarium*, among which *F. culmorum* is a major pathogen (Xu and Nicholson, 2009).

Many *Fusarium* species that infect wheat can produce trichothecene mycotoxins (Desjardins, 2006), which are considered strong protein inhibitors (Snijders, 1994). Deoxynivalenol (DON), mainly produced by *F. graminearum* and *F. culmorum* (Desjardins, 2006), is the most common trichothecene contaminant of wheat, and appears to play an important role in the aggressiveness of both species toward wheat (Muthomo *et al*., 2000; Mesterhazy, 2002).

Crop residues, such as wheat straw, are reported as the major sources of the primary inoculum for species involved in FHB epidemics (Dill-Macky and Jones, 2000). Fungal inoculum in infested debris in soil can survive either as saprotrophic mycelium or as thick-walled resting clamydospores, depending on the species, and may result in the infection of seedlings (Miedaner *et al*., 2008). Their survival in residues can be affected by other microbes, either

Corresponding author: G. Vannacci
Fax: +39 050 2216109
E-mail: g.vannacci@agr.unipi.it

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directly by competition, parasitism and predation, and/or indirectly by influencing the rate of residue decomposition (Perez *et al*., 2008).

The major part of the life cycle of FHB-causing species is saprotrophic and depends on retaining occupation of colonized plant debris in competition with numerous other microorganisms (Bruehl, 1987), but knowledge about the ecological role of DON during this critical growth stage is poor (Lutz *et al*., 2003). The primary role of the compound may be related to competition with other fungi and bacteria and it is known that the biosynthesis of secondary metabolites, including mycotoxins, probably occurs at a significant cost for the fungus (Xu and Nicholson, 2009). Mycotoxin productivity increases dramatically in co-inoculation, by as much as 1000 times, suggesting that competition results in greater production of trichothecene mycotoxins (Xu *et al*., 2007).

Aims of this study were the exploitation of mycobiota of wheat haulms buried in natural soil as a source of potential antagonists of Fusarium Head Blight causal agents, and evaluation of the effects of DON on composition of mycobiota of soil-buried wheat haulms. In order to test the effects of selected components of mycobiota on pathogenic and saprotrophic colonizers of wheat crop residues, 5 *Pythium* spp. isolates were employed in preliminary biological control tests against mycotoxigenic *F. culmorum* and against *F. oxysporum*, one of its principal fungal competitors for crop residues.

Materials and methods

Effect of DON on mycobiota colonizing wheat haulms

To evaluate the effects of DON on ability of soilborne fungi to colonize crop residues, dry wheat haulms, collected from a field near Pisa (Italy), were employed as bait. Haulms were cut in pieces 3 mm long. 60 pieces were dipped in 1 mL of a methanol:H_2O (85:15 vol/vol) solution supplemented by DON (Sigma, St. Louis, MO, USA) at the final concentration of 25 ppm. Haulm pieces immersed in a solution containing only 85:15 methanol:H_2O were used as controls. Baits were left in the solution for 24 hours at 24°C. Afterwards, pieces were let to dry under a flow cabinet at room temperature for 24 hours, then buried in pots containing 150 g of each of three different natural soils (sandy, clayey and mixed, Table 1), all with a previous history of cereal cultivation, collected around Pisa (Italy). Sixty haulm pieces were buried in each pot. Three replicates for each soil/treatment (with or without DON) combination were prepared. Water was added to reach field capacity. The amount of DON after desiccation was quantified on 180 haulm pieces, treated and untreated by DON as described above, before burying. DON extraction was performed by MycoSep 22 5 Trich Push Columns (Romer Labs Diagnostic GmbH, Tulln, Austria), according to manufacturer's instructions and quantified by HPLC (Omurtag *et al*., 2007). The amount of mycotoxin on treated haulms was 40 ppm (w/w) while no mycotoxin was detected in control haulms. The concentration of DON on wheat haulms was similar to the amount of mycotoxin produced in our experiments by *F. culmorum* strains on rice.

After 7 d of incubation in soil at room temperature, haulms pieces were collected from each pot by sieving, rinsed under tap water, superficially sterilized for 10 sec in a water solution of NaClO (1% active chloride) and 50% ethanol, then washed three times in sterile distilled water. Thirty sterilized pieces, randomly chosen, were plated on Water Agar (bacteriological agar 20 g L^{-1}; Difco, Detroit, MI, USA) added with Streptomycin Sulphate (50 ppm) and Bacitracin (7500 i.u. L^{-1}), 5 pieces for each plate. Plates were incubated at 24°C (12h/12h light/darkness) and

Table 1. Soils used to evaluate the effect of DON on mycobiota colonizing wheat haulms.

Soil	pH	Clay (%)	Lime (%)	Sand (%)	Organic matter (%)	P (ppm)	Cationic exchange ability
Clayey	7.9	35.28	28.15	36.57	2.32	21.5	27.04
Mixed	8.4	25.68	31.70	42.62	2.25	17.2	20.35
Sandy	8.5	19.98	18.10	61.92	1.01	8.3	15.03

observed daily. From the seventh day, every colony developing from each haulm piece was transferred to PDA (Potato Dextrose Agar, 39 g L^{-1}, Difco) plates, incubated at 24°C (12h/12h light/darkness) and, where reproductive structures were differentiated, submitted to microscopic identification at least at genus level. After attempts to induce sporulation by growing isolates on different substrates or by changing incubation conditions, non sporulating-colonies were classified as mycelia sterilia and maintained in collection for future analysis.

All isolates were stored at 4°C on PDA under mineral oil and included in the fungal collection of the Department of Tree Science, Entomology and Plant Pathology "G. Scaramuzzi" of the University of Pisa, Italy.

Evaluation of DON production by *Fusarium culmorum* isolates

Five *Fusarium culmorum* strains (SD3-19b, SD2-7a and MD3-23a from treated haulms, SC2-21a and SC1-20b from untreated haulms) selected for antagonistic and mycoparasitic tests, were investigated for DON production. One hundred g of rice kernels, adjusted to 45% moisture, were left overnight in 500 mL Erlenmeyer flasks then autoclaved for 20 minutes at 120°C. Kernels were inoculated with 2 mL of an aqueous suspension containing 10^6 spore mL^{-1} of each isolate, two flasks for each isolate. Flasks were incubated in the dark at 25°C for 3 weeks. At the end of incubation time, harvested cultures were dried in a forced-draft oven at 60°C for 48 h, finely ground and stored at 4°C until used. Non-inoculated kernels were used as control. Evaluation of DON content was performed according to Quarta *et al.* (2005) assuming 0.5 μg g^{-1} of the mycotoxin as limit of detection.

Antagonistic activity

To evaluate the antagonistic activity of five *Pythium* spp. strains isolated from wheat haulms mycobiota (SD3-21a, SD2-17b and MD3-9a from DON-treated haulms and SC2-1a and SC1-14a from untreated haulms) against mycotoxigenic *F. culmorum*, *in vitro* tests were performed. PDA disks of 6 mm diameter, cut from the edge of actively growing colonies of both antagonist and pathogen, were placed on opposite sides (at 7.5 cm from each other) of a sterilized cellophane membrane laid down on PDA in 100 mm diam. plates. Plates were incubated in darkness at 24°C and radial growth of the pathogen in the direction of the antagonist (Ra) and in a control direction (Rc; perpendicular to Ra) were registered at regular times till colonies' contact. Antagonist/pathogen combinations were as follows: SD3-21a/SD3-19b, SC2-1a/SC2-21a, SC1-14a/SC1-20b, SD2-17b/SD2-7a and MD3-9a/MD3-23a.

To confirm the activity of the two best *Pythium* spp. strains (SC2-1a and SC1-14a) against *F. culmorum*, additional combinations were tested: SC2-1a against SD3-19b, SC1-20b, SD2-7a and MD3-23a; SC1-14a against SD3.19b, SC2-21a, SD2-7a and MD3-23a.

To evaluate possible antagonistic activity against *F. oxysporum*, an effective colonizer of cultural debris, both SC2-1a and SC1-14a were tested by the same protocol against *F. oxysporum* MD2-24a, MD1-15b, MC3-22a, isolated from mycobiota of DON treated and untreated wheat haulms.

Mycoparasitic activity

Mycoparasitic activity of *Pythium* spp. towards macroconidia of *F. culmorum* and production of oogonia in the presence and in the absence of *Fusarium*, were evaluated for the four best *Pythium* spp./*F. culmorum* combinations from the first set of antagonistic tests (SD3-21a/SD3-19b, SC2-1a/SC2-21a, SC1-14a/SC1-20b and MD3-9a/MD3-23a).

The ability of *Pythium* to parasitize *Fusarium* conidia and the reproductive capacity of *Pythium* in the presence/absence of *Fusarium* were assessed on WA. The effects of *Pythium* on sporulation capacity of *Fusarium* were visually assessed on PDA plates. One hundred μL of an aqueous suspension of 10^7 macroconidia mL^{-1} of each *F. culmorum* isolate was uniformly spread on Petri plates containing a very thin layer of PDA or WA. Each plate was then inoculated with 5 PDA disks of 6 mm diameter, cut from the edge of actively growing colony of each *Pythium* spp. strain. One disk was placed at the centre, and four others were placed equidistantly around it and at about 2 cm from the edge of the plate. Plates containing only disks of *Pythium* spp. and plates inoculated with both macroconidia of *F. culmorum* and disks of uninoculated PDA were used as controls. Three replicates for each combination were arranged. After one week of incubation at 24°C, PDA plates were examined for the presence of a halo of reduced mycelial growth and absence of sporulation around *Pythium* inoculum. Af-

ter 48 h at 24°C, strips of WA (10 mm wide) were cut between the central and peripheral *Pythium* inoculation disks, mounted on glass slides and stained with Cotton blue-Sudan III. Percentages of parasitized empty *Fusarium* conidia were calculated out of 100 macroconidia for each replicate. The number of oogonia per microscopic field (10× magnification; 26.4 mm^2) was recorded and data from 20 microscopic fields per each replicate were pooled. Numbers of oogonia produced in presence of the pathogen were compared with those produced in control plates. In addition, presence of coilings by *Pythium* spp. around *F. culmorum* macroconidia was recorded.

Statistical analysis

Results involving number of isolates, percentages of empty macroconidia (after angular transformation) and number of oogonia were subjected to analyses of variance (ANOVA) by SYSTAT 10 package. Radial growth values, collected from antagonistic tests, were subjected to regression analysis by Prism GraphPad 5.0. $P<0.05$ was assumed as significant level.

Results

Effect of DON on mycobiota colonizing wheat haulms

The list of all fungal strains isolated from wheat haulms (in presence and in absence of DON) incubated in three different soils is reported in Table 2. A total of 578 colonies were isolated, around 40% was represented by *Fusarium* spp. and another 40% by mycelia sterilia. *Pythium* spp. and *Chaetomium* spp., each respresented 7% of total isolates. All other genera were very rarely recovered, in total corresponding to about 5% of all isolates.

In order to evaluate the influence of DON on the number and species profiles of fungi isolated from haulms buried in the three soils, a statistical analysis was performed, using soil type, treatment (presence/absence of DON) and their interactions as sources of variation. When total number of fungal isolates was submitted to ANOVA, statistical differences emerged in the interaction between soil type and presence/absence of DON on cultural debris. Number of strains isolated from wheat haulms buried in mixed soil, both with or without DON, was statistically higher than from sandy soil in presence of the mycotoxin (Table 3). From pairwise comparisons (Tukey' tests) no clear effect of DON or soil type could be detected, showing an apparent lack of effect of the mycotoxin in defining the total amount of fungi in wheat haulms.

The same general observation could be made when differences among soil and treatments were analyzed in detail for single genera, species or groups (*Fusarium* spp., *F. oxysporum*, mycelia sterilia, *Pythium* spp. and *Chaetomium* spp). Only sources of variation showing significant differences are further discussed. The type of soil statistically influenced the amount of *Fusarium* spp. which was significantly higher in mixed soil (17.8 averaged number of isolates) than in clayey soil (8.8 averaged number of isolates), showing an intermediate value in sandy soil (11.3 averaged number if isolates). Mycelia sterilia was significantly higher in sandy soil (16.33 averaged number) compared to clayey (9.2 averaged number). When *Chaetomium* spp. was considered, interaction between soil type and treatment was statistically significant. Pairwise comparisons (Table 4) showed that in clayey soil the presence of DON resulted in the isolation of a higher number of *Chaetomium* spp. when compared with the same soil in absence of mycotoxin and if compared with sandy soil, with or without DON. No significant differences between soils, presence/absence of DON and their interaction emerged when *F. oxysporum* and *Pythium* spp. were considered.

Deoxynivalenol production by *Fusarium culmorum* isolates

Evaluation of DON production on rice kernels by the five *F. culmorum* strains was performed by HPLC. All strains were able to produce the mycotoxin in a range of 21 to 35 μg g^{-1}. *F. culmorum* MD3-23a produced the lowest amount of DON and *F. culmorum* SD3-19b was the highest producer, whereas *F. culmorum* SC1-20b, SD2-7a and SC2-21b produced 25.0 μg g^{-1}, 25.5 μg g^{-1} and 30.2 μg g^{-1} of DON, respectively. No production of DON was detected in the controls.

Antagonistic activity

Fungal genera and species isolated from wheat debris suggested the mycobiota as a source of potential antagonists of *F. culmorum*. Tests were performed in order to investigate the ability of *Pythium* spp., isolated from straw treated and not treated with DON, to inhibit growth of *F. culmorum* and *F. oxysporum*,

Table 2. Number of fungal isolates from wheat haulm pieces, treated/not treated (Ctrl.) with DON, and buried in three different soils.

Fungi	Mixed soil		Clayey soil		Sandy soil	
	Ctrl.[a]	DON[b]	Ctrl.	DON	Ctrl.	DON
Fusarium species (total)	55	52	26	30	45	24
F. equiseti	14	21	12	10	12	6
F. oxysporum	29	21	8	13	14	8
F. sciirpi	0	2	0	0	2	0
F. solani	4	4	3	1	15	5
F. lateritium	2	0	0	1	0	0
F. culmorum	1	2	2	3	2	2
F. tricinctum	0	0	1	0	0	0
Fusarium sp.	5	2	0	2	0	3
Pythium spp.	6	6	0	5	17	5
Chaetomium spp.	8	10	6	17	0	0
Trichoderma spp.	1	0	1	0	0	0
Acremonium falciforme	0	0	1	0	0	2
Acremonium strictum	0	0	0	0	0	1
Stachybotris chartarum	0	1	0	0	1	1
Alternaria sp.	0	0	2	0	0	0
Myrothecium spp.	2	3	0	0	0	0
Clonostachys rosea	0	0	0	1	0	0
Sclerotium sp.	0	0	0	3	3	0
Cunninghamella elegans	2	0	0	0	1	0
Mucoraceae	0	0	0	1	0	0
Mycelia sterilia	35	50	57	42	33	22
Total	109	122	93	99	100	55

[a] Wheat haulms without DON.
[b] Wheat haulms with DON (40 ppm).

a well known competitor of FHB agents. In a first experiment 5 *Pythium* strains were challenged with 5 *F. culmorum* strains isolated from the same haulm samples (as shown by the first 3 digits of strains number). Growth rates of each *F. culmorum* and *F. oxysporum* strain were evaluated along the Rc and the Ra radii, resulting all in highly significant equations ($P<0.0001$; the lowest R^2, 0.824, was recorded for a *F. oxysporum* isolates, data not shown) when submitted to regression analysis.

Within *Pythium* spp./*F. culmorum* combinations (Table 5), four (SC2-1a, SC1-14a, SD3-21a and MD3-9a) out of the five antagonistic strains significantly reduced pathogen growth, as revealed by comparing slope values ($P\leq0.0003$).

With the aim of confirming these results, *Pythium* spp. SC2-1a and SC1-14a, the most effective strains against the pathogen, were submitted to an additional test against other *F. culmorum* and *F. oxysporum* isolates. As shown in Table 5, *Pythium* sp.

Table 3. Statistical analysis (ANOVA) of the number of fungal isolates from three different soils, using pieces of wheat haulm (treated/untreated with DON) as baits. Significant sources of variation (interaction soil type × presence/absence of DON) only are shown.

Soil	DON[a]	Number of isolates[b]
Mixed	+	40.7 a
Mixed	-	36.3 a
Sandy	-	33.3 ab
Clayey	+	31.7 ab
Clayey	-	31.3 ab
Sandy	+	19.0 b

[a] +, haulm with DON at 40 ppm; -, haulm without DON.
[b] Averaged number of isolates from three replicates, each of 30 wheat haulm pieces. At different letters correspond values statistically different (P=0.05, Tukey test).

Table 4. Statistical analysis (ANOVA) of the number of *Chaetomium* spp. isolated from wheat haulms (treated/untreated with DON) buried in three different soils. Significant sources of variation (interaction Soil type × presence/absence of DON) only are shown.

Soil	DON[a]	Number of *Chaetomium* spp.[b]
Clayey	+	5.66 a
Mixed	+	3.33 ab
Mixed	-	2.33 ab
Clayey	-	2.00 b
Sandy	+	0.00 b
Sandy	-	0.00 b

[a] See Table 3.
[b] See Table 3.

SC2-1a significantly inhibited growth of all four *F. culmorum* strains (P≤0.0127) while *Pythium* sp. SC1-14a showed the same ability in three out of the four tested combinations (P≤ 0.0331). No antagonism occurred against *F. culmorum* SD2-7a (P=0.1785), the same strain against which *Pythium* sp. SD2-17b did not show any effect (P=0.8083).

When the antagonistic activity of these two *Pythium* spp. strains was evaluated against *F. oxysporum*, no significant growth inhibition was recorded (data not shown).

Mycoparasitic activity

Parasitized conidia of *Fusarium* were easily detected as they appeared empty when mounted in Cotton blue. Percentages of apparently healthy macroconidia were reduced in all the four *Pythium* spp./*F. culmorum* combinations (Table 6), and 100% of macroconidia appeared healthy in control plates.

In all tested combinations, *Pythium* strains produced a significantly higher number of oogonia on WA in the presence of the pathogen than in control plates (Table 6). Coilings around empty macroconidia could be detected by microscopic observation, as shown in Figure 1.

On PDA, reduction of macroconidial germination and of mycelial growth of *F. culmorum* strains was evident in all combinations as shown in *Pythium* sp. SC2-1a/*F. culmorum* C2-21a (Figure 2A) and *Pythium* sp. SC1-14a/*F. culmorum* SC1-20b combinations (Figure 2B).

Discussion

Fusarium culmorum is an important species involved in FHB and produces DON, the mycotoxin most frequently occurring in wheat grains. Since *F. culmorum* survives saprotrophically on plant residues that serve as sources of inoculum for the subsequent year (Sutton, 1982), competition for their possession against other fungi and bacteria is important for the disease (Xu and Nicholson, 2009). The potential impact of DON on *Fusarium* competition with other microorganisms has not been thoroughly described. In this study, we showed that DON did not seem to affect the quantity and the profile of fungal isolates on the whole, occurring on wheat debris. The only significant activity emerged from the interaction between DON and soil type, suggesting a slight influence of the mycotoxin on mycobiota from sandy soil. This suggests that these soilborne saprotrophic fungi are in large part able to grow in presence of DON and this could be ascribed to different mechanisms, including resistance, detoxification or degradation of this compound (Karlovsky, 1999). However, to

Table 5. Linear regression analysis of radial growth of *Fusarium culmorum* in dual cultures against *Pythium* spp. Ra, radius towards *Pythium* colony; Rc, control radius and significativity (*P**) of differences between the two (a) radial growth rates.

Dual culture	Radius	a	R^2	P	P*
Pythium sp. SD3-21a vs *F. culmorum* SD3-19b	Rc	0.423	0.926	< 0.0001	< 0.0001
	Ra	0.179	0.927	< 0.0001	
Pythium sp. SC2-1a vs *F. culmorum* SC2-21a	Rc	0.378	0.990	< 0.0001	< 0.0001
	Ra	0.125	0.909	0.0032	
Pythium sp. SC1-14a vs *F. culmorum* SC1-20b	Rc	0.479	0.939	< 0.0001	< 0.0001
	Ra	0.083	0.933	0.0017	
Pythium sp. SD2-17b vs *F. culmorum* SD2-7a	Rc	0.431	0.952	< 0.0001	0.8083
	Ra	0.421	0.966	< 0.0001	
Pythium sp. MD3-9a vs *F. culmorum* MD3-23a	Rc	0.298	0.922	< 0.0001	0.0003
	Ra	0.155	0.886	0.0002	
Pythium sp. SC2-1a vs *F. culmorum* SD3-19b	Rc	0.407	0.911	< 0.0001	0.0127
	Ra	0.310	0.8534	< 0.0001	
Pythium sp. SC2-1a vs *F. culmorum* SC1-20b	Rc	0.480	0.973	< 0.0001	0.0001
	Ra	0.385	0.947	< 0.0001	
Pythium sp. SC2-1a vs *F. culmorum* SD2-7a	Rc	0.405	0.928	< 0.0001	0.0034
	Ra	0.312	0.893	< 0.0001	
Pythium sp. SC2-1a vs *F. culmorum* MD3-23a	Rc	0.313	0.972	< 0.0001	< 0.0001
	Ra	0.221	0.873	< 0.0001	
Pythium sp. SC1-14a vs *F. culmorum* SD3-19b	Rc	0.404	0.958	< 0.0001	0.0331
	Ra	0.354	0.951	< 0.0001	
Pythium sp. SC1-14a vs *F. culmorum* SD2-7a	Rc	0.359	0.953	< 0.0001	0.1785
	Ra	0.329	0.953	< 0.0001	
Pythium sp. SC1-14a vs *F. culmorum* SC2-21a	Rc	0.485	0.982	< 0.0001	< 0.0001
	Ra	0.370	0.909	< 0.0001	
Pythium sp. SC1-14a vs *F. culmorum* MD3-23a	Rc	0.331	0.971	< 0.0001	0.0003
	Ra	0.265	0.936	< 0.0001	

confirm this hypothesis, more information on the effects of DON on single saprotrophic fungal species need to be collected.

Because of the low effectiveness of fungicides to control members of the Fusarium head blight complex, there have been several efforts to identify biological antagonists, which could be used in biological or integrated pest management strategies (Wagacha and Muthomi, 2007). Several fungal species have been examined for the ability to reduce the potential inoculum of *Fusarium* pathogens, mainly by reduction of biomass in plant residues colonized by *Fusarium* (Dawson *et al*., 2004; Luongo *et al*., 2005). The mycobiota we collected from baits, both in the presence or absence of DON, represents an interesting and potentially useful source of antagonistic and competitive isolates to be used as biocontrol agents in the management of FHB. *Fusarium oxysporum* predominates among the species we recovered from baits. This is not surprising since this species is an

Table 6. Percentages of full, apparently healthy macroconidia of *F. culmorum* and numbers of oogonia of *Pythium* spp. in dual cultures on WA.

Pythium spp./ *F. culmorum*	Full conidia[a] %	Oogonia	
		Control plates[b]	Dual culture[b]
SD3-21a/SD3-19b	34.66 ± 4.50	5.90 ± 1.90 a	10.20 ± 2.29 b
SC2-1a/SC2-21a	27.00 ± 8.48	3.05 ± 2.62 a	4.93 ± 2.18 b
SC1-14a/SC1-20b	43.66 ± 3.78	7.46 ± 2.10 a	11.20 ± 0.58 b
MD3-9a/MD3-23a	30.66 ± 4.16	9.33 ± 2.41 a	15.76 ± 3.58 b

[a] Numbers represent the average (± SD) of three replicates of 100 macroconidia each. 100% full macroconidia were recorded in control plates for each antagonist/pathogen combination

[b] Numbers represent the average (± SD) of three replicates of the number of oogonia per microscopic field (26.4 mm^2). On the same row at different letters correspond statistically significant differences (P=0.05, Tukey test).

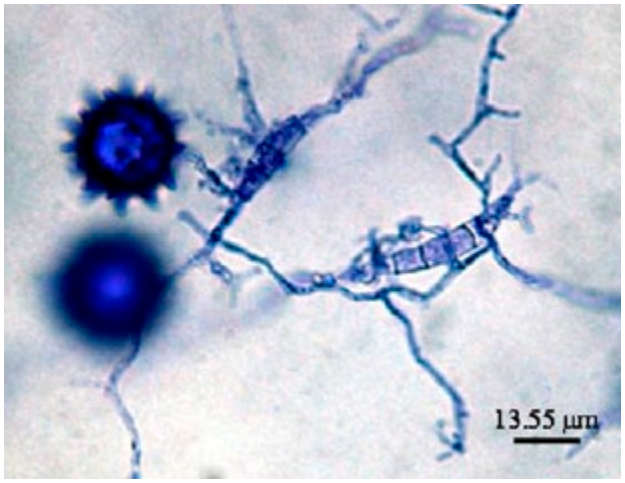

Figure 1. Coilings of *Pythium* sp. SC1-14a around *F. culmorum* SC1-20b macroconidia.

effective colonizer of partially decomposed wheat residues and has been reported to have a greater saprophytic ability than common FHB causal agents (Pereyra and Dill-Macky, 2004).

In our experiment, a large number of *Chaetomium* spp. was isolated, possibly due to the well known cellulosolytic and competitive abilities of species belonging to this genus (Sandhu and Puri, 1988; Umikalsom *et al*., 1997). Knudsen *et al*. (1995) found *Chaetomium* species as the most successful antagonists against seedborne *F. culmorum* on winter wheat. Interestingly, fungi belonging to this genus seems to take advantage, at least in clayey soil, from the presence of DON in haulms.

Although *Trichoderma* spp. are among the principal competitors that aggressively colonize crop residues of maize and wheat throughout the decomposition process (Broder and Wagner, 1988), only two *Trichoderma* strains were isolated and only from untreated wheat haulm pieces. Lutz *et al*. (2003) examined the effect of DON on interactions between pathogenic *Fusarium* strains and *T. atroviride* strain P1, demonstrating that the pathogen down-regulates chitinase genes in this fungal BCA and provided evidence that DON may have an ecological function as a factor increasing *Fusarium* competitiveness.

A highly represented, but probably heterogeneous, group of fungi was what we called mycelia sterilia. Indeed, some members of mycelia sterilia, such as *Rhizoctonia* and *Sclerotium*, are important plant pathogens worldwide while some others, associated with roots, have been reported as efficient plant growth promoters on many crops (Jacobs, 1994). Moreover, several isolates have also been found to act as biocontrol agents against pathogenic fungi (Dewan and Sivasithamparam, 1991). Our frequent recovery of such fungi suggests a strong competition ability for wheat residues and a slight, if any, sensitivity to DON. Considering their potential antagonistic activity, we are carrying out further investigations aimed to characterize the isolate collection, to identify representative isolates by molecular methods and to evaluate this large component of wheat haulm mycobiota for their potential use as biocontrol agents.

Pythium spp. is well represented within the mycobiota of buried haulms. In fields where the main crops are wheat, rye, beet and carrot, colony forming unit counts of *Pythium* are generally high (Brozova, 2002). All our strains are characterized by spiny oogonia, a character typical of mycoparasitic *Pythium* species. Mycoparasitism, in addition to competition for nutrients and space, are mechanisms associated with the biocontrol activity of *Pythium* species (Davanlou *et al*., 1999). Four out of the five strains tested against DON-producing *F. culmorum* isolates significantly reduced growth rate of the pathogen up to 80% but only when tested against isolates of *F. culmorum* collected from the same sample of haulms. When the two best isolates were confronted with pathogen iso-

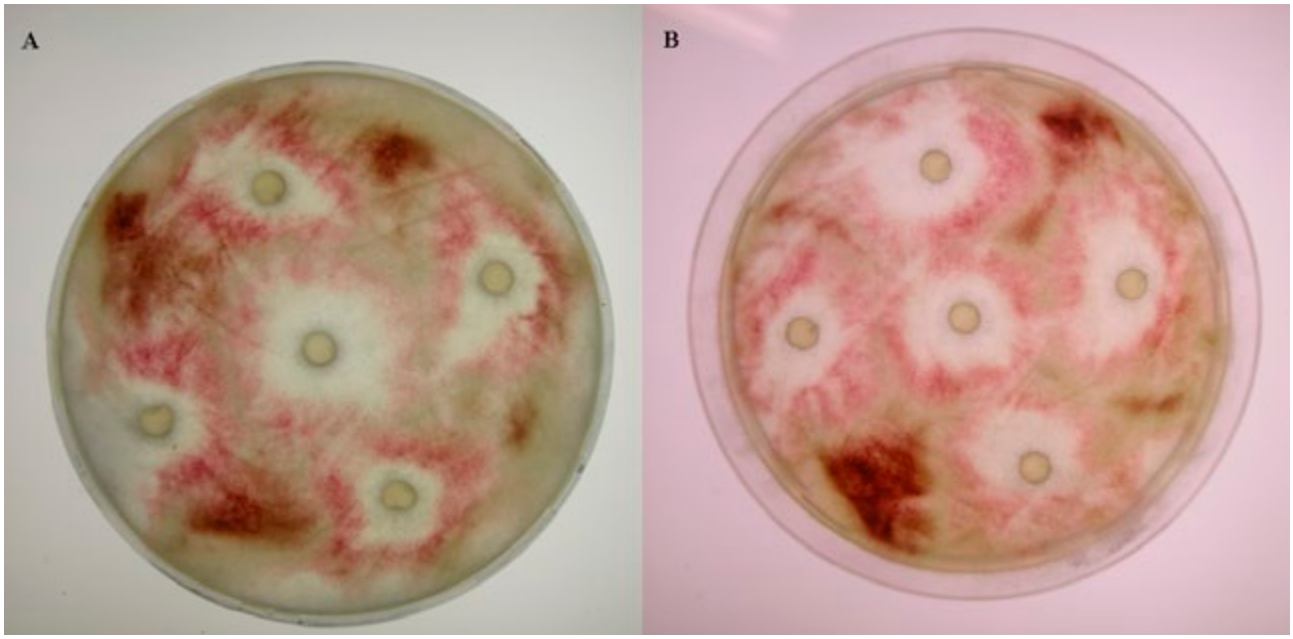

Figure 2. Inhibition of conidia germination and mycelium growth of *F. culmorum* by *Pythium* spp. A, *Pythium* SC2-1a against *F. culmorum* C2-21a; B, *Pythium* SC1-14a against *F. culmorum* SC1-20b.

lates coming from a different haulm sample the maximum reduction of growth of the pathogen was 30%. If we also consider that one isolate of *F. culmorum* was not affected by the two isolates of *Pythium* tested against it, we must conclude that antagonistic interactions between *Pythium* and *F. culmorum* depend on the pathogenic isolate tested, and this trait would require more attention in evaluating this antagonist/pathogen system. *Pythium* isolates were able to parasitize and to inhibit germination of *Fusarium* macroconidia and they were able to produce a significant higher number of oogonia when in presence of DON-producing *Fusarium* strains. Working with *P. oligandrum*, Hocknehull and co-authors (1995) found a significant correlation between the formation of zoospores and the degradation of *Fusarium* conidia. Random contact between hyphae of the mycoparasite and those of the host was followed by cessation of growth of the latter, lysis or vacuolation and coagulation of the cytoplasm, and penetration and growth of the mycoparasite within affected hyphae. Parasitism of conidia was seen as a rapid loss of cytoplasm followed by degradation of the cell walls. In addition, *P. oligandrum* produced abundant oogonia on parasitized macroconidia. Similar effects were recorded in our system, as we found a higher production of oogonia following parasitisation of *Fusarium* conidia and a rapid loss of cytoplasm in parasitized conidia frequently coiled by mycoparasite hyphae.

With the aim to employ our *Pythium* strains in the biocontrol of *F. culmorum* in a multitrophic system, we investigated the effect of these potential biological control agents against also *F. oxysporum* isolated from wheat. No inhibition activity resulted against what is considered one of the most competitive saprotrophic species for cultural debris against FHB agents (Pereyra *et al*., 2004).

Our results suggest that DON has no effects on the whole on mycoparasites and saprotrophic fungi natural inhabitants of wheat residues in the soil, but we cannot exclude a specific effect of DON on single genets of the saprotrophic/mycoparasitic species tested. A combined use of two or more different fungi could be an effective strategy to reduce growth of DON producing *Fusarium*, provided that screening procedures to select the best antagonists must include an evaluation step against several isolates representing the natural population of the pathogen. This will avoid the use of inefficient isolates, as it has been shown by our experiments.

One *Pythium* sp. showed antagonistic and mycoparasitic activity against all *F. culmorum* isolates

tested, without affecting the growth of *F. oxysporum* isolates. These results open the possibility to use a multitrophic strategy as a tool for a biological control of FHB agents, directed against pathogen primary inoculum in crop residues.

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