Acta Phytopathologica et Entomologica Hungarica 38 (3–4), pp. 275–280 (2003)

Effect of Antagonistic Fungi against *Fusarium graminearum* and *F. culmorum* on Stubble of Different Cereals and at Different Temperatures

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Bioassays were carried out with antagonists to suppress sporulation by *F. culmorum* and *F. graminearum* on cereal debris. A differential effect was found for temperatures on the effect of antagonistic fungal isolates. Isolates 10 and 11 were more effective at low temperature of 5 °C, while isolate 2 works well at higher temperature of 15 °C and fails at 5 °C. On the other hand, antagonism varied little when antagonists were tested on stubble from different cereals.

Keywords: Antagonisti saprophytes to *Fusarium,* cereals.

Fusarium ear blight (FEB) or scab affects the ears (heads) of wheat, barley, oats, rye and triticale. The majority of reports of the disease concern wheat. Most records of the disease are associated with *F. culmorum, F. avenaceum, F. graminearum, F. poae* and *Microdochium nivale.* The disease occurs in most areas of the world where small grain cereals are grown. Epidemics of FEB occur sporadically, and are usually associated with warm, wet weather around anthesis (Parry et al., 1995).

Control of the disease by the use of fungicides often is inconsistent in the field. Biocontrol of FEB using antagonistic fungi may be an alternative control option. The development of both pathogen and antagonist in aboveground plant parts is determined by several a biotic factors such as the availability of nutrients, temperature, water availability, UV radiation and the deposition of agrochemicals. Temperature and water availability in the boundary layer of green leaves (Burrage, 1971) and in necrotic leaf tissues determine microbial growth. Rapid fluctuations in water availability and temperature are characteristic for these niches and are the main factors limiting the development of microbial populations. Therefore, selection of potential antagonists depends on ecological competence. Antagonists must reach high growth rates under favorable conditions during leaf wetness at moderate temperatures and with sufficient nutrient supply as well as under marginal conditions (e.g. at low temperatures or at low water potentials). Pathogens and their potential antagonists can markedly differ in their activity at low water potentials and low temperatures. Isolates of *T. viride* grew better at low temperatures than those of other *Tricho-*

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0238–1249/2003/$ 20.00

derma spp., and destroyed sclerotia of *Botrytis cinerea* even at 5 °C in experiments under controlled conditions (Köhl and Schlösser, 1989). In bioassays with dead onion leaves, the antagonist *Ulocladium atrum* suppressed sporulation of *B. cinerea* by more than 90% in a broad temperature range between 6 °C and 24 °C, whereas an isolate of *Gliocladium roseum* was not effective at temperatures under 18 °C (Köhl et al., 1999). In this paper, our study was focused on the interaction between *F. graminearum* and *F. culmorum* and fungal antagonists at different temperatures as well as on different stubble of different cereals.

Material and Methods

Candidate antagonists

Six isolates of saprophytic antagonistic fungi were tested. Candidate antagonists, isolates no. 01, 02, 03, 09, 10 and 11, were originated from the collection of Plant Research International, Wageningen, The Netherlands.

Fusarium

Tests were carried out with isolates of *F. culmorum* (isolated from wheat ear variety Vivant in Mookhoek, The Netherlands in 1998) and *F. graminearum* (isolated from ear of the same wheat variety in Zuid Flevoland, The Netherlands in 1999).

Spore suspension

Fusarium isolates were grown on SNA medium (1 g KH_2 PO_4, 1 g KNO_3, 0.5 g $MgSO_4$, $7H_2O$, 0.5 g KCL, 0.2 g glucose, 0.2 g saccharose, 1 l distilled water) for 14 days at 18 °C with 12 h blacklight per day. All candidate antagonists were grown on oatmeal agar, except no. 11 which was grown on PDA, at 20 °C for 14 days in the dark. Slow growing isolates, nos 09 and 10 were incubated for 28 days. To obtain spore suspensions, cultures were flooded with sterile tap water containing 0.01% Tween 80. After gently rubbing with a rubber spatula to remove spores from the fungal cultures, suspensions were filtered through sterile nylon gauze with a mesh of 200 µm. For candidate antagonists the concentrations were adjusted 1×10^6 spores per ml as determined with the help of a haemocytometer. In the same way conidial suspensions of *Fusarium* spp. were adjusted to a concentration of 1×10^4 conidia per ml.

Bioassay

Bioassays were carried out following the protocol of Köhl and de Haas (unpublished; Dawson et al., 2002) on straw segments of wheat, barley and oat which had not been sprayed with pesticides during the growing season. Straw segments were prepared by cutting straw stems into 4 cm segments. Each segment had an intact node (not being split during combining) in the middle. Straw segments were than sealed in plastic bags and sterilized by 4 Mrad gamma irradiation (100 segments per bag). Before use in bioassays,

sterile straw segments were put into bottles (100 segments per 500 ml bottle) containing 500 ml sterile tap water and left for 6 h at room temperature. Three water-soaked segments were transferred to a sterile moist chamber consisting of a Petri dish (9 cm) containing one sterile 1.5 mm thick filter paper (8 cm) with a sterile filter paper (8 cm) on top of it and 10 ml sterile tap water. Spore suspensions were sprayed under sterile conditions using sterile atomizers with approximately 5 µl per cm^2. Two different tests have been carried out. The first *(a)* was with wheat straw (variety Tambor) only and the second *(b)* was carried out with three different straw types, wheat (variety Tambor), barley and oat (variety Valiant). Straw segments were sprayed first with conidial suspensions of *F. culmorum* or *F. graminearum* at a concentration of 1×10^4 conidia per ml. After 6 h of incubation at 15 °C in the dark the following treatments were applied for the two previous tests:

Bioassay *(a):* control (sterile tap water containing 0.01% Tween 80); six different suspensions containing spores of the following candidate antagonists: 01, 02, 03, 09, 10 and 11. Petri dishes sealed with parafilm were further incubated (completely randomized) at 5 °C for five weeks and 10 °C and 15 °C for three weeks with 12 hrs blacklight per day. Five replicates were used.

Bioassay *(b):* control (sterile tap water containing 0.01% Tween 80); six different suspensions containing spores of the following candidate antagonists: 01, 02, 03, 09, 10 and 11. Petri dishes sealed with parafilm were further incubated (completely randomized) at 15 °C with 12 h blacklight per day for three weeks. Five replicates were used.

After the incubation periods, straw segments of each Petri dish were put into an Erlenmeyer (100 ml) containing 10 ml of a washing liquid (water with 20% ethanol and 0.01% Tween 80). Erlenmeyers were shaken with the help of a reciprocal shaker (Flask Shaker sf1, Sturt Scientific, U.K.) at speed 9 out of maximum 10 for 10 min and conidial concentrations of *Fusarium* were determined using a haemocytometer.

Statistics

Data (numbers of spores of *Fusarium* spp. per ml) were log transformed and analyzed by Genstat 5 ANOVA.

Results

Temperature effect on antagonistic fungal isolates against Fusarium *spp.*

Data in *Table 1* show the effect of the antagonistic fungal isolates 01, 02, 03, 09, 10, 11 on *Fusarium* spp. sporulation at three different temperatures, 5 °C and 10 °C and 15 °C. At 5 °C isolates 09, 10 and 11 show the highest effect in sporulation suppression of both *F. culmorum* and *F. graminearum.* Isolates no. 01, 02 and 03 have little effect. At 10 °C isolates 02, 10 and 11 are effective in spore suppression of both *Fusarium* spp. but isolate 10 is the best with respect to *F. culmorum.* Isolate 09 suppresses sporulation of *F. culmorum,* but not of *F. graminearum.* Isolate no. 02 is much more effective on both *Fusarium*

Table 1

Effect of antagonistic fungal isolates on sporulation of *F. culmorum* and *F. graminearum* on wheat straw pieces at different temperatures

Isolates	Log. number of conidia of		Log. number of conidia of		Log. number of conidia of	
	F. culmorum at 5 °C	*F. graminearum* at 5 °C	*F. culmorum* at 10 °C	*F. graminearum* at 10 °C	*F. culmorum* at 15 °C	*F. graminearum* at 15 °C
01	5.736	5.684	6.542	6.062	6.789	6.413
02	5.804	5.693	6.139	5.379	5.753	5.242
03	5.794	5.682	6.457	6.119	6.796	6.398
09	5.261	4.964	5.730	6.070	6.705	6.496
10	4.577	4.423	5.197	5.451	5.949	5.912
11	4.474	3.921	6.035	5.083	6.375	5.987
Water	5.949	5.578	6.610	6.317	6.761	6.397
LSD ($\alpha = 0.05$)	0.4137		0.4137		0.3219	

spp. at 10 °C than 5 °C. However isolate 10 has more or less the same effect at both temperatures. At 15 °C isolate no. 02 is better than at 5 °C or 10 °C and has a stronger effect than isolate 10 (significantly only for *F. graminearum*). Isolate 09 has no effect at 15 °C, and isolate 11 is only effective at 5 °C.

Substrate effect on antagonistic fungal isolates against Fusarium *spp.*

Data in *Table 2* show the effect of the antagonistic fungal isolates, 01, 02, 03, 09, 10, 11 on *Fusarium* spp. sporulation on straw of three different host plants, barley, oat and wheat. Isolate no. 02 is the most effective on the three different host plants in spore suppression of both *Fusarium* spp. followed by isolate no. 10. There is a significant interaction effect of antagonistic isolates and the three different host plants. Isolate 02 works more or less the same on the three different host plants, but 10 fails on oat, whereas it is second to 02 on wheat and barley. Isolates 01, 03, 09 and 11 have hardly any effect in sporulation suppression of both *Fusarium* spp. on each host plant, and sometimes even stimulate *Fusarium* sporulation.

Discussion

The experiments have shown that a number of potential antagonists is capable of suppressing the sporulation of potentially toxigenic *Fusarium* spp. on debris from cereal crops. In this way their intentional application might limit the carry-over of *Fusarium* from preceeding cereal crops to new crops (Köhl et al., 2003).

During the selection of antagonists it must be considered that field conditions are strongly divergent from normal laboratory conditions. The differential effect of temperature on some of the tested fungi is highly relevant with respect to survival and coloniza-

Table 2

Effect of antagonistic fungal isolates on sporulation of *F. culmorum* and *F. graminearum* on three different cereal substrates at 15 °C

Isolates	Log. number of conidia of		Log. number of conidia of		Log. number of conidia of	
	F. culmorum on wheat	*F. graminearum* on wheat	*F. culmorum* on barley	*F. graminearum* on barley	*F. culmorum* on oat	*F. graminearum* on oat
01	6.789	6.413	6.591	6.010	6.363	5.922
02	5.753	5.242	5.180	4.332	4.990	4.327
03	6.796	6.398	6.531	6.387	6.463	6.121
09	6.705	6.496	6.525	6.390	6.372	6.349
10	5.949	5.912	5.911	6.107	6.263	5.692
11	6.375	5.987	6.529	6.047	6.259	6.205
Water	6.761	6.397	6.585	6.538	6.397	5.665
LSD ($\alpha = 0.05$)	0.3219		0.3219		0.3219	

tion of both the pathogens and the antagonists during growing seasons in Western Europe. Although isolate 02 is the most promising at 15 °C, it fails at 5 °C. At low temperature isolates 10 and 11 are much more effective than other antagonists tested. More research is necessary to understand the differential effect of cereal crop on antagonism in stubble.

The application of antagonists to out-compete *Fusarium* spp. in straw debris in the field seems feasible with a deliberate choice of antagonists.

Acknowledgement

The financial support by the Netherlands Organization for International Cooperation in Higher Education (NUFFIC) for the first author is acknowledged.

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