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Interactions between fungi colonizing the stem base of winter wheat

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In vitro conditions, the interactions between the fungi most frequently isolated from the stem base of winter wheat were determined. These were the species from genus *Fusarium* (*F. culmorum, F. avenaceum* and *F. poae*) and *Rhizoctonia cerealis, Pseudocercosporella herpotrichoides, Alternaria alternata* and *Aureobasidium bolleyi*. Some saprotrophes showed stimulating effect on *R. cerealis, P. herpotrichoides* and *F. poae*. Certain species in combined cultures showed exceptionally favourable relationships.

Key words: pathogens, stem base diseases, interactions.

INTRODUCTION

Studies conducted in 1989–1992 on health status of winter wheat grown on 24 fields in north-eastern Poland showed a negative correlation in the frequency of occurring the particular stem base diseases (Wójcik 1993). Moreover, the mycological analysis of the collected stems showed that wheat is commonly infected by several fungal species, which sometimes leads to simultaneous appearance of several kinds of disease symptoms (Wachowska 1998). Reinecke and Fehrmann (1979) were first to pay attention to the competition between *Rhizoctonia cerealis* and *Pseudocercosporella herpotrichoides* underlying that after application of fungicides controlling eyespot, the number of stems with the symptoms of infection by the first pathogen increased. On the other hand, Mańka et al. (1983) observed a negative correlation between the frequency of occurrence of symptoms due to infection by *Fusarium* and the frequency of occurring the symptoms of sharp eyespot caused by *R. cerealis*.

An attempt was made to evaluate interactions among 8 fungal species, most numerously isolated from the infested stem base of winter wheat. Saprotrophic

fungi were also included to elucidate their role in the disease process. It seems possible then in case of the combined infections they can quicken destruction of the infested tissues (Ł a c i c o w a and W a g n e r 1989).

MATERIALS AND METHODS

Analysis included the fungi that are most frequently isolated from wheat stems showing root rot symptoms (W a c h o w s k a 1998). They were: *Acremonium strictum* W. Gams, *Alternaria alternata* (Fr.) Keisler, *Aureobasidium bolleyi* Sprague, species from the genus *Fusarium* (*F. avenaceum* (Fr.) Sacc., *F. culmorum* W. G. Smith Sacc., *F. poae* (Peck) Wollenw.) and *Pseudocercosporella herpotrichoides* (Fron.) Deighton and *Rhizoctonia cerealis* v. d. Hoeven.

Isolates were taken from the stems of winter wheat harvested from the fields in six localities of the Olsztyn province. Samples were divided into 5 groups, from each 5 culms were chosen a piece. There were distinguished 4 kinds of foot rot diseases and the stems with symptoms of at least two diseases (W a c h o w s k a 1998). Mycological analysis was performed as recommended by R e i n e c k e and F e h r m a n n (1979) and R a s h i d and S c h l ö s s e r (1977).

To determine the biotic effect of species, the methods described by M a ń k a (1974) and K o c z o w s k a and W i w a r t (1988) were used.

Isolated of fungi were put by pairs into Petri dishes (of 9 cm diameter) onto acidified medium PDA. Inoculum constituded 5 mm disks overgrown with the analyzed fungus, which were placed in the middle part of the dish, 20 mm apart. All possible combinations of pairs were performed. Control dishes were inoculated with the disks of the single fungal species. Fungi were incubated at 21°C in dark. The experiment was replicated 5 times.

Size of a colony was determined by measuring after 3, 4, 5 and 6 days of incubation (dates I—IV) two perpendicular diameters of colony. After calculating the average diameters of a colony, the results were analyzed statistically, employing the test of Newman-Keuls.

In addition, after 6 days of incubation, the biotic effect was estimated using the scale elaborated by M a ń k a (1974) and photographs were taken.

RESULTS AND DISCUSSION

Differences in the size of colonies of the fungal species resulting from the effect of the other species underwent changes in the course of the experiment. The most conspicuous differences were in the sixth day (Tables 1—8), which is in accordance with the observations of others (K o c z o w s k a and W i w a r t 1988).

Fusarium avenaceum was not found to respond to the presence of most fungi studied. Only *Acremonium strictum*, beginning with the second date of

measurement, markedly stimulated its growth (16% on an average); *Fusarium culmorum* inhibited its development as early as with the first date of observation (47% on an average). The differences were significant (Table 1, Fig. 1). In the study by T r u s z k o w s k a et al. (1988) *F. avenaceum* was inferior to the rapidly growing *R. cerealis*.

T a b l e 1

The size of *Fusarium avenaceum* colonies depending on biotic effect of accompanied fungus

Species of fungi	Diameter of colony in mm				Mean
	3. day	4. day	5. day	6. day	
Alternaria alternata	17 abc*	26 abcdef	32 efghi	42 i	29 b
Aureobasidium bolleyi	17 abc	25 abcde	31 defgh	41 i	28 b
Acremonium strictum	18 abc	36 fghi	55 j	61 j	42 d
Rhizoctonia cerealis	20 abcd	26 abcdefg	32 efghi	38 hi	29 b
Fusarium culmorum	16 a	18 abc	21 abcd	20 abcd	19 a
Fusarium poae	20 abcd	26 abcdefg	33 efghi	37 hi	29 b
*P. herpotrichoides***	17 ab	23 abcde	29 cdefgh	33 efghi	25 b
Control	21 abcd	28 bcdefgh	36 ghi	61 j	36 c
Mean	18.5 a	26 b	34 c	42 d	

Explanations: * – values marked by same letters do not significantly according to Newman-Keuls test; ** – *Pseudocercosporella herpotrichoides*

Fusarium culmorum responded with a marked retardation of growth to the presence of *Acremonium strictum* (by 28%) and with a slight growth checking to *A. bolleyi, F. avenaceum, F. poae* and *P. herpotrichoides*. These differences were significant and were present especially with the third day of experiment. A similar reaction of that species to the presences of *F. avenaceum* was found by K o c z o w s k a and W i w a r t (1988) in the study on relationships among pathogens isolated from the ears of rye. In the other cases, *F. culmorum* fully overgrew the mycelium of the accompanying fungal species (Fig. 3), which was also observed by B r ü c k and S c h l ö s s e r (1982).

T a b l e 2

The size of *Fusarium culmorum* colonies depending on biotic effect of accompanied fungus

Species of fungi	Diameter of colony in mm				Mean
	3. day	4. day	5. day	6. day	
Alternaria alternata	56 abcde*	77 fghi	90 i	90 i	78 d
Aureobasidium bolleyi	59 cde	65 def	69 efgh	90 i	71 c
Acremonium strictum	24 a	50 bc	65 def	90 i	57 a
Fusarium avenaceum	46 bc	71 efgh	82 ghi	80 fghi	69 c
Fusarium poae	44 b	56 bcde	68 efg	84 hi	63 b
Rhizoctonia cerealis	58 cde	75 fghi	83 ghi	84 hi	75 cd
*P. herpotrichoides***	52 bcd	65 def	78 fghi	84 hi	70 c
Control	57 bcde	80 fghi	90 i	90 i	79 d
Mean	50 a	67 b	78 c	86 d	

Explanations: * – values marked by same letters do not significantly according to Newman-Keuls test; ** – *Pseudocercosporella herpotrichoides*

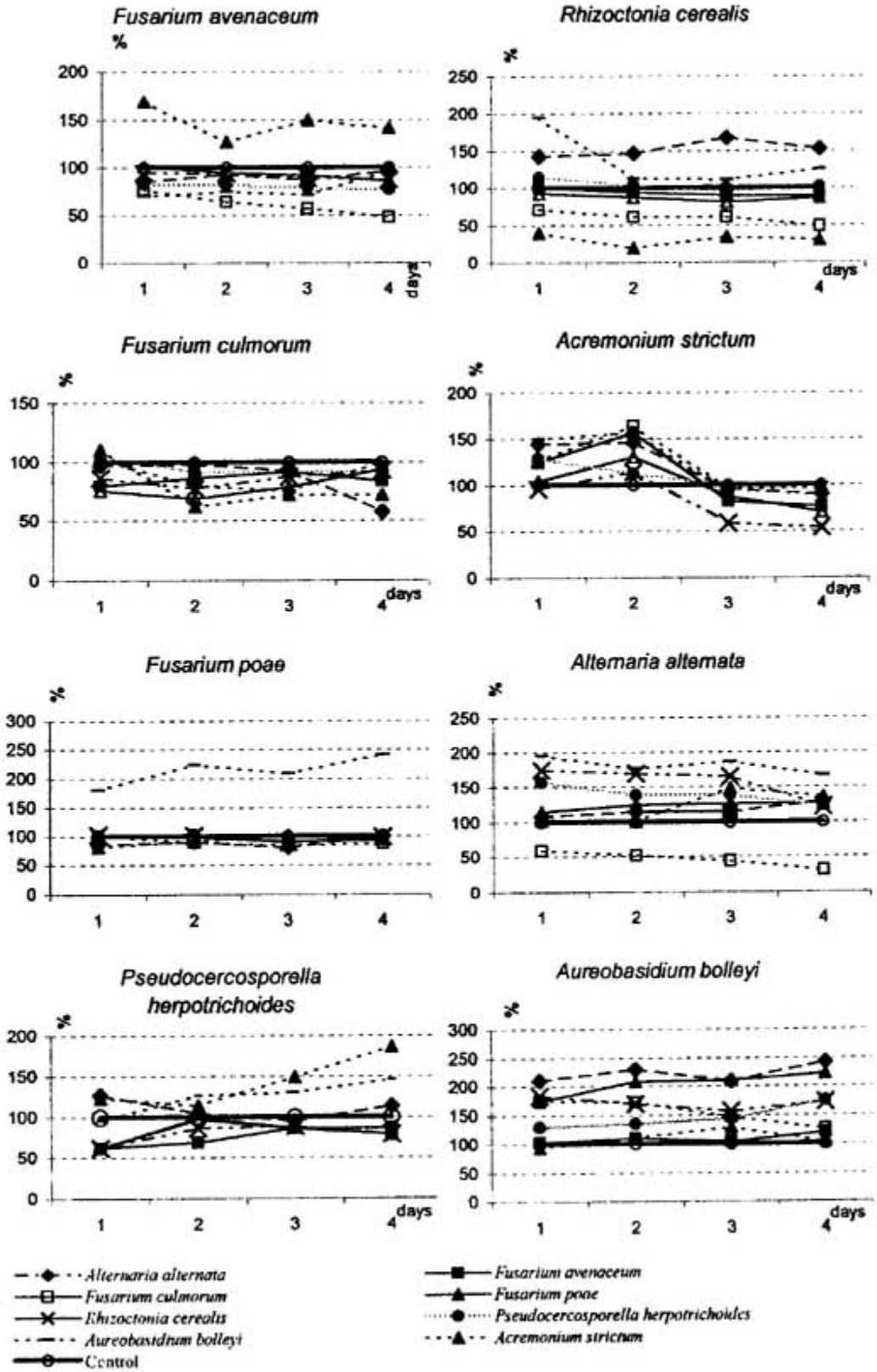

Fig. 1. Antagonistic effect of fungus (*F. avenaceum*, *R. cerealis*, *F. culmorum*, *A. strictum*, *F. poae*, *A. alternata*, *T. yallundae*, *A. bolleyi*) against:

The species *Fusarium poae* distinguished itself a high tolerance to the presence of most of the species studied. Differences in the size of colony growing in company of other fungi were not higher than 12% as compared with control. An exception was *Aureobasidium bolleyi* that stimulated the development of *F. poae* increasing twofold its growth rate, which was significant (Table 3, Fig. 1).

Table 3

The size of *Fusarium poae* colonies depending on biotic effect of accompanied fungus

Species of fungi	Diameter of colony in mm				Mean
	3. day	4. day	5. day	6. day	
Alternaria alternata	12 ab	14 abcde	16 abcdef	22 efg	16 a
Aureobasidium bolleyi	21 cdefg	36 h	45 i	53 j	38 b
Acremonium strictum	7,8 a	13 abcd	21 cdefg	25 g	16 a
Fusarium culmorum	12 abc	15 abcdef	19 bcdefg	21 defg	17 a
Fusarium culmorum	13 abcd	14 abcde	17 bcdefg	20 bcdefg	16 a
Rhizoctonia cerealis	12 abcd	16 abcdef	20 bcdefg	24 fg	18 a
*P. herpotrichoides***	13 abcd	16 abcdef	18 bcdefg	22 efg	17 a
Control	12 abc	15 abcdef	20 bcdefg	23 fg	18 a
Mean	13 a	17 b	22 c	26 d	

Explanations: * – values marked by same letters do not significantly according to Newman-Kuels test; ** – *Pseudocercosporella herpotrichoides*

The fungus *Pseudocercosporella herpotrichoides* in the presence of other potential pathogens of cereals grain crops grew worse. Saprophytes markedly stimulated its growth throughout the experiment, but most effectively in the sixth day. When saprophytes were present on dishes, the growth of *P. herpotrichoides* was faster then in control. The differences were significant (Table 4, Fig. 1). This relationship may be of significance in natural condition

Table 4

The size of *Pseudocercosporella herpotrichoides* colonies depending on biotic effect of accompanied fungus

Species of fungi	Diameter of colony in mm				Mean
	3. day	4. day	5. day	6. day	
Alternaria alternata	10 abcd	10 abcd	12 de	13 de	11 b
Aureobasidium bolleyi	8 abc	11 bcde	12 cde	15 e	11 b
Acremonium strictum	7 abc	10 abcd	14 e	19 f	13 b
Fusarium avenaceum	5 a	5 a	6 ab	7 ab	6 a
Fusarium culmorum	5 a	5 a	7 ab	7 ab	6 a
Fusarium poae	5 a	5 a	5 a	6 ab	5 a
Rhizoctonia cerealis	5 a	5 a	5 a	6 ab	6 a
Control	5 a	7 ab	8 abc	8 abc	7 a
Mean	6 a	7 a	9 b	10 b	

Explanations: * – values marked by same letters do not significantly according to Newman-Keuls test

as *A. bolleyi*, *A. alternata* and *A. strictum* are often isolated in different regions of the country and their role in the disease process has not been elucidated yet (M a j c h r z a k and M i k o ł a j s k a 1982, T r u s z k o w s k a et al. 1988).

The fungus *Rhizoctonia cerealis* responded with a strong decline of the rate of colony growth to the presence of fast growing *Fusarium culmorum* and *Acremonium strictum*. The colony of *R. cerealis* reached in the sixth day of the experiment 50% and 35% of the diameter as compatred with control and the differences were significant (Table 5, Fig. 1) T r u s z k o w s k a et al. (1988) noted down different dependence as the isolates of *R. cerealis* studied by them were characterized by the faster growth and constituded competition for *F. culmorum*. The species *Alternaria alternata* and *Aureobasidium bolleyi* stimulated development of the said pathogen speeding up its growth by 44% and 22%, on an average, as compared with control. The presented differences were noted throughout the experiment and were significant (Fig. 2). The other species slightly modified the rate growth of *Rhizoctonia cerealis*. *Pseudocercosporella herpotrichoides* in spite of a show growth limited the growth of that fungus in the first day of the experiment (Table 5, Fig. 1). This was probably associated with the formation of inhibiting substances by *P. herpotrichoides*, which was reported by B r ü c k and S c h l ö s s e r (1992).

T a b l e 5

The size of *Rhizoctonia cerealis* colonies depending on biotic effect of accompanied fungus

Species of fungi	Diameter of colony in mm				Mean
	3. day	4. day	5. day	6. day	
Alternaria alternata	25 defghi*	36 hijk	44 kl	52 l	39 e
Aureobasidium bolleyi	25 defghi	27 efghij	38 ijk	42 jkl	33 d
Acremonium strictum	6 a	7 ab	8 abc	11 abcd	8 a
Fusarium avenaceum	17 abcdef	23 cdefghi	28 efghij	30 fghijk	25 c
Fusarium poae	16 abcdef	21 bcdefgh	25 defghi	30 fghijk	23 c
Fusarium culmorum	12 abcde	15 abcdef	18 abcdefg	17 abcdef	16 b
*P. herpotrichoides***	20 abcdefgh	25 defghi	30 fghijk	34 ghijk	27 c
Control	17 abcdef	24 defghi	31 fghijk	34 ghijk	27 c
Mean	17 a	22 b	27 c	31 d	

Explanations: * — values marked by same letters do not significantly according to Newman-Kuels test; ** — *Pseudocercosporella herpotrichoides*

In the first two days of the experiment, the growth rate of *Acremonium strictum* grown in the presence of most fungi was higher as compared with control (Table 6, Fig. 1). Beginning with the second date of measurements, *A. strictum* grew worse when accompanied by *Rhizoctonia cerealis* (Fig. 4). A negative effect on its growth was also exerted by *Fusarium poae* and *F. avenaceum*, beginning with the third date of measurement. These notices were significant.

Fig. 2. *Alternaria alternata* (on the left) and *Rhizoctonia cerealis* (on the right)

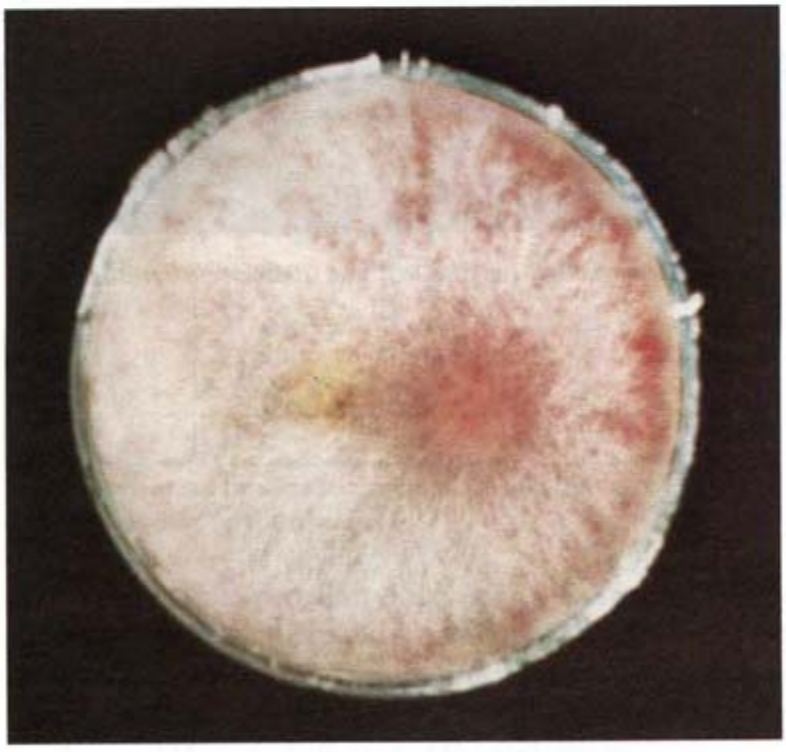

Fig. 3. *Rhizoctonia cerealis* (on the left) and *Fusarium culmorum* (on the right)

Fig. 4. *Aureobasidium bolleyi* (on the left) and *Rhizoctonia cerealis* (on the right)

Table 6

The size of *Acremonium strictum* colonies depending on biotic effect of accompanied fungus

Species of fungi	Diameter of colony in mm				Mean
	3. day	4. day	5. day	6. day	
Alternaria alternata	7 ab*	10 cd	16 f	18 fgh	13 c
Aureobasidium bolleyi	7 ab	10 cd	17 fg	20 hi	13 c
Fusarium avenaceum	7 ab	10 cd	16 f	18 fgh	13 c
Fusarium culmorum	7 ab	10 cd	17 fg	16 fg	13 c
Fusarium poae	5 a	8 bc	12 de	13 e	10 b
*P. herpotrichoides***	7 ab	10 cd	17 fg	21 i	13 c
Rhizoctonia cerealis	5 a	6 ab	10 cd	11 de	8 a
Control	5 a	7 ab	17 fg	21 i	12 c
Mean	6 a	9 b	16 c	17 d	

Explanations: * – values marked by same letters do not significantly according to Newman-Kuels test; ** – *Pseudocercosporella herpotrichoides*

The species *A. alternata* and *A. bolleyi* performed better when accompanied by most of species studied. The first of them behaved indifferently in the presence of *F. culmorum* and the second one did not respond to *A. strictum* and *F. avenaceum* (Tables 7, 8, Fig. 1).

Table 7

The size of *Alternaria alternata* colonies depending on biotic effect of accompanied fungus

Species of fungi	Diameter of colony in mm				Mean
	3. day	4. day	5. day	6. day	
Aureobasidium bolleyi	26 cdef*	34 efgh	42 ghi	53 j	39 d
Acremonium strictum	15 abc	20 abcd	33 efgh	41 ghi	27 c
Fusarium avenaceum	12 ab	16 abc	19 abcd	23 bcde	17 b
Fusarium culmorum	8 a	10 ab	10 ab	10 ab	10 a
Fusarium poae	12 ab	15 abc	17 abc	22 abcde	16 b
*P. herpotrichoides***	21 abcde	31 defg	38 fghi	48 ij	35 d
Rhizoctonia cerealis	23 bcde	32 efgh	38 fghi	44 hij	35 d
Control	8 a	10 ab	12 ab	17 abc	12 a
Mean	16 a	21 b	26 c	32 d	

Explanations: * – values marked by same letters do not significantly according to Newman-Keuls test; ** – *Pseudocercosporella herpotrichoides*

In some cases, the mutual proximity of colonies was exceptionally favorable to the two growing side by side fungi. In combined cultures, the colonies of *A. alternata attained* 325% of colony size in control and those of *Aureobasidium bolleyi* 226%. Similar reaction to their proximity showed pairs of: *A. bolleyi* (186%) and *F. poae* (211%); *A. bolleyi* (173%) and *Rhizoctonia cerealis* (122%) (Fig. 4); *A. bolleyi* (140%) and *P. herpotrichoides* (157%); *A. alternata* (292%) and *P. herpotrichoides* (157%) and *A. alternata* (291%) and *R. cerealis* (144%) (Fig. 2).

Table 8

The size of *Aureobasidium bolleyi* colonies depending on biotic effect of accompanied fungus

Species of fungi	Diameter of colony in mm				Mean
	3. day	4. day	5. day	6. day	
Alternaria alternata	24 cdefgh*	30 ghi	35 i	45 j	34 d
Acremonium strictum	10 a	13 abc	19 abcdef	19 abcdef	15 a
Fusarium avenaceum	10 a	14 abcd	17 abcde	23 cdefg	16 a
Fusarium culmorum	17 abcde	21 bcdefg	25 defgh	24 defgh	22 b
Fusarium poae	19 abcdef	27 efghi	25 defgh	42 j	28 bc
*P. herpotrichoides***	15 abcd	22 cdefg	15 abcd	32 hi	21 b
Rhizoctonia cerealis	20 abcdefg	22 cdefg	28 fghi	32 hi	26 c
Control	11 ab	13 abc	17 abcde	19 abcdef	15 a
Mean	15 a	20 b	23 c	29 d	

Explanations: * – values marked by same letters do not significantly according to Newman-Keuls test; ** – *Pseudocercosporella herpotrichoides*

The evaluation of individual biotic effects showed that *F. culmorum* markedly limited the growth of the other species, particularly *A. strictum* (Table 9). Similarly, strongly inhibiting effect on this species was found to exert *F. avenaceum* and *A. alternata*. The fungus *P. herpotrichoides* appeared a weak competitor to most of the partners grown with it in Petri dishes. In the remaining combined cultures the mutual interaction of the colonies, estimated according to the 8-point scale of M a ń k a (1974), was small.

Table 9

The estimation of ineraction of eight fungal species (according of Mańka's scale from −8 to 8)

Testing fungus	Fungus under test							
	Acremonium strictum	*Alternaria alternata*	*Aureobasidium bolleyi*	*Fusarium avenaceum*	*Fusarium culmorum*	*Fusarium poae*	*Rhizoctonia cerealis*	*Pseudocercosporella herpotrichoides*
Acremonium strictum	–	−6	0	−1	−2	−2	−2	0
Alternaria alternata	6	–	3	1	−6	3	2	4
Aureobasidium bolleyi	0	0	–	1	−4	3	0	0
Fusarium avenaceum	1	−2	−1	–	−5	2	−1	−1
Fusarium culmorum	2	6	4	4	–	5	6	5
Fusarium poae	2	−3	−3	−2	−5	–	0	−2
Rhizoctonia cerealis	2	1	−3	1	−6	0	–	2
Pseudocercosporella herpotrichoides	1	−4	−2	1	−5	2	−2	–

The results of the employed methods in some cases are not unanimous. It is true when one of the partners grows very dynamic in the first stage of the experiment and then undergoes competition of accompanying fungi. Such a dynamics of growth showed *A. strictum*, which gave way to *F. culmorum* not before six days and to *F. avenaceum* after five days. Therefore, the mathematical calculations, contrary to the estimation of biotic effect according to the Maqka's scale emphasized its competitiveness to those species. Also, a strong mutual stimulation of some pairs of fungal species (*A. bolleyi* and *A. strictum*, *A. bolleyi* and *R. cerealis*, *A. bolleyi* and *P. herpotrichoides*) estimated by the Mańka's method may be interpreted as a lack of reaction to the matual vicinity as the colonies meet along to straight line and are not inhibited in growth. However, in the evaluation of a complex phenomenon, which is biotic effect of fungal communities on soil pathogens, a more complete picture, no doubt, gives this last method.

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Interakcje między grzybami zasiedlającymi podstawę źdźbła pszenicy ozimej

Streszczenie

W warunkach *in vitro* analizowano wzajemne oddziaływania między grzybami zasiedlającymi podstawę źdźbła pszenicy ozimej: *Fusarium culmorum*, *F. avenaceum F. poae*, *Rhizoctonia cerealis*, *Pseudocercosporella herpotrichoides*, *Alternaria alternata* oraz *Aureobasidium bolleyi*. Niektóre saprotrofy stymulowały, w warunkach laboratoryjnych rozwój *R. cerealis*, *P. herpotrichoides* i *F. poae*.