

Mycoparasitic fungi reducing the incidence and virulence of *Bipolaris sorokiniana*

ELIŠKA ONDRÁČKOVÁ^{1*}, MICHAL ONDŘEJ¹, EVŽENIE PROKINOVÁ², MILOSLAV NESRSTA³

¹Agritec, Research, Breeding and Services, Ltd., Zemědělská 2520/16, CZ-787 01 Šumperk, Czech Republic;
ondrackova@agritec.cz

²Czech University of Life Sciences Prague, Kamýčká 1, CZ-165 21 Praha 6, Czech Republic;
prokinova@af.czu.cz

³Fytovita Ltd., Ostrožská Lhota 413, CZ-687 23 Ostrožská Lhota, Czech Republic;
fytovita@ostrozsko.cz

*corresponding author

Ondráčková E., Ondřej M., Prokinová E., Nesrsta M. (2013): Mycoparasitic fungi reducing the incidence and virulence of *Bipolaris sorokiniana*. – Czech Mycol. 65(1): 103–112.

The mycoparasitic efficiency of 28 strains/isolates of *Clonostachys rosea* f. *rosea* and *Clonostachys rosea* f. *catenulata* against the pathogenic fungus *Bipolaris sorokiniana* was determined in dual cultures on Czapek-Dox nutrient agar. Strains with low and medium efficiency were antagonistically inhibited by *B. sorokiniana*, and inhibitory zones were formed between the colonies of both fungi. The mycelium of *Clonostachys* strains with high efficiency overgrew and degraded *B. sorokiniana* colonies without formation of an inhibitory zone. In dual cultures, *Trichoderma* sp. and the most effective *Clonostachys* strains degraded *B. sorokiniana* colonies within 3 to 5 and 12 to 15 days, respectively. When rye seeds were treated with a mixture of *C. rosea* f. *rosea*, *Trichoderma* sp. and *B. sorokiniana*, development of *B. sorokiniana* on both seeds and seedlings was reduced when compared with a *B. sorokiniana* treatment. The treatment of rye seeds with *Clonostachys* and *Trichoderma* had a positive effect on seed germination and seedling length.

Key words: mycoparasitic activity, mycoparasitic fungi, *Clonostachys*, *Trichoderma*, *Bipolaris sorokiniana*.

Ondráčková E., Ondřej M., Prokinová E., Nesrsta M. (2013): Mykoparazitické houby redukující výskyt a škodlivost patogenní houby *Bipolaris sorokiniana*. – Czech Mycol. 65(1): 103–112.

V podvojných kulturách na živné půdě Czapek-Dox byla studována mykoparazitická účinnost 28 kmenů *Clonostachys rosea* f. *rosea* a *Clonostachys rosea* f. *catenulata* proti patogenní houbě *Bipolaris sorokiniana*. Kmeny s nízkou a střední účinností houba *B. sorokiniana* antagonisticky inhibovala za tvorby inhibiční zóny. Kmeny s vysokou účinností nebyly inhibovány při styku okrajů kolonií obou hub, mycelium *Clonostachys* přerůstalo kolonie *B. sorokiniana* a degradovalo je. Nejrychlejší destrukce kolonií *B. sorokiniana* byla v podvojných kulturách zjištěna u houby *Trichoderma* sp., která kolonie totálně degradovala do 3–5 dnů. Nejúčinnější kmeny *Clonostachys* degradovaly kolonie *B. sorokiniana* pomaleji do 12–15 dnů. Ošetření osiva žita směsí *B. sorokiniana* + *C. rosea* + *Trichoderma* sp. eliminovalo výskyt a škodlivost patogenní houby *B. sorokiniana*. Ošetření osiva žita houbami *Clonostachys* a *Trichoderma* mělo pozitivní vliv na klíčivost a délku klíčnicích rostlin.

INTRODUCTION

The fungus *Cochliobolus sativus* (S. Ito et Kurib.) Drechsler ex Dastur [anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker] is an important pathogen of cereals (*Hordeum*, *Triticum*, *Secale*) and grasses (*Poa*, *Agrostis*, *Lolium*, etc.). The fungus is a part of the soil pathogen complex and is both soil-borne and seed-borne. *B. sorokiniana* reduces germination of seeds and causes root rot and leaf yellowing, necrosis of emerging plants and reduces growth and yield. Overall reduction in yield can reach up to 30 % (Welling et al. 1957, Straube & Fritsche 1978, Al-Sadi & Deadman 2010).

The fungus survives saprotrophically in the soil for a long time on plant debris. Infected seed is the main source of plant infection. *B. sorokiniana* is the only pathogen consistently isolated from seed, crown rot and root rot diseases of barley, wheat and rye cultivars. Yield losses are significantly correlated with occurrence of *B. sorokiniana* on seeds. Frequency of natural infection of seed with *Bipolaris* ranges from 0 to 13 % (Knudsen et al. 1995, Al-Sadi & Deadman 2010).

B. sorokiniana varies greatly in pathogenicity, growth rate and in production of phytotoxic and fungistatic metabolites which effectively inhibit growth of many antagonistic microorganisms in the soil (Harding 1984, Kachlicki 1995). To date, more than 20 different metabolites have been identified, among which sorokinianin (sesquiterpen), prehelminthosporol, helminthosporopsid (α -D-galaktosid 12-Dihydrocyclopropan), steroids, terpenoids, glycosides, and pentapeptides are the most recorded ones (Straube & Fritsche 1978, Nakajima et al. 1994, Kachlicki 1995, Ghazvini & Tekauz 2007, Friesen et al. 2008, Han et al. 2010).

Besides fungicides, treatment with biological products based on mycoparasitic fungi and chomista (*Trichoderma*, *Clonostachys*, *Chaetomium*, and *Pythium oligandrum*) can be used to reduce incidence of *B. sorokiniana* in the soil. Only highly active strains of these organisms have the ability to overcome the fungistatic effects of the metabolites of *B. sorokiniana* and to decompose its mycelium in the soil (Tveit & Moore 1959, Lockwood 1964, Chinn 1967, Fokkema 1973, Lockwood 1977, Katznelson 1980, Epstein & Lockwood 1984, Knudsen et al. 1995).

Mycoparasitic fungi of the genera *Trichoderma* and *Clonostachys* significantly reduce incidence of *B. sorokiniana* and damages caused by it. *C. rosea* f. *rosea* and *Trichoderma* spp. are common saprotrophic and antagonistic soil-borne fungi worldwide, which have in addition an antagonistic and mycoparasitic effect against other pathogenic fungi. Currently the mode of action responsible for their antagonism is not well understood. Mycoparasitism, substrate competition, antibiosis and induced resistance can all play a role in antagonistic relationships. Clonostachin (peptaibol) is an antagonistic metabolite produced by some highly active strains of *C. rosea* f. *rosea* (Barnet & Lilly 1962, Pachenari & Dix 1980, Chikanishi et al. 1996, Schroers et al. 1999, Domsch et al. 2007, Rodriques 2011).

The aim of the present study was to determine the mycoparasitic activity of *Clonostachys* strains available in the Czech fungal collections against *B. sorokiniana*. Another objective of this work was to compare the effectiveness of the most active *Clonostachys* strains and *Trichoderma* sp., *Chaetomium globosum*, *Chaetomium cochliodes*, and *Pythium oligandrum* against *B. sorokiniana*.

MATERIAL AND METHODS

Mycoparasitic organisms. To test their mycoparasitic activity against *B. sorokiniana*, 28 *Clonostachys* strains were obtained from the Culture Collection of Fungi (CCF) in Prague, Czech Collection of Microorganisms (CCM) in Brno and Agritec Ltd. in Šumperk (AGT isolates). The origins of the isolates are shown in Tab. 1. *Chaetomium globosum* and *Ch. cochliodes* strains were obtained from the Culture Collection of Fungi (CCF), *P. oligandrum* was isolated from the bio-product Polyversum (Biopreparáty Ltd., Czech Republic) and *Trichoderma* sp. from the bio-product Gliorex – mixture of the mycoparasitic fungi *C. rosea* f. *rosea* and *Trichoderma* sp. (Fytovita Ltd. Ostrožská Lhota, Czech Republic).

Pathogenic fungus. *Bipolaris sorokiniana* was isolated in 2009 from diseased rye plants (*Secale cereale*) grown at Šumperk-Temenice, Czech Republic.

Culture conditions. All fungi and chromista used in the experiments were grown at 20–25 °C with a natural photoperiod of day/night cycle in 9 cm diameter Petri dishes on Czapek-Dox nutrient agar.

In vitro methods. Mutual interactions between *B. sorokiniana* and the antagonistic organisms were determined in dual cultures in which mycelial disks of 0.5 cm in diameter of both the antagonistic fungus and *B. sorokiniana* (from 14-day cultures) were inoculated 4–5 cm opposite each other in Petri dishes. There were four replicates of each treatment. The inoculated dishes were incubated at 20–25 °C. The inhibitory zones created between both the pathogen and the bio-agent colonies were measured 5 days after inoculation. After mutual contact of both pathogen and bio-agent colonies, the ability of the bio-agent to overgrow and to degrade the pathogen colony was recorded. The rate of degradation was expressed as the visually degraded area of the pathogen colony in mm² 20 days after mutual contact. Digital image analysis (Nikon DS-U1 digital camera and Pentax macro optics; Cosmocar zoom objective, Nikon Instruments, CS-Optoteam, Prague, Czech Republic), and NIS Elements AR 2.30 software (LIM, Laboratory Imaging Prague, Czech Republic) were used to that aim.

The bio-agent's ability to inhibit the growth of the pathogen was determined using a mixed-test method in which mycelial disks 1 cm in diameter with both the antagonistic fungus and *B. sorokiniana* (both from pure cultures) were ground together using a mortar and pestle. The homogenous mixture of these fungi was

Tab. 1. Evaluation of *Clonostachys rosea* f. *rosea* and *C. rosea* f. *catenulata* activities against the pathogenic fungus *Bipolaris sorokiniana* in dual cultures 20 days after contact of the colony edges.

Strain	Origin	Colony degradation of <i>Bipolaris</i> (%)	Mycoparasitic activity
<i>C. rosea</i> f. <i>rosea</i> CCF 4182	<i>Glycine</i> – root	99.0 l	very high
<i>C. rosea</i> f. <i>catenulata</i> CCF 4184	<i>Ribes</i> – root	98.3 kl	very high
<i>C. rosea</i> f. <i>catenulata</i> AGT 12	<i>Malus</i> – leaf	98.0 kl	very high
<i>C. rosea</i> f. <i>rosea</i> CCF 4183	<i>Linum</i> – root	97.0 jkl	very high
<i>C. rosea</i> f. <i>rosea</i> AGT 4	<i>Ribes</i> – twigs	96.2 jkl	high
<i>C. rosea</i> f. <i>rosea</i> CCF 4181	<i>Claviceps</i> – sclerotium	96.0 jkl	high
<i>C. rosea</i> f. <i>catenulata</i> CCM 8071	<i>Pinus</i> – cone	94.0 jkl	high
<i>C. rosea</i> f. <i>rosea</i> AGT 16	<i>Faba</i> – root	93.5 jk	high
<i>C. rosea</i> f. <i>catenulata</i> CCF 3686	<i>Betula</i> – leaf	92.2 j	high
<i>C. rosea</i> f. <i>rosea</i> AGT 7	<i>Thuja</i> – root	92.2 j	high
<i>C. rosea</i> f. <i>rosea</i> AGT 11	<i>Faba</i> – root	86.5 i	medium
<i>C. rosea</i> f. <i>rosea</i> CCF 3532	soil	84.7 i	medium
<i>C. rosea</i> f. <i>rosea</i> AGT 17	<i>Faba</i> – root	82.3 i	medium
<i>C. rosea</i> f. <i>rosea</i> AGT 5	<i>Phaseolus</i> – root	73.7 h	medium
<i>C. rosea</i> f. <i>rosea</i> AGT 8	<i>Glycine</i> – root	71.2 gh	medium
<i>C. rosea</i> f. <i>rosea</i> AGT 6	<i>Phaseolus</i> – root	66.8 g	medium
<i>C. rosea</i> f. <i>catenulata</i> AGT 15	<i>Ribes</i> – root	50.0 f	medium
<i>C. rosea</i> f. <i>rosea</i> AGT 10	<i>Solanum</i> – tuber	40.7 e	low
<i>C. rosea</i> f. <i>rosea</i> AGT 9	<i>Glycine</i> – root	40.0 e	low
<i>C. rosea</i> f. <i>catenulata</i> AGT 14	<i>Sclerotinia</i> – sclerotium	38.8 e	low
<i>C. rosea</i> f. <i>rosea</i> CCF 3222	wheat	37.3 e	low
<i>C. rosea</i> f. <i>rosea</i> CCF 1495	air	30.2 d	low
<i>C. rosea</i> f. <i>catenulata</i> CCF 1494	eggs of geohelminths	27.3 d	low
<i>C. rosea</i> f. <i>rosea</i> CCF 2813	dead stem	26.0 d	low
<i>C. rosea</i> f. <i>rosea</i> CCF 2814	unknown	20.3 c	low
<i>C. solani</i> CCF 923	soil	14.2 b	low
<i>C. rosea</i> f. <i>rosea</i> CCF 2500	<i>Platanus</i> – bark	6.3 a	low
<i>C. rosea</i> f. <i>rosea</i> AGT 18	<i>Betula</i> – leaf	3.7 a	low

ANOVA, Tukey LSD test ($P = 0.05$). Values for each strain followed by the same letter are not significantly different at $P = 0.05$.

placed into the centre of a Petri dish (4 replicates). Interaction of both fungi was evaluated 5 days after inoculation.

Seed treatment of rye. Rye seeds (cv. Selgo) were inoculated with spore suspensions of 10^7 spores of *B. sorokiniana*, *C. rosea* f. *rosea* and *Trichoderma* sp. per ml at a rate of 10 ml per kg of seed. To obtain a fungus suspension, two 15-day-old cultures of each fungus were mixed with 100 ml of water. In the laboratory experiment, six seed treatments were used, i.e. *B. sorokiniana*, *C. rosea* f. *rosea*,

Trichoderma sp., *B. sorokiniana* + *C. rosea* f. *rosea*, *B. sorokiniana* + *Trichoderma* sp., and an untreated control.

In vitro laboratory tests. Four layers of filter paper in each Petri dish 12 cm in diameter were moistened with 6 ml of distilled water and 50 treated (as described above) rye seeds were placed into each dish. Each treatment was replicated three times. Five days later, the frequency of seed germination and the length of coleoptiles and roots were measured. All laboratory tests were repeated three times.

Statistical analysis. The experimental data were subjected to analysis of variance (ANOVA) using the STATISTICA 10 software. The treatment means were compared using the Tukey LSD test or Kruskal-Wallis test at $p = 0.05$.

RESULTS

Based on their mycoparasitic activities against *B. sorokiniana*, the *Clonostachys* strains were divided into four groups, i.e. strains with very high, high, medium, and low activity.

The *Clonostachys* strains with high to very high mycoparasitic activity did not form inhibitory zones in dual cultures on Czapek-Dox nutrient agar. Mycelia of these strains overgrew and degraded *B. sorokiniana* after the *Bipolaris* and *Clonostachys* colonies had made contact. *B. sorokiniana* inhibited the growth of *Clonostachys* strains with low to medium mycoparasitic activity, inhibitory zones of 1–5 mm were formed and degraded *B. sorokiniana* colonies partially there. *Clonostachys* strains with high, medium, and low mycoparasitic activity degraded *B. sorokiniana* colonies by 92.2 to 99 %, 50 to 86.5 %, and 3.7 to 40.7 %, respectively when compared with the untreated control (Tab. 1).

Mycoparasitic activity of *Clonostachys* strains (CCF 4181, CCF 4182, CCF 4183 and CCF 4184) and other mycoparasitic organisms (*P. oligandrum*, *Trichoderma* sp., *Ch. globosum* and *Ch. cochliodes*) against *B. sorokiniana* was assessed in both dual and mixed cultures.

Mycoparasitic activity was not detected in dual cultures of *P. oligandrum*, *Ch. globosum* (CCF 2785) and *Ch. cochliodes* (CCF 1447). The growth inhibition between the *Ch. globosum* CCF 3429 and *B. sorokiniana* strains was mutual in both dual and mixed tests. Of all tested strains of mycoparasitic organisms, the *Trichoderma* sp. strain rapidly and totally degraded the *B. sorokiniana* colonies. *B. sorokiniana* colonies were degraded within 3 to 5 days after both colonies made contact. Tested strains of *Clonostachys* overgrew and degraded the *B. sorokiniana* colonies more slowly (CCF 4182 up to 12–15 days, CCF 4184 16–18 days, CCF 4183 20–21 days and CCF 4181 24–26 days), see Tab. 2.

Tab. 2. Mycoparasitic activities of fungi involved in the growth reduction and colony degradation of the fungus *Bipolaris sorokiniana* in dual tests on Czapek-Dox agar. Evaluated after 20 days of mutual contact.

Variants of fungi in dual test	<i>Bipolaris</i> colony size (mm ²)	% of control	Mycoparasitic fungus colony size (mm ²)	Degraded area of <i>Bipolaris</i> colony (%)
<i>Bipolaris</i> – control	1737 a	100.0 a	–	0.0 a
<i>Bipolaris</i> + <i>Chaetomium globosum</i> CCF 2785	1793 a	101.4 a	2663 a	0.0 a
<i>Bipolaris</i> + <i>Pythium oligandrum</i>	1715 a	97.0 a	2034 e	0.0 a
<i>Bipolaris</i> + <i>Chaetomium cochliodes</i> CCF 1447	1679 a	95.0 a	3740 b	0.0 a
<i>Bipolaris</i> + CCF 4181 ¹	1344 d	76.0 d	2710 a	28.5 c
<i>Bipolaris</i> + CCF 4183 ¹	1238 d	70.0 d	3938 b	44.4 d
<i>Bipolaris</i> + CCF 4184 ²	1085 c	61.4 c	4804 c	77.7 e
<i>Bipolaris</i> + CCF 4182 ¹	1024 c	57.9 c	4883 c	100.0 b
<i>Bipolaris</i> + <i>Trichoderma</i> sp.	114 b	6.5 b	6085 f	100.0 b
<i>Bipolaris</i> + <i>Chaetomium globosum</i> CCF 3429	86*b	4.9 b	93*d	0.0 a

* mutual inhibition of both fungi; ANOVA Tukey LSD test ($P = 0.05$). Data in a column for each variant of fungi followed by the same letter are not significantly different at $P = 0.05$.

¹ *Clonostachys rosea* f. *rosea* strain; ² *Clonostachys rosea* f. *catenulata* strain

Mutual growth inhibition was observed between *B. sorokiniana* and *Ch. globosum* CCF 3429 in the test with mixed cultures. Of mixed cultures, *B. sorokiniana* + *Ch. globosum* CCF 2785, *B. sorokiniana* + *P. oligandrum*, and *B. sorokiniana* + *Ch. cochliodes*, only *B. sorokiniana* grew. In mixed cultures of *B. sorokiniana* with *Trichoderma* sp., only *Trichoderma* sp. grew. With *Clonostachys* strains, *B. sorokiniana* increased initially and *Clonostachys* strains started growing after 3 days and then rapidly degraded *B. sorokiniana* colonies (Tab. 3).

The mycoparasitic efficacy of *C. rosea* f. *rosea* CCF 4181 and *Trichoderma* sp. (included in the bio-product Gliorex) in reducing the occurrence of *B. sorokiniana* has been investigated under laboratory conditions on rye seeds (cv. Selgo).

Germination of seeds treated with *B. sorokiniana* decreased by 27.5 %, and both root length and shoot length by 28.9 and 36.0 %, respectively, in comparison with an untreated control (Tab. 4). *C. rosea* f. *rosea* CCF 4181 and *Trichoderma* sp. strains completely inhibited the incidence of *B. sorokiniana* on seedlings and increased both root length and shoot length by 15.8 and 19.2 % on average, respectively, compared with the untreated control and by 62.8 and 78.3 %, respectively, compared with the *B. sorokiniana* treatment (Tab. 4).

Tab. 3. Mycoparasitic activities of fungi and chromista involved in the growth reduction and colony degradation of *Bipolaris sorokiniana* in mixed tests at a 1:1 ratio. Evaluated after 5 days.

Combination of fungi or chromista in mixed test	<i>Bipolaris</i> colony size (mm ²)	% of control	Mycoparasitic fungus colony size (mm ²)	Degraded area of <i>Bipolaris</i> colony (%)
<i>Bipolaris</i> – control	5030	100.0 b	–	0.0 a
<i>Bipolaris</i> + <i>Pythium oligandrum</i>	4999	99.4 b	0 a	0.0 a
<i>Bipolaris</i> + <i>Chaetomium cochliodes</i> CCF 1447	4855	96.5 b	0 a	0.0 a
<i>Bipolaris</i> + <i>Chaetomium globosum</i> CCF 2785	4770	94.8 b	0 a	0.0 a
<i>Bipolaris</i> + CCF 4183 ¹	684	13.6 d	3557 b	94.6 c
<i>Bipolaris</i> + CCF 4181 ¹	497	9.9 ad	3537 b	94.3 c
<i>Bipolaris</i> + CCF 4184 ²	452	9.0 a	3958 b	98.6 b
<i>Bipolaris</i> + CCF 4182 ¹	436	8.7 a	3983 b	98.3 b
<i>Bipolaris</i> + <i>Trichoderma</i> sp.	14	0.3 c	7451 c	100.0 b
<i>Bipolaris</i> + <i>Chaetomium globosum</i> CCF 3429	4*	0.1 c	4* a	100.0 b

* mutual inhibition of both fungi; ANOVA, Tukey LSD test (P = 0.05). Data in a column for each combination of fungi followed by the same letter are not significantly different at P = 0.05.

¹ *Clonostachys rosea* f. *rosea* strain; ² *Clonostachys rosea* f. *catenulata* strain

Tab. 4. Effect of seed inoculation of rye (cv. Selgo) with spore suspension of *Bipolaris sorokiniana* and with mixtures of spore suspensions of *Clonostachys rosea* + *B. sorokiniana* and *Trichoderma* sp. + *B. sorokiniana* on germination, root length and shoot length. In vitro test in Petri dishes. Evaluated 5 days after the establishment of the experiment.

Type of inoculation	Seed germination (%)	Seed germination (% of control)	Root length (mm)	Root length (% of control)	Shoot length (mm)	Shoot length (% of control)
Control – without inoculation	92.7	100.0	25.3	100.0	50.1	100.0
<i>Bipolaris sorokiniana</i>	67.2	72.5	18.0	71.1	32.1	64.0
<i>C. rosea</i> f. <i>rosea</i>	90.8	98.0	28.7	113.4	54.9	109.6
<i>Trichoderma</i> sp.	91.7	98.9	29.8	117.8	60.0	119.8
<i>B. sorokiniana</i> + <i>C. rosea</i> f. <i>rosea</i>	87.2	94.1	28.8	113.8	59.4	118.5
<i>B. sorokiniana</i> + <i>Trichoderma</i> sp.	90.2	97.3	29.8	117.7	55.1	109.9

DISCUSSION

Differences in mycoparasitic activity of 28 *Clonostachys* strains against the pathogenic fungus *B. sorokiniana* were demonstrated in this work (Tab.1). Most *Clonostachys* strains were inhibited by *B. sorokiniana* in dual cultures and formed inhibition zones 1–5 mm in size. Due to formation of inhibitory zone, the

overgrowth of *B. sorokiniana* colony by the *Clonostachys* colony was reduced. When overgrowth of *B. sorokiniana* occurred, colony degradation by *Clonostachys* was slow and partial, ranging from 3.7 to 26 % of degraded colony area. However, two *Clonostachys* strains (CCF 4182, CCF 4184) were not inhibited by *B. sorokiniana*, but rapidly overgrew and degraded the entire *B. sorokiniana* colonies within a few days.

As previously reported, *B. sorokiniana* produces at least 20 different metabolites with phytotoxic, fungistatic and bacteriostatic effects protecting itself against soil antagonists and mycoparasitic microorganisms (Katznelson 1940, Lockwood 1964, 1977, Epstein & Lockwood 1984, Harding 1984, Kachlicki 1995). The fungistatic metabolites of *B. sorokiniana* which inhibit the growth of some *Clonostachys* strains are still unknown. Also the mode of deactivation of the fungistatic metabolites of *B. sorokiniana* by *Clonostachys* strains with high mycoparasitic activity is unknown. Both production of similar enzymes as produced by *Trichoderma* fungi and production of the fungistatic substance clonostachin can play a role in the deactivation process of *B. sorokiniana* metabolites (Pachenari & Dix 1980, Barnet & Lilly 1962, Chikanishi et al. 1996, Rodriguez et al. 2011, Bulat et al. 2000).

The present study demonstrated that in dual and mixed in vitro tests the highest rates of degradation of *B. sorokiniana* colonies were achieved by the fungus *Trichoderma* sp. A similar effect was found in some *Clonostachys* strains but with a slight delay. Growth dynamics of *Clonostachys* strains were four to five times slower than that of *Trichoderma* (Ondřej et al. 2010).

The use of other antagonistic organisms such as *P. oligandrum*, *Ch. cochliodes* and *Ch. globosum* in biological control of cereals is unlikely because they are inhibited by *B. sorokiniana*.

Gliorex, a commercial product used to reduce soil infestation with sclerotia of *Claviceps purpurea*, *Sclerotinia sclerotiorum*, *Sclerotium cepivorum* and *Botrytis cinerea* in the Czech Republic, could be a highly effective biological product for seed treatment of cereals in plant protection against *B. sorokiniana* (Ondřej et al. 2010).

Mycoparasitic fungi contained in Gliorex reduced pathogen occurrence on artificially infected rye seeds and seedlings in laboratory experiments and positively influenced germination of seeds and overall root and shoot growth. Our results confirm the results of Knudsen et al. (1995) in which growth dynamics and yield parameters increased and the occurrence of the pathogens *Fusarium culmorum* and *B. sorokiniana* decreased after seed treatment with the *C. catenulata* J1446 strain.

ACKNOWLEDGEMENTS

Supported by the National Agency for Agriculture Research (NAAR), Project No. QI111C039, and by RO0111. The authors thank Iva Smýkalová, Agritec Plant Protection Ltd., Czech Republic, for carrying out the digital image analysis, Mr. Jack Sutherland, Canada, and Mr. Miroslav Griga, Agritec Plant Protection Ltd., Czech Republic, for language corrections to the manuscript.

REFERENCES

- AL-SADI A.M., DEADMAN M.L. (2010): Influence of seed-borne *Cochliobolus sativus* (anamorph *Bipolaris sorokiniana*) on crown rot and root rot of barley and wheat. – J. Phytopathol. 158: 683–690.
- BARNET H.L., LILLY V.G. (1962): A destructive mycoparasite *Gliocladium roseum*. – Mycologia 54: 72–77.
- BULAT S.A., LÜBECK M., ALEKHINA I.A., JENSEN D.F., KNUDSEN I.M., LÜBECK P.S. (2000): Identification of a universally primed-PCR-derived sequence-characterized amplified region marker for an antagonistic strain of *Clonostachys rosea* and development of a strain-specific PCR detection assay. – Appl. Environ. Microb. 66(11): 4758–4763.
- DOMSCH K.H., GAMS W., ANDERSON T.-H. (2007): Compendium of soil fungi. 2nd edition – 672 p. Eching.
- EPSTEIN L., LOCKWOOD J.L. (1984): Effect of soil microbiota on germination of *Bipolaris victoriae* conidia. – Trans. Br. Mycol. Soc. 82: 63–69.
- FOKKEMA N.J. (1973): The role of saprophytic fungi in antagonism against *Drechslera sorokiniana* (*Helminthosporium sativum*) on agar plates on rye leaves with pollen. – Physiol. Plant Pathol. 3: 195–205.
- FRIESEN L., FARIS J.D., SOLOMON P.S., OLIVER R.P. (2008): Host-specific toxins: effectors of necrotrophic pathogenicity. – Cell. Microbiol. 10(7): 1421–1428.
- GHAZVINI H., TEKAUZ A. (2007) Virulence diversity in the population of *Bipolaris sorokiniana*. – Plant Dis. 91: 814–821.
- HAN Q., HUANG L., BUCHENAUER H., WANG C., KANG Z. (2010): Cytological Study of wheat spike infection by *Bipolaris sorokiniana*. – J. Phytopath. 158: 22–29.
- HARDING H. (1984): Spore colour mutants of *Bipolaris sorokiniana*. – Can. J. Plant Pathol. 6: 273–279.
- CHIKANISHI T., HASUMI K., HARADA T., KAWASAKI N., ENDO A. (1996): Clonostachin a novel peptaibol that inhibits platelet aggregation. – J. Antibiot. 50: 105–118.
- CHINN S.H.F. (1967): Differences in fungistasis in some Saskatchewan soil with special references to *Cochliobolus sativus*. – Phytopathology 57: 224–226.
- KACHLICKI P. (1995): Metabolites of *Helminthosporium* – In: Chelkowski J., ed., Helminthosporia – Metabolites, biology, plant diseases – *Bipolaris*, *Drechslera*, *Exserohilum*. p. 1–26, Poznań.
- KATZNELSON H. (1940): Survival of microorganisms introduced into soil. – Soil Sci. 49: 283–293.
- KNUDSEN I.M.B., HOCKENHULL J., JENSEN D.F. (1995): Biocontrol of seedling diseases caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: effect of selected fungal antagonists on growth and yield components. – Plant Pathol. 44: 467–471.
- LOCKWOOD J.L. (1964): Soil fungistasis. – Ann. Rev. Phytopathol. 2: 341–362.
- LOCKWOOD J.L. (1977): Fungistasis in soil. – Biol. Rev. 52: 1–43.
- NAKAJIMA H., ISOMI K., HAMASAKI T., ICHINOE M. (1994): Sorokinianin: a novel phytotoxin produced by the phytopathogenic fungus *Bipolaris sorokiniana*. – Tetrahedron Lett. 35: 9597–9600.

- ONDŘEJ M., CAGAŠ B., ONDRÁČKOVÁ E. (2010): Effect of mycoflora of ergot (*Claviceps purpurea*) sclerotia on their viability. – Plant Protect. Sci. 46 (2): 66–71.
- PACHENARI A., DIX N.J. (1980): Production of toxin and wall degrading enzymes by *Gliocladium roseum*. – Trans. Br. Mycol. Soc. 74: 561–566.
- RODRIGUEZ M.A., CABRERA G., GOZZO F.C., EBERLIN M.N., GODEAS A. (2011): *Clonostachys rosea* BAFC3874 as a *Sclerotinia sclerotiorum* antagonist: mechanisms involved and potential as a biocontrol agent. – J. Appl. Microbiol. 110: 1177–1186.
- SCHROERS H.J., SAMUELS G.J., SEIFERT K.A., GAMS W. (1999): Classification of the mycoparasite *Gliocladium roseum* in *Clonostachys rosea*, its relationship to *Bionectria ochroleuca*, and notes on other *Gliocladium*-like fungi. – Mycologia 91: 365–385.
- STRAUBE G., FRITSCHÉ W. (1978): Phytopathogene Toxine-Struktur, Wirkungsweise und mögliche Bedeutung als Unkrautbekämpfungsmittel. – Biologische Rundschau 16: 232–243.
- TVEIT M., MOORE M.B. (1954): Isolates of *Chaetomium* protect oats from *Helminthosporium victoriae*. – Phytopathology 44: 686–689.
- WELLING J.L., JENSEN S.G., HAMILTON R.L. (1957): *Helminthosporium sativum*, a destructive pathogen of bluegrass. – Phytopathology 47: 744–746.