Effect of the Mycoflora of Ergot (*Claviceps purpurea*) Sclerotia on their Viability

Michal ONDŘEJ[1], Bohumír CAGAŠ[2] and Eliška ONDRÁČKOVÁ[1]

[1]AGRITEC, Research, Breeding and Services, Ltd., Šumperk, Czech Republic;
[2]OSEVA PRO, Ltd., Grassland Research Station Rožnov-Zubří, Zubří, Czech Republic

Abstract

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Twenty fungal species were isolated from sclerotia of ergot (*Claviceps purpurea)* originating from rye. In *in vitro* tests with the anamorphic stage of ergot (known as *Sphacelia segetum)* high mycoparasitic activity was exhibited only by two of them – *Clonostachys rosea* and *Trichoderma harzianum,* moderate mycoparasitic activity was detected in *Ulocladium* sp., *Clonostachys catenulata*, *Trichoderma hamatum,* and *Trichothecium roseum*. In *in vitro* tests of mycoparasitic activity with sclerotia of the fungus *Sclerotinia sclerotiorum* their rapid and total destruction was brought about only by the fungi *Clonostachys rosea* and *Trichoderma harzianum*. The viability of overwintering ergot sclerotia in the soil was influenced by their placement, age, depth of placement and treatment with a conidial suspension of the fungus *Clonostachys rosea*. The highest viability values were reported in fresh one-year-old sclerotia. In two-year-old sclerotia their viability was reduced and their sensitivity to microbial activity was higher. The sclerotia aged 3 years or more did not germinate and were all microbiologically degraded in the soil. More than 80% of degradation of sclerotia which were 1 to 2 years old was caused by the fungus *Clonostachys rosea*. The degradation of sclerotia aged 3 to 4 years was brought about by bacteria, soil edafauna (mites, nematodes) and fungi of the genera *Trichoderma*, *Fusarium*, *Clonostachys,* etc.

Keywords: sclerotia; *Claviceps purpurea*; mycoflora of sclerotia; mycoparasitic degradation of sclerotia; *Clonostachys rosea*

No literary data have been found about the practical use of mycoparasitic fungi to reduce the ergot of rye (*Secale cereale*). The occurrence of mycoflora on ergot (*Claviceps purpurea*) sclerotia was studied by Krebs (1936). He reported a few mycoses which he believed that they caused destruction of sclerotia in the soil (*Clonostachys rosea*, *Trichoderma* sp., and *Fusarium heterosporum*).

In the past the mycoparasitic activity of fungi of the genera *Trichoderma* and *Clonostachys* was tested and compared predominantly under *in vitro* conditions on nutrient medium. The highest efficacy was found against the fungi *Sclerotinia sclerotiorum*, *Sclerotinia cepivorum*, *Botrytis cinerea*, *Rhizoctonia solani*, etc. (Weidling 1937; Barnett & Lilly 1962; Burgess *et al*. 1977; Davet 1985; Papavizas 1985; Idemitsu Kosan 1997; Zazzerini & Tosi 1985; Köhl *et al*. 1999). The patented strain of the fungus *Clonostachys rosea* (ACM 941 – ATCC 7447, US Patent 6495133) has a very broad spectrum of efficacy against almost the majority of pathogenic fungi (*Alternaria*, *Aphanomyces*, *Ascochyta*, *Bipolaris*, *Fusarium*, *Pythium*, *Rhizopus*, *Rhizoctonia*, *Sclerotinia*, etc. – Xue Allen 2002).

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The objective of the study was to determine the species spectrum of fungi on ergot sclerotia and to test their mycoparasitic effect on overwintering sclerotia in the soil. Another objective was to verify the conditions affecting the viability of sclerotia in the soil over winter (effect of age, depth of placement and location).

MATERIAL AND METHODS

Isolation of fungi from ergot sclerotia. The isolation of mycoflora contaminating ergot sclerotia was carried out from 114 ergot sclerotia coming from the soil surface (collected 14 days after the harvest of rye) and from 72 ergot sclerotia placed in the soil during winter (depth 5–15 cm, placed in mesh bags with the pore size 0.5 cm). Sclerotia were washed with tap water and after drying they were placed on the surface of nutrient agar (Czapek-Dox) – 5 sclerotia per dish. After 10 days of incubation (20 ± 3°C) the number of fungal colonies around sclerotia was evaluated. The detection of individual fungi was realised microscopically after isolation and obtaining pure cultures. All fungi produced conidia spontaneously.

Evaluation of the mycoparasitic activity of fungal isolates against the anamorphic stage of ergot – *Sphacelia segetum*. The activity of fungal isolates was evaluated in dual culture in Petri dishes (9 cm in diameter) on Czapek-Dox medium. The disks (0.5 cm in diameter) of both mycoparasitic fungi and the conidial stage of *Sphacelia* were inoculated to the rim of Petri dishes (within 3 cm of the rim) against each other (4 replications). After 5 days of incubation (incubation temperature 20 ± 3°C) there occurred interactions between the *Sphacelia* colony and the colony of tested fungi (colony area of *Sphacelia segetum* was 4 ± 0.5 cm in diameter). Two types of interaction were observed: stoppage of growth and non-overgrowth of *Sphacelia* colony or overgrowth of *Sphacelia* colony and its lytic degradation. Within 30 days after inoculation (depending on the growth dynamics of the tested fungus) lytic degradation (overgrowth of the tested fungi over *Spacelia* colony) of *Sphacelia* colony in mm^2 was measured and expressed in percentage.

Evaluation of the mycoparasitic activity of selected fungal isolates against sclerotia of *Sclerotinia sclerotiorum in vitro.* A method developed by Zazzerini and Tosi (1985) was used for evaluation. The test involves sclerotia of the fungus *Sclerotinia sclerotiorum* (they germinate rapidly and dynamics of mycelium growth is high). The ergot sclerotia were not suitable for *in vitro* tests because they germinated unevenly and after a longer period they produced only the anamorph of *Sphacelia segetum*. A group of test isolates was complemented by a different isolate of the fungus *Clonostachys rosea* from dead roots of broad bean (*Vicia faba*) and an isolate of the fungus *Coniothyrium minitans* (origin – sclerotia of the fungus *Sclerotinia sclerotiorum* from rapeseed stand treated with the biopreparation Contans). Fresh *Sclerotinia sclerotiorum* sclerotia from nutrient agar (Czapek-Dox) were shortly soaked in a conidial suspension of the test fungi (titrant 10^5) and after drying they were placed on agar (6 sclerotia per dish, replicated 6 times, control – untreated sclerotia). After 3–6 days the number of germinated sclerotia was counted and the percentage of inhibition (destruction) was determined. The data were statistically evaluated by ANOVA (Kruskal-Wallis test, $P = 0.05$).

Evaluation of the mycoparasitic activity of selected fungal isolates against sclerotia of ergot (*Claviceps purpurea*) ***under field conditions***. After treatment with the conidial suspension of the fungi *Fusarium heterosporum*, *Trichoderma harzianum* and *Clonostachys rosea* some fifty fresh ergot sclerotia from the 2005 harvest were put into textile mesh bags (about 50 sclerotia per bag) and buried in the soil (at a depth of ca 8 cm) in different locations over winter (November 2005–April 2006). Various locations: 1 = edge of the field under the trees, 2 = middle of the field, 3 = near the wall – south exposure, 4 = greenhouse. Retrieved from the soil and washed with water, sclerotia were placed on moistened filter paper in Petri dishes. The germinating ability of sclerotia and the degree of microbial destruction were evaluated. The data were statistically evaluated by ANOVA (Kruskal-Wallis test, $P = 0.05$) and *t*-test for independent samples.

Evaluation of the effect of age and depth of placement of ergot sclerotia in the soil over winter on their viability. Fifty ergot sclerotia of different ages (harvested during the years 2004–2007 and stored at room temperature) were put into textile mesh bags and buried at different soil depths (2 cm, 8 cm and 15 cm) over the winter season (November 2007–April 2008). Retrieved from the soil and washed with water, sclerotia were placed

on moistened filter paper in Petri dishes. The germinating ability of sclerotia, number of apothecia produced per sclerotium, microbial destruction and the causal agent of destruction were evaluated. The data were statistically evaluated by ANOVA (Kruskal-Wallis test, $P = 0.05$).

RESULTS

A total of 20 fungal isolates were obtained from ergot sclerotia on the soil surface after rye harvest and from sclerotia retrieved from the soil after overwintering (Table 1). The highest frequency of occurrence was reported in the strains of *Clonostachys rosea* (39%), *Trichoderma harzianum* (16%), *Fusarium heterosporum* (7%) and in a group of three strains accounting for 5% (*Fusarium oxysporum*, *Stachybotrys alternans* and *Ulocladium* sp.). In dual culture with the anamorphic stage of ergot – *Sphacelia segetum* only two strains showed *in vitro* high efficacy (100% colony degradation). These were *Clonostachys rosea,* and *Trichoderma harzianum*. Moderate mycoparasitic activity (less than 50% colony degradation) was found in the fungal strains of *Trichoderma hamatum*, *Clonostachys catenulata*, *Trichothecium roseum* and *Ulocladium* sp. The remaining 14 fungal strains showed very low or no activity.

Table 1. Different fungi isolated from ergot sclerotia and their mycoparasitic activity in *in vitro* tests (Czapek-Dox) against *Sphacelia segetum*

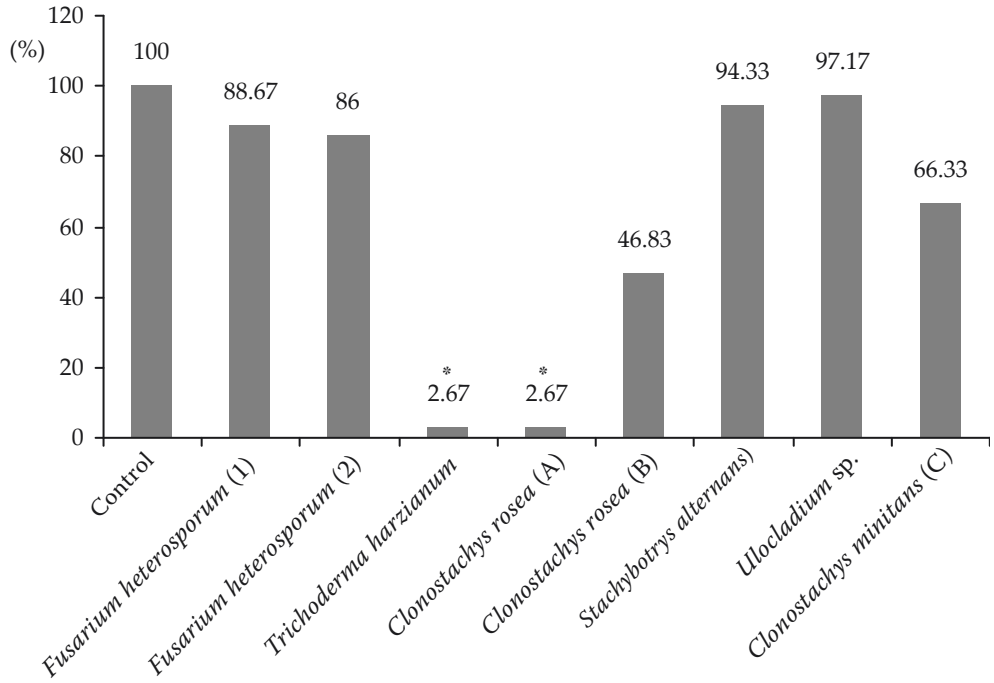
Fungus	Frequency of occurrence (%)	Mycoparasitic activity (%)
Clonostachys rosea	39	100
Trichoderma harzianum	16	100
Fusarium heterosporum	7	3.5
Fusarium oxysporum	5	3
Ulocladium sp.	5	26.3
Stachybotrys alternans	5	2.7
Clonostachys catenulata	3	48.5
Alternaria alternata	3	0
Cladosporium cladosporioides	3	0
Trichoderma hamatum	2	48.3
Fusarium avenaceum	2	0
Mucor mucedo	2	0
Rhizopus nigricans	2	0
Cladosporium herbarum	1	0
Fusarium equiseti	1	0
Clonostachys sp.	1	0
Acremonium sp.	1	3.5
Penicillium sp.	1	1.8
Trichothecium roseum	0.5	21
Aspergillus sp.	0.5	0

The ability of mycoparasitic degradation of sclerotia under *in vitro* conditions was studied in some fungal isolates using fresh sclerotia of the fungus *Sclerotinia sclerotiorum* (Figure 1). Sclerotia of this fungus germinate on nutrient medium very rapidly and evenly.

Sclerotia treated with a conidial suspension of the fungi *Trichoderma harzianum* and *Clonostachys rosea* (isolate A) germinated only sporadically (2.7%). Sclerotia became rapidly covered with sporulating mycoparasites and the sporadically growing mycelium of the fungus *Sclerotinia sclerotiorum* was gradually vacuolised, slowly degraded, and the formation of new sclerotia was sporadic. The germination percentage of the sclerotia treated with the fungus *Coniothyrium minitans* was 66% on average. The growing mycelium rapidly covered the total surface of the Petri dish forming new sclerotia profusely. The degradation of the mycelium of the fungus *Sclerotinia sclerotiorum* by the fungus *Coniothyrium* proceeded very slowly. The other test fungi did not show any mycoparasitic activity and the germinating ability of sclerotia was high (over 85%).

Fresh ergot sclerotia from the 2005 harvest were treated with a conidial suspension of the fungi *Trichoderma harzianum*, *Clonostachys rosea* and *Fusarium heterosporum* and were buried in textile mesh bags in the soil over winter (November 2005–April 2006) in different locations at a depth of 8 cm (Table 2).

The germinating ability of untreated ergot sclerotia stored in the greenhouse was very low and microbial degradation was very high (Table 2). Besides the applied mycoparasitic fungi the degradation process was enhanced by bacteria and microfauna (mites, nematodes and larvae of delicate Diptera). The highest germinating ability was found in sclerotia from a shaded place where snow lay 6 days longer. Relatively low germinating ability was in sclerotia placed near the wall (southern



(1) Isolate from sclerotia of ergot from an ear of rye; (2) Isolate from sclerotia of ergot from the soil; (A) Isolate from sclerotia of ergot from the soil; (B) Isolate from dead roots of *Vicia faba*; (C) Isolate from sclerotia of the fungus *Sclerotinia sclerotiorum*; *$P < 0.05$

Figure 1. Effect of selected fungal isolates on the germinating ability of sclerotia of *Sclerotinia sclerotiorum* treated with spore suspension in *in vitro* tests (Czapex Dox)

exposure). Compared with the control there were no differences in germinating ability between the sclerotia treated with the fungi *Fusarium heterosporum* and *Trichoderma harzianum* from the shaded place. Both fungi were apparently involved in sclerotia degradation but only slightly. Sclerotia treated with the fungus *Clonostachys rosea* were highly degraded at all places.

Ergot viability is influenced by the depth of placement in the soil and age (Table 3). The sclerotia placed near the surface are subjected to microbial degradation more than sclerotia at a greater depth. With greater depth the spectrum of the surface (contaminating) mycoflora of sclerotia decreased. In sclerotia placed near the surface 12 strains of fungi were found, at a depth of 8 cm there were

Table 2. Effect of treatment of ergot sclerotia (*Claviceps purpurea*) with conidial suspension of selected fungi on their viability after burial in the soil over the winter period of 2005/2006 at a depth of 8 cm under different conditions

Location	Germinating ability of sclerotia (%)			
	control (no treatment)	*Fusarium heterosporum*	*Trichoderma harzianum*	*Clonostachys rosea***
1	94.3	90.6	93.0	2.6
2	87.5	78.7	66.2	0
3	32.0	20.4	24.4	0
4	2.5*	0.0*	0.0*	0

1 = edge of the field, shaded by the trees; 2 = middle of the field, no shading; 3 = near the wall, south exposure; 4 = greenhouse; values marked with * are significantly different ($P < 0.05$) from values of location 1; **germinating ability of sclerotia treated with conidial suspension of *Clonosachys rosea* is significantly different ($P < 0.05$) from control, *F. heterosporum* and *T. harzianum* (*t*-test for independent samples)

Table 3. Effect of age and depth of placement of ergot sclerotia (*Claviceps purpurea*) burried in the soil on their germinating ability and microbial destruction

Age of sclerotia (year of collection)	Depth of placement in soil (mm)	Germinating ability of sclerotia (%)	Number of apothecia per sclerotium	Microbial destruction
	20	54.6	8.3	22.0
1 (2007)	80	87.5	14.2	4.5
	150	93.6*	24.5*	1.4*
	20	4.1	3.3	60.0
2 (2006)	80	8.3	5.5	38.7
	150	15.2*	16.7*	14.2*
	20	0.0	0.0	100.0
3 (2005)**	80	0.0	0.0	100.0
	150	2.4	3.3	96.7
	20	0.0	0.0	100.0
4 (2004)**	80	0.0	0.0	100.0
	150	0.0	0.0	100.0

Values marked with * are significantly different ($P < 0.05$) from values of depth 20 mm; **statistical significat ($P < 0.05$) from years 2006 and 2007 for all evaluations (germinating ability, number of apothecia per sclerotium, microbial destruction)

only 5 strains and at a depth of 15 cm only 2 strains. High viability of sclerotia was determined in fresh sclerotia. Sclerotia aged 3 and 4 years did not germinate any more and were subjected to microbial degradation in the soil over winter. The degradation of sclerotia 3–4 years old was enhanced by bacteria, soil edafauna – mites, nematodes and the fungi *Trichoderma harzianum*, *Fusarium oxysporum*, *Fusarium heterosporum*, *Clonostachys rosea*, and *Clonostachys catenulata*.

DISCUSSION

The obtained results confirmed the data provided by Krebs (1936) about the spectrum of the mycoflora of ergot sclerotia and mycoses involved in their degradation (*Clonostachys rosea*, *Trichoderma* spp. and *Fusarium heterosporum*). Of the fungal species mentioned above *Fusarium heterosporum* was not effective in sclerotia degradation tests (Table 2) even though it often colonises ergot sclerotia (Table 1). In *in vitro* tests the highest rates of degradation of sclerotia and the anamorphic stage of ergot (*Sphacelia segetum*) were achieved by the fungus *Trichoderma harzianum*. A similar effect was found in *Clonostachys rosea* (Tables 1 and 2; Figure 1) but with a slight delay (growth dynamics of *Clonostachys* is about five times slower than that of *Trichoderma*). In tests under field conditions over winter, however, the mycoparasitic effect of the fungus *Trichoderma harzianum* on degradation and viability of fresh ergot sclerotia was almost negligible in comparison with the untreated control and with mycoparasitic efficacy of the fungus *Clonostachys rosea*. Under field conditions over the winter season the fungus *Clonostachys rosea* ensured high degradation of fresh sclerotia at all depths of placement in the soil (Table 2). Differences in the mycoparasitic activity of fungi apparently lie in different requirements for soil temperature. According to literary data fungi of the genus *Trichoderma* exhibited mycoparasitic activity only when the soil temperature was above 10–15°C (Papavizas 1985). No data on the effect of temperature on the mycoparasitic activity of the fungus *Clonostachys rosea* have been found in literature. Our results (Table 2) indicated that the fungus was involved in mycoparasitic activity even at lower soil temperatures (evidently also below 10°C).

Clonostachys rosea applied in *in vitro* tests to sclerotia of the fungus *Sclerotinia sclerotiorum* prevented their germinating ability and caused their degradation like the fungus *Trichoderma*

harzianum (Figure 1). The obtained results have confirmed the literary data (Barnett & Lilly 1962; Papavizas 1985; Zazzerini & Tosi 1985; Idemitsu Kosan 1997; Xue Allen 2002). The mycoparasitic activity of different isolates of *Clonostachys rosea* is likely variable. This is supported by the fact that the isolate obtained from the dead roots of broad bean had a lower capacity of degrading sclerotia than the isolate obtained from ergot sclerotia (Figure 1). Similar differences in mycoparasitic activity were reported in the study by Zazzerini and Tosi (1985).

The rate and efficacy of mycoparasitic degradation of ergot sclerotia in the soil are affected by their age and the depth of their placement in the soil. Deep burial of ergot sclerotia in the soil prolongs their viability. Fresh sclerotia are more resistant to microbial destruction. Sclerotia over two years old lose their viability and in the soil they rapidly undergo degradation in which bacteria and a broad spectrum of fungi (*Fusarium*, *Ulocladium*, *Stachybotrys*, *Acremonium*, *Penicillium*, *Trichothecium*, *Clonostachys*, T*richoderma*, etc.) are involved.

To reduce the occurrence of sclerotia in the soil (*Claviceps* and *Sclerotinia*) over the winter season the application of the fungus *Clonostachys rosea* is very promising and was recommended even in studies by Idemitsu Kosan (1997) and Xue Allen (2002).

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Corresponding author:

Doc. Ing. Bohumír Cagaš, CSc., OSEVA PRO, Ltd., Rožnov-Zubří, Hamerská 698, 756 54 Zubří, Česká republika
tel.: + 420 571 658 294, e-mail: cagas@oseva.cz