Bohumila Voženílková  
Jan Moudrý & Bohuslav Čermák

The University of South Bohemia Faculty of Agriculture, Studentská 13, 370 05 České Budějovice, Czech Republic

SURFACE MYCOFLORA IN STORED GRAINS OF NAKED OATS

ABSTRACT: In the framework of small-site experiments we examined the biopreparation Supresivit S 2 containing the propagules of the antagonistic fungus *Trichoderma harzianum*.

The small-site experiments, which were carried out in the experimental plot of the Department of Crop Production (University of South Bohemia in České Budějovice, Faculty of Agriculture), were aimed at impairing the biotic fungistasis by biological means applied to the grains of naked oats, variety Adam. Our objective was to accomplish surface microbiological analysis of stored naked oats grains, variety Adam, after the application of the biofungicide Supresivit S 2 in combination with surface treatment of the grains and biological screen applied during vegetation period.

In particular variants of the experiment we estimated the values of the levels of bacteria and fungi representation in the surface parts of stored grains and analysed the contents of some mycotoxins produced by the fungi of the genus *Fusarium*.

The highest numbers of bacteria and fungi on the surface of the investigated grains were found in the variant treated by the bioagent (Supresivit S 2), namely 82,000,000 spores of bacteria and 110,000 spores of fungi (*Cladosporium, Verticillium, Coelomycetum*) per 1 g of grain. Also the fungi of the genus *Fusarium* were most numerous in the variant oats — bioagent, namely 250 spores per 1 g of grain.

In all particular variants the amounts of mycotoxins were considerably below the known effective doses for animals and plants. The largest amount of vomitoxin was found in the variant chemical standard (Koval-TS, effective substance Carbendazim 17.5% and Iprodion 35%) — 18 μg. Zearalenon was found in all variants below 5 μg.

Lower doses of ergosterin were found in the variant treated by the bioagent 4.7 mg/kg.

KEY WORDS: grains, mycotoxins, naked oats

INTRODUCTION

The aim of small-plot experiments carried out in university experimental field belonging to the Department of Plant Production, University of South Bohemia, was to disturb biotic fungistasis by means of a biological agent (Supresivit S 2-antagonistic fungus *Trichoderma harzianum*), applied to the grains
of naked oats, variety Adam. We respected the fact that the most important component of the environment influencing the survival of pathogene development stages seems to consist in biological factors. Authors Sivan & Chet (1989) carried out the planting of T. harzianum fungus in the roots of the grown plants and thus reduced the amount of fungi Fusarium in the rhizosphere.

In our operational experiments we applied the bioagent in the form of solution during vegetation period with the aim to influence the microflora of phyloplan. Also the authors Okrouhlij (1993) & Michalíková (1994) investigated the protection of plants by antagonistic fungus T. harzianum against pathogens attacking the above-ground parts of plants — phyloplan protection.

Surface microbial analyses of grains after naked oats harvest were carried out in cooperation with the Microbiological Institute in Linz (Lew 1995).

MATERIAL AND METHODS

Naked oats, variety Adam, was sown in the operational area in the plot of the department of plant production by means of small-plot sowing machine. The experiment took place in 12 small plots, each with the area of 6 square meters. In variant no. 2 (oats, chemical standard) the grains were soaked with Rovral TS (effective substance was Carbendazim 17.5% and Iprodione 35%).

In variant no. 3, oats were dusted with Supresivit S 2 and, during vegetation period at the beginning of wax ripeness in phase 83 DC, it underwent a single treatment with Supresivit S 2. The amount of sprinkling solution was about 2 litres per variant and was provided by hand pressure sprinkler Pilmet (see Table 1).

Table 1: Organization of the small-plot experiment

<table>
<thead>
<tr>
<th>Variant no</th>
<th>Dose of Supresivit per 2 kg of grains</th>
<th>Dose of Rovral per 1 kg of grains</th>
<th>Supresivit application (phase DC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. oats control</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2. oats chemical standard</td>
<td>—</td>
<td>10 g</td>
<td>—</td>
</tr>
<tr>
<td>3. oats bioagent</td>
<td>20 g</td>
<td>—</td>
<td>83</td>
</tr>
</tbody>
</table>

During vegetation period we evaluated the health state of the observed cereals. The development of diseases was assessed according to subjective scale 1—9 (9-healthy plants with no symptoms of disease).

The harvest of naked oats, variety Adam, took place at the beginning of full ripeness in phase 91 DC, during sunny, warm weather. The harvest itself was done in the individual small plots. Particular variants were hand-mown and individual yields were threshed by the stationary thrasher Veb Fortschnitt K-119. In the presence of Dr. H. Lew & Dr. A. Adler (Bundesanstalt für Agrarbiologie, Linz), 1 kg oats specimens of each observed variety were taken and transported to Linz for microbial analysis of fungi and bacteria colonizing
grain surfaces in stored variants, as well as for the assessment of the occurrence of Fusaria secondary metabolites. Bacteria were investigated by cultivation on IAG — bacterial agar; the representation of Verticillium, Cladosporium, Coelomycetum fungi was tested on IAG — fungal agar, and fungi of the genus Fusarium by cultivation on modified nutritional substance according to Papavizas (1985). Under laboratory conditions the average yield characteristics were investigated (weight of one thousand grains and bulk weight).

**RESULTS AND DISCUSSION**

Macroscopic evaluation of the growth from the viewpoint of the attack of serious pathogens (Fusarium sp. div., B. graminis, P. graminis, U. avenae) took place at the end of tasseling in phase 59 DC. The development of fungal disease was assessed according to the scale of 1 to 9 points. In oats we observed moderate development of the fungus P. graminis in variant no. 2, chemical standard (8 points); in variants no. 1 (control) and no. 3 (bioagent) the intensity of the disease was classified with 7 points (as shown in Table 2). Further attacks of infection were stopped by high temperatures due to intense sunshine in summer months. Fungi of Fusarium genus, powdery mildew of cereals B. graminis and loose smut of oats U. avenae were not observed in our small-plot experiments.

Table 2: Evaluation of naked oats plants (variety Adam) attacked by particular pathogens

<table>
<thead>
<tr>
<th>Variant no</th>
<th>Fusarium sp. div.</th>
<th>Blumeria graminis</th>
<th>Puccinia graminis</th>
<th>Ustilago avenae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. oats control</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>2. oats chemical standard</td>
<td>—</td>
<td>—</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>3. oats bioagent</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>—</td>
</tr>
</tbody>
</table>

The highest numbers of bacteria and fungi on the surface of the observed grains were found in variant no. 3, oats, bioagent, namely 82.000.000 spores of bacteria and 110.000 spores of fungi (Cladosporium, Verticillium, Coelomycetum) in 1 g of oats. Also Fusarium fungi were most numerous in variant no. 3, oats, bioagent, namely 250 spores in 1 g of grains (Table 3).

Table 3: Quantities of fungi and bacteria on the surface of naked oats

<table>
<thead>
<tr>
<th>Variant no</th>
<th>Bacteria</th>
<th>Fungi (total)</th>
<th>Fusarium sp. div.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. oats control</td>
<td>65.000.000</td>
<td>40.000</td>
<td>150</td>
</tr>
<tr>
<td>2. oats chemical standard</td>
<td>60.000.000</td>
<td>85.000</td>
<td>150</td>
</tr>
<tr>
<td>3. oats bioagent</td>
<td>82.000.000</td>
<td>110.000</td>
<td>250</td>
</tr>
</tbody>
</table>

The table gives numbers of spores per 1 g of grains

The values of mycotoxin levels (Table 4) in particular variants were in all cases deep below the known effective levels for animals and plants. The lar-
gest amount of Vomitoxin was found in variant no. 2, oats, chemical standard — 18 µg. Zearalenon was detected in all variants at levels below 5 µg.

Higher amounts of Ergosterin were found in variants no. 1, oats, control — 5.3 mg, and oats, chem. standard — 5.2 mg, compared to variant no. 3, oats, bioagent — 4.7 mg/kg of grains where the measured values were lower (Table 4).

<table>
<thead>
<tr>
<th>Variant no</th>
<th>Vomitoxin mg/kg</th>
<th>Zearalenon mg/kg</th>
<th>Ergosterin mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. oats control</td>
<td>11</td>
<td>&lt; 5</td>
<td>5.3</td>
</tr>
<tr>
<td>1. oats chemical standard</td>
<td>18</td>
<td>&lt; 5</td>
<td>5.2</td>
</tr>
<tr>
<td>3. oats bioagent</td>
<td>7</td>
<td>&lt; 5</td>
<td>4.7</td>
</tr>
</tbody>
</table>

In laboratory conditions we examined basic yield characteristics, namely the weight of 1.000 grains and bulk weight (Table 5). The highest values were found in the variant treated with the bioagent (Supresivit S 2).

According to Hausvater & Trnková (1993), Voženílková (1993) and Dušková (1994), the stimulating effects of the fungi of Trichoderma genus in plants manifest themselves by better and faster germination and sprouting, faster and entirely bigger growth, increased formation of leaves, the root system is more powerful, and the total yield is increasing.

In our experiments the stimulating effects of the used biopreparation were apparent in variant no. 3, oats, bioagent, the weight of 1.000 grains 33.0 g, and bulk weight 703.3 g .1⁻¹ in comparison with the control untreated variant where the values were considerably lower (var. no. 1, oats, control, HTZ 30.6 g, OH 672.0 g .1⁻¹). The variant treated with Rovral TS showed lower weight of 1.000 grains, and also bulk weight was lower compared to the variant with bioagent treatment (var. no. 2, oats, chemical standard, HTZ 31.2 g, OH 688.5 g .1⁻¹), as shown in Table 5.

<table>
<thead>
<tr>
<th>Variant no</th>
<th>Oats-variety Adam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>1. Control</td>
<td>672.0</td>
</tr>
<tr>
<td>2. Chemical standard (Rovral TS)</td>
<td>688.5</td>
</tr>
<tr>
<td>3. Supresivit S (T. harzianum)</td>
<td>703.3</td>
</tr>
</tbody>
</table>

### CONCLUSION

In small-plot experiments we tested the biopreparation Supresivit S 2 containing the propagules of fungus T. harzianum utilized against selected diseases in cereals (Fusarium sp. div., B. graminis, P. graminis, U. avenae). The aim consisted in surface microbial analysis of the grains of naked oats var. Adam after the application of the biofungicide Supresivit S 2 in combination
with surface modification of the grains and biological screen applied during vegetation period. Owing to high temperatures and intense sunshine during summer months the infection causing the observed diseases was hindered and thus it was difficult to precisely assess the efficiency of the applied bioagent against selected pathogenes.

We examined the basic yield characteristics, the weight of 1,000 grains and bulk weight. The highest values within the experiment were found in the variant treated by the bioagent (Supresivit S 2-naked oats, variety Adam).

Very hot weather during maturing period and harvest influenced the quality of harvested grains of naked oats (var. Adam). In all observed variants, the levels of mycotoxins were considerably lower than the known effective doses for animals and plants.

The contribution was supported by project MSM: J06/98: 122200002

LITERATURE


ПОВРШИНСКА МИКОФЛОРА У УСКЛАДИШТЕНИМ ЗРНИМА ГОЛОСЕМЕННОГ ОВСА

Бохумила Воженилкова, Јан Мудри и Бохуслав Чермак
Пољопривредни факултет Универзитета Јужне Чешке,
Студентска 13, 37005 Чешке Буђејовице, Чешка Република

Резиме

У оквиру експеримената на малим огледним пољима проучавали смо био-
препарат Supersvit S 2 који садржи честице антагонистичке гљивице Trichoderma
harzianum.

Експерименти на малим огледним пољима који су спроведени на огледној
парцели Департмана за ратарство (Универзитет Јужне Бохемије у Чешким Буђе-
јовицама, Пољопривредни факултет) били су усмерени ка спречању биотичке
фунгистазе применом биолошких средстава на зрна голосеменог овса, варијетета
Адам. Наш циљ је био површинска микробиолошка анализа ускладиштеног зрна
gолосеменог овса, варијетет Адам, након примене биофунгицида Supersvit S 2 у
комбинацији са површинским третманом зрна и биолошким заслоном примење-
ним током вегетацијског периода.

Код посебних варијаната овог експеримента одредили смо вредности нивоа
појаве бактерија и гљивица на површинским деловима ускладиштених зрна и
анализирали садржaj неких микотоксина узрокованих гљивицама из рода Fusar-
ium.

Највећи броj бактерија и гљивица на површини испитиваних зрна саста-
tован је код варијанте третиране биоагентом (Supersvit S 2), и то 82.000.000 спо-
ра бактерија и 110.000 спора гљивица (Cladosporium, Verticillium, Coelomycetum)
по једном граму зрна. Такође, гљивице из рода Fusarium биле су најмногоброj-
није у варијанти овас — биоагент, и то 250 спора по 1 граму зрна.

У свим посебним варијантама количине микотоксина биле су знатно ниже
од познатих ефективних доза за животиње и биљке. Наведена количина вомиток-
сина констатована је код варијанте хемиских стандарда (Rovral TS, ефективна
ступица Carbendazim 17,5%, и Iprodion 35%) — 18 μг. Задржено је констатован
код свих варијаната количини мањоj од 5 μg.

Ниже дозе ергостерина констатоване су код варијаната третираних биоаген-
tома 4.7 mg/kg.