Antagonistic Microorganisms and Medium Moisture as possible Sources of Variation in Common Bunt (*Tilletia tritici*) Incidence*

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Abstract

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Antagonistic soil microorganisms decreased the germination of *Tilletia tritici* teliospores in dependence on the concentration of three formulations (Polyversum®, Supresivit® and Ibefungin®) used and added in or on water agar. A higher content of water in the agar also decreased the germination, and water drops on the agar surface inhibited it entirely. The presence and concentration of antagonistic microorganisms and soil moisture can vary in the soil and it is supposed that this may cause the variation in bunt incidence in field. This variation has to be taken into consideration in particular in variety trials. Polyversum® decreased the incidence of bunt in a field trial (efficacy 60%).

Key words: *Tilletia tritici*; *Pythium oligandrum*; *Trichoderma harzianum*; *Bacillus subtilis*; water agar assays; field trials

Field infection trials have shown a high variation in incidence of common bunt caused by *Tilletia tritici* (Bjerk.) Wint. (POLIŠENSKÁ *et al.* 1998; POSPÍŠIL *et al.* 1999). Since the inoculation and plant growing conditions were the same, it was necessary to study the reasons of the variation *in vitro*. The study mainly focused on the influence of three known biocontrol agents *Pythium oligandrum, Trichoderma harzianum* and *Bacillus subtilis* on the disease incidence. *Pythium oligandrum* and *Trichoderma harzianum* are common in most soils (FOLEY & DEACON 1985). Moreover, the effectiveness of a product containing *P. oligandrum* in a field trial will be shown in this paper. The antagonistic activity of *B. subtilis* was tested in our field trial some years ago and the disease incidence was reduced by about 50% (unpublished results).

MATERIALS AND METHODS

The following commercial formulations of biological agents were used in the experiments:

1. Polyversum®: Formulation containing 1×10^6 oospores of *Pythium oligandrum* Drechsler in 1 g talc powder, with at least 70% germination power, purchased from Biopreparáty Ltd., Horoměřice, CZ.
2. Supresivit®: Formulation containing 1.4×10^{10} conidia of *Trichoderma harzianum* Rifai in 1 g talc powder, with at least 70% germination power, purchased from Fytovita Ltd., Blatnice, CZ.
3. Ibefungin®: Suspension containing 1×10^9 spores of *Bacillus subtilis* Cohn IBE 711, purchased from PPS Agro Company, Strachotín, CZ.

Laboratory experiments: Polyversum® and Supresivit® were added to 1% water agar at concentration rates of 10 g, 1 g and 0.1 g/l prior to solidification of the medium and immediately poured out to Petri dishes. Since *Bacillus subtilis* is strictly aerobic, Ibefungin® suspended in water in above concentrations (ml) was added to the surface of solid water agar (1 ml per dish) so that it covered its whole surface. The Petri dishes were then left open for evaporation of surplus water. Water agar without addition of microorganisms served as control. The surface of all agar plates was then dusted with *T. tritici* teliospores (10 mg for one dish 8 cm in diameter). After an incubation of 7 d at 20°C, the germination rate of teliospores was determined in 10 fields of view of a light microscope (objective magnif. 20). Teliospores were considered germinated when basidiospores or germ tubes were visible. All experiments were conducted in three replicates and repeated several times. However, similar trends were observed in all and therefore the results of one trial are presented.

Similar assays were prepared with different concentrations of agar in water agar (0.5, 1 and 2%) to study the influence of agar dilution on teliospore germination.

Field experiment: The seed of winter wheat cv. Ina was inoculated with teliospores of *T. tritici* at a rate of 1 g

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per kg and dressed with Polyversum® at rates of 2, 1 or 0.5 kg/t and drilled in field microplots (2 m^2) with four replicates. At maturity about 100 ears were harvested by hand from the centre of each plot and scored for infection.

Data analysis: Percent germination and diseased ear data were transformed with arcsin according to BLISS (1952) prior to analysis of variance followed by Tukey grouping. The differences in percent germination were also tested by *t*-test. Means in % in tables followed by the same letter did not differ significantly in the Tukey test ($P_{0.05}$).

RESULTS

Laboratory experiments: The antagonistic soil microorganisms decreased the germination of teliospores of *T. tritici* in dependence on the concentration of the three formulations used (Table 1). Higher concentrations of Polyversum® and Supresivit® were more efficient to decrease teliospore germination than lower ones. With Ibefungin® it was the opposite: the higher concentration was less effective than the lower ones.

Table 1. Influence of Polyversum®, Supresivit® and Ibefungin® on the level (in %) of teliospore germination of *Tilletia tritici*

	Concentration of formulations [g/l]		
Treatment	10	1	0.1
Polyversum®	1.3 c	14.2 bc	28.1 ab
Supresivit®	8.5 bc	14.6 abc	23.3 ab
Ibefungin®	12.3 bc	5.9 bc	3.2 c
Control	40.1 a		

Similarly, the difference in concentration of the water agar also influenced the germination (Table 2). The lowest level of germination was observed on medium with the highest water content. When some water appeared by condensation on the surface of the agar in form of drops, the germination stopped completely. A good indicator for free water on the agar surface was the movement of protozoa in it. In this case, the teliospores did not germinate at all.

Table 2. Germination of *Tilletia tritici* teliospores on water agar with increasing concentrations of agar

Concentration [%]	0.5	1	2
Germination [%]	15.1 a	37.6 b	40.6 b

Field experiment: There were only small differences in the effects of different rates of Polyversum® on the infection level of *T. tritici*, nevertheless the bunt incidence was higher in the control (Table 3).

Table 3. Influence of Polyversum® seed dressing of winter wheat cv. Ina on the incidence of *Tilletia tritici* in a field trial (% diseased ears)

Rate [kg/t]	0 (control)	0.5	1	2
% disease	39.2 a	15.2 b	15.9 b	15.6 b

DISCUSSION

Several authors have observed the variation in the incidence of bunt infection in field trials, but the causative factors remain still unknown. BECKER and WELTZIEN (1993) report results obtained in seed dressing with organic substances rich in nutrients, which effects both great increases in soil bacteria and inhibition of teliospore germination. There are several informations on biological control of wheat bunt by seed bacterization (e.g., JOHNSSON *et al.* 1997; RONQUIST 1994; SOLINGER *et al.* 1997). Nevertheless the effect of soil microorganisms on bunt incidence variation in field trials was not considered.

The inhibition of teliospore germination on potato dextrose agar contaminated with saprophytic fungi was also observed in our laboratory. Our assays show that some common soil microorganisms in form of commercial products such as Polyversum®, Supresivit® and Ibefungin®, can decrease the teliospores germination of *T. tritici* on water agar. The protective effect of Polyversum® against bunt infection was shown also in field trials. A similar effect was observed with Ibefungin® in our field trials some years ago (unpublished results). SYCHROVÁ (unpublished results) likewise reported Polyversum® to be effective in decreasing bunt incidence in field trials. The incidence of antagonistic microorganisms in the soil is not expected to be regular. The hypothesis that the disease variation of *T. tritici* or other bunts is due to antagonistic microorganisms or the water content in the soil is supported by the fact that the distribution of diseased plants in a field may be uneven, or appear in pockets. Since teliospores adhere to the seeds they are exposed to and influenced by the various soil conditions during their germination.

It is interesting that in the trial with Polyversum® the level of infection does not correspond to the applied rates. Higher efficacy could not be achieved by doubling the recommended rate (1 kg/t). This demands further investigations. The antagonistic microorganisms of the formulations used cannot compete with the effectiveness of chemical seed dressing and, therefore, are not recommended for bunt control. On the other hand, they are widely distributed in the soil and can contribute to and explain the variation in bunt infection in field trials. In contrast, in trials with recommended rates of chemical seed dressings there was no variation in bunt incidence or a very low one (unpublished results). While the chemical treatments are effective, there was in each trial variation in disease level in the controls (microplots without seed treatment). The

explanation is that both antagonistic and parasitic microorganisms are very sensitive to environmental factors. Even the water content in the medium can influence teliospore germination and consequently the infection. The bunt teliospores are strictly aerobic organisms and require air for germination.

It was observed that the effectiveness of Ibefungin® increased with decreasing concentrations (Table 1). The reasons of this phenomenon are to be cleared in further experiments. When Ibefungin® was mixed with water agar, it was completely ineffective against *T. tritici*. It is supposed that *Bacillus subtilis* has to multiply and this can be achieved only in aerobic conditions. Recently BENHAMOU *et al.* (1997) claimed that *Pythium oligandrum* induced defence related reactions in plants and in this way decreased the level of infection by *Fusarium*.

To conclude, the concentration of antagonistic microorganisms and soil moisture can vary in a field and may thus cause the variation in bunt incidence. This effect must be taken into consideration, particularly in field trials of the resistance of varieties.

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Souhrn

BENADA J., POSPÍŠIL A. (1999): **Antagonistické mikroorganismy a obsah vody v agarové půdě jako možné zdroje variability výskytu sněti mazlavé (*Tilletia caries*)**. Pl. Protect. Sci., **35**: 121–123.

Byl ověřován vliv různých koncentrací komerčně vyráběných preparátů (Polyversum®, Supresivit® a Ibefungin®) přidaných do vodního agaru nebo na jeho povrch na klíčení teliospor sněti mazlavé (*Tilletia caries*). Klíčení teliospor bylo sníženo. Podobně snižoval klíčení i vyšší obsah vody ve vodním agaru. Vodní kapky na povrchu agaru zabránily klíčení teliospor úplně. Poněvadž obsah antagonistických organismů v půdě stejně jako půdní vlhkost mohou značně kolísat, tyto faktory mohou způsobovat i variabilitu napadení v polních pokusech. Bylo zjištěno, že preparát Polyversum® snižoval podstatně napadení pšenice sněti i v polních podmínkách (účinnost 60 %).

Klíčová slova: *Tilletia tritici*; *Pythium oligandrum*; *Trichoderma harzianum*; *Bacillus subtilis*; vodní agar; polní pokusy

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