SHORT COMMUNICATION

Effect of Inoculum Doses on Common Bunt Infection on Wheat Caused by *Tilletia tritici* and *T. laevis*

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Abstract: In the trial with different doses of common bunt teliospores used for inoculation of wheat the number of bunted ears increased with the increasing dose of inoculum. This increase was small (statistically insignificant) in the resistant cv. Bill and high (statistically significant) in the susceptible cv. Samanta. The effect of inoculation on the vigour of inoculated plants was estimated according to the number of tillers per plot in summer in cultivars inoculated with different inoculum doses sown at the same sowing rate in autumn. The number of tillers was decreasing with the increasing inoculum dose. This was less pronounced in the resistant cv. Bill than in the susceptible cultivar Samanta. Competition between *Tilletia tritici* and *Tilletia laevis* during infection was studied by inoculation with a 1:1 mixture of teliospores of these species and scoring bunted ears. Ears bunted with *T. laevis* prevailed in almost all trials. The germination test of teliospores showed a higher speed of germination of *Tilletia laevis* teliospores. The number of teliospores per seed after inoculation was counted. No significant difference in the number of teliospores per seed after inoculation with *T. tritici* or *T. laevis* was ascertained.

Keywords: common bunt; inoculum doses; competition between *T. tritici* and *T. laevis*

In organic farming the use of synthetic pesticides is not allowed and for this reason the disease resistance of grown cultivars is particularly important. Wheat breeding for resistance to leaf diseases is a routine breeding procedure. Less common is breeding for resistance to common bunt *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn because this disease can be controlled by chemical seed treatment. However, experience with the use of untreated seed demonstrated the need for resistance breeding to avoid common bunt of wheat in organic farming. In traditional farming bunt resistant cultivars can help to decrease the application of pesticides. This short contribution deals with technical experience obtained in the tests for resistance to common bunt.

Effect of inoculum doses on bunt incidence. Seed inoculation with teliospores of common bunt is usually carried out with dry spores by shaking the inoculum with the tested seed. Less frequently a water suspension of teliospores is applied to seeds. Inoculum doses applied in field tests for resistance to common bunt usually vary from 0.2 g (Pospíšil *et al.* 1997) through 5 g (Liatukas & Ruzgas 2007) to 10 g of teliospores (Goates 1996) per 1000 g of seed, the most common dose being 1–2 g teliospores per 1000 g of seed. Koch and Spiess (2002) used 1 g of teliospores for a greenhouse trial and 2 g for a field trial, later (Koch *et al.* 2004) 2–3 g per 1000 g of seed. Bänziger *et al.* (2003) found the dose 0.5 g per 1000 g of seed too low and doubled it in the subsequent trials. Several authors tested the effect of different doses on the level of bunt incidence. Pospíšil *et al.* (2000) tested three doses 1 g, 5 g and 10 g of teliospores per 1000 g of seed but found no significant difference in the bunt incidence. Benada *at al.* (1995) applied very low doses of teliospores, 1 mg and 10 mg per 50 g of seed for the inoculation of 13 cultivars in 1993 and 22 cultivars in 1994. The average bunt incidence was 14.8% and 2.5%, respectively. In the trials of Polišenská *et al.* (1998) with the inoculum dose 0.2 g of teliospores per 1000 g of seed bunt incidence up to 45% (cv. Ina) has been achieved. In most trials with different inoculum doses the higher the dose the higher the bunt incidence (up to certain level).

We applied the inoculum doses 0.5 g, 0.25 g, 0.05 g and 0.005 g teliospores per 250 wheat seeds. The field trial had 4 replications. Three cultivars differing in resistance to common bunt were inoculated. In previous trials (Dumalasová & Bartoš 2006 a, b) cv. Bill was assessed as resistant, cv. Brea as medium susceptible and cv. Samanta as susceptible. These cultivars showed different bunt incidences after inoculation with different inoculum doses. None of the inoculum doses caused bunt incidence on the cv. Bill higher than 2.4%; the lowest dose did not cause any infection. On the cv. Brea bunt incidence increased stepwise from 5.8% to 27.4%, on the cv. Samanta from 0.6% to 37.4% with the increasing dose of the inoculum. Table 1 presents data after the angular transformation of percents of bunted ears (for ANOVA). The bunt incidence depended upon the inoculum dose and the degree of resistance of the cultivar. The higher is the resistance of the cultivar, the lower increase in the bunt incidence with increasing inoculum doses can be expected. In our trial this was demonstrated by the span between the number of bunted ears after inoculation with the lowest and highest inoculum dose. The smallest difference in the number of bunted ears after the lowest and highest inoculum dose was in the resistant cultivar Bill (statistically not significant), higher in the medium susceptible cv. Brea (but statistically not significant either) and the largest in the susceptible cv. Samanta (statistically significant). Similar results were obtained earlier by other authors, e.g. by Fadrhons (1955) or Geshele (1978).

Table 1. Effect of different inoculum doses on bunt incidence in three winter wheat cultivars; statistically significant differences (LSD, $P < 0.05$) in bunt incidence are indicated by different letters in columns

Inoculum dose[1]	Bunt incidence/cultivar[2]					
	Bill		Brea		Samanta	
0.5	7.1	a	31.8	a	37.6	a
0.25	7.0	a	27.6	a	31.0	ab
0.05	2.7	a	21.2	a	28.5	b
0.005	0.0	–	12.8	a	3.2	c

[1]grams per 250 seeds; [2]percentage of bunted ears after angular transformation

Effect of inoculation on the plant vigour. The effect of bunt infection on the plant vigour as it is expressed in the overwintering of winter wheat has been described by several authors. Older results were summarized by Fischer and Holton (1957). Recently the effect of bunt on the overwintering of wheat was recorded by Veisz *et al.* (1997).

We have estimated the effect of seed inoculation with bunt teliospores in four doses (0.5 g, 0.25 g, 0.05 g and 0.005 g per 250 seeds; in cv. Brea without the highest dose) on plant vigour according to the number of ears in summer on field plots sown with winter wheat in autumn. Cultivars Samanta, Bill and Brea were tested. Though variety differences in tillering may have affected the results, inoculum doses seem to have played the major role. The highest number of ears was found after the inoculation with the lowest inoculum dose (Figure 1). Differences in the number of tillers were significant between the lowest and highest dose of inoculum in medium susceptible cv. Brea and susceptible cv. Samanta.

Competition between *Tilletia tritici* and *Tilletia laevis* in mixed inoculum. For inoculation a mixture of teliospores of *Tilletia tritici* and *T. laevis* (if present) of different proveniences is usually applied in variety tests. However, there exists a competition during the infection process between the above mentioned species and also different bunt races. For this reason we tested this phenomenon with a mixture of *T. tritici* and *T. laevis* in several spring wheat cultivars with the following results. When cv. Vinjett was inoculated only with *T. tritici* bunt incidence was 48.4% and teliospores of all examined 14 ears belonged to *T. tritici*. When the

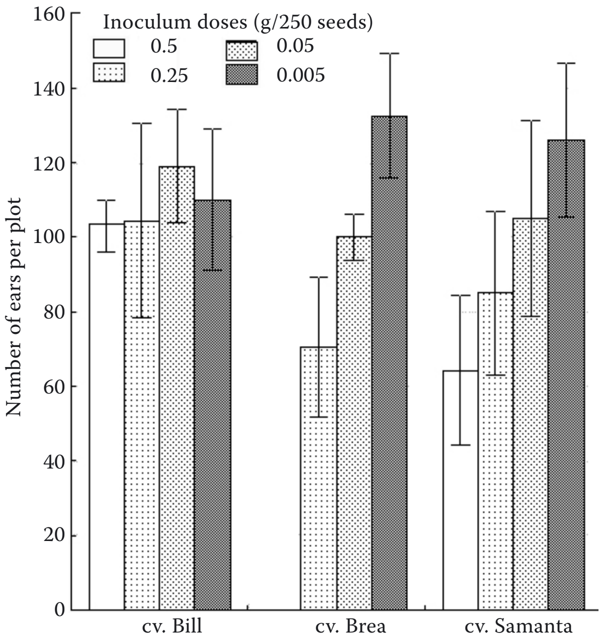

Figure 1. Effect of bunt infection on the overwintering of three winter wheat cultivars; the bars are 95% confidence interval

same cultivar was inoculated only with *T. laevis,* bunt incidence was 57.5% and all examined ears showed only *T. laevis*. Bunt incidence in the same cultivar inoculated with the 1:1 mixture of the both bunt species was 60.0% but all examined ears contained only *T. laevis*. This shows a high competitiveness of *T. laevis* applied in our trial. In another experiment inoculated with a mixture of *T. tritici* and *T. laevis* in cultivars Corso, Bruncka and Munk only *T. laevis* was determined. In the cv. Kadrilj the proportion of *T. laevis* was 93.3%, in another trial with cv. Vinjett 85.7%, in cv. Aranka 80% and only in cv. Linda *T. tritici* prevailed. In a similar trial with the winter wheat cv. Trintella the proportion of *T. laevis* was 71.4%. Similar results after inoculation with mixtures of different physiological races may appear. This was demonstrated by Rodenhiser and Holton (1953) who analyzed changes in different mixtures of physiological races of *T. tritici* and *T. laevis* for several years. In general *T. laevis* races were more aggressive than those of *T. tritici*. In some mixtures *T. laevis* prevailed after the first passage in others more passages were necessary for predominance. The nature of the observed differential survival ability in mixtures was further studied. Experiments with the cultivation of *T. tritici* and *T. laevis in vitro* in close vicinity did not indicate any detrimental or antagonistic effects on the mycelial development of one race toward another. When the rate of spore germination as a basis for differential survival was investigated, only one consistent difference in the rate of germination was observed. Spores of two *T. laevis* races germinated about 48 hours earlier than a *T. tritici* race. The same authors revealed natural hybridization in passages of spore mixtures of the above mentioned bunt species. Their experiments were carried out at two locations. For the major part of experiments there was a close agreement in the results from the both locations. However, a notable exception was observed in passages of one mixture. This difference could have been caused either by differences in the environment or by modification of the original mixture through hybridization.

Nevertheless no general explanation could be offered for the demonstrated differential ability of species and races of *T. tritici* and *T. laevis* to survive in mixture in the experiments by Rodenhiser and Holton (1953).

To find out the reason for the prevalence of *T. laevis* in our trials when inoculated in a 1:1 mixture with *T. tritici* we investigated the speed of germination of the applied bunt samples. Germination speed was higher in teliospores of *T. laevis* than in those of *T. tritici* (Figure 2). In the sample of *T. laevis* a small number of germinating teliospores was recorded already on the second day and 98.9% germination was reached already after five days. Germination of *T. tritici* teliospores started between the third and fourth day and reached 76.6% on the fifth day. Faster germination of *T. laevis* than of *T. tritici* may be the possible reason for the prevalence of *T. laevis* after inoculation with the 1:1 mixture of these bunt species. The result obtained with cv. Linda suggests that the genotype of the cultivar may also play a role in the competition of bunt species.

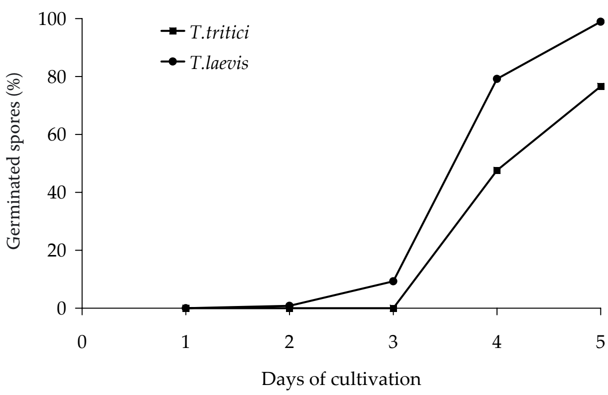

Figure 2. Speed of teliospore germination

Number of teliospores on seed after inoculation. In the literature substantial differences exist in the data on the number of teliospores on seeds after inoculation. Polišenská *et al.* (1998) found only 176 teliospores per one seed after inoculation with the dose 0.2 g teliospores per 1000 g of seed. When a surplus of teliospores was applied and "the seed was almost black" only 434 spores were registered per one seed. Under similar conditions (2–4) × 10^5, i.e. 200 000–400 000 spores per seed were reported by Sholberg *et al.* (2006). Heald (1921) found 533 and 5333 teliospores per one seed after inoculation with the doses 0.01 g and 0.1 g per 100 g of seed, respectively. Jahn *et al.* (2004) reported 13 188 spores per one seed after inoculation with 500 mg spores per 1000 g of seed.

The number of spores per one seed is an important piece of information for the bunt control in practice because treatment tresholds are based on this number. According to Waldow and Jahn (2007) the proposed number and/or prescribed tresholds range from 20 spores/seed in Germany and 10 spores/seed in Austria and Switzerland to only one spore/seed in Scotland. In the Czech Republic the maximum number of spores is 10/300 seeds for seed grades SE, E, and C. Waldow and Jahn (2007) proved that 5–20 spores/seed were sufficient to produce a distinct infection and concluded that a treshold of 20 spores/seed as proposed in Germany is too high for more susceptible cultivars. Susceptible cultivars should be treated from a treshold of 1–5 spores/seed. The Czech regulation cited above seems to be adequate to this recommendation.

Number of spores/seed is counted according to the International Seed Testing Association (ISTA) or modification of this method. We applied the modification used by Waldow and Jahn (2007).

Comparison of results obtained with different doses of inoculum on different amounts of seeds, expressed either in grams or in numbers of seeds is difficult. The linear conversion of acquired data can be one source of discrepancies in the comparison of the results. Discrepancies can also be caused by differences in the procedures of analysis. Another reason can be different surface and humidity of analyzed seeds and the applied bunt sample. Some authors apply inoculum as water suspension. Jahn *et al.* (2004) experienced that wet application of the same amount of teliospores led to a lower number of teliospores left on the seed than dry application.

To find out how many teliospores adhere on one seed after inoculation in our trials and whether there is a difference when *T. tritici* or *T. laevis* is applied we inoculated 250 seeds with 0.1 g of teliospores. Ten seeds were individually washed in 1 ml of water by vortexing in 2 ml tubes. Ten 0.5 μl drops of spore suspension from each tube were evaluated under a microscope and numbers of spores were counted. We considered the different surface of teliospores of *T. tritici* and *T. laevis* as a possible cause of recorded differences in the amount of teliospores on seeds after inoculation. However, we found no significant difference in the number of teliospores per seed after inoculation with the same inoculum dose of *T. tritici* and *T. laevis*. At the inoculum dose 0.1 g teliospores per 250 seeds 257 000 spores per one seed were found on the average when teliospores of *T. tritici* were applied. When teliospores of *T. laevis* were applied for inoculation at the same rate, 259 000 teliospores per seed on average were ascertained. The difference was not statistically significant.

Conclusion. The results summarized in this short communication may be useful for experiments with common bunt. Field trials were carried out only in one year, however, other authors cited in the paper obtained similar results as we have achieved with three wheat cultivars registered in the Czech Republic.

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