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Investigations in the regulation of common bunt (*Tilletia tritici*) of winter wheat with regard to threshold values, cultivar susceptibility and non-chemical protection measures

Untersuchungen zur Bekämpfung von Steinbrand (*Tilletia tritici*) an Winterweizen unter Berücksichtigung von Bekämpfungsschwelle, Sortenanfälligkeit und alternativen Behandlungsmethoden

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Summary

The objectives of the present study were the determination of treatment threshold values for control of common bunt of wheat (*Tilletia tritici*) and evaluation of alternative seed treatments. Wheat seed inoculated with the fungus was sown in fields at three sites in Germany from 2002–2004. Seeds of three cultivars – 'Naturastar' (highly susceptible), 'Aron' (moderately susceptible), and 'Batis' (highly susceptible) – were first inoculated with 20, 100 and 1000 spores/seed and treated with Tillecur®, a natural plant strengthener, or hot water before planting. Untreated inoculated seeds were used as control. Bunt infection rates in the resulting wheat plants varied according to inoculum size, cultivar and treatment method, with additional variation between the respective years and sites. Regarding inoculum size, 20 spores/seed sufficed for bunt development in the highly susceptible cultivars. In the moderately susceptible cultivar, 1000 spores/seed were required in the first year compared to only 20 spores/seed in the following years. Tillecur® provided the most effective bunt control: the number of infested ears was low to nil for all sites and inoculum sizes. Hot water was less effective and its effect was not significant except at the high inoculum level. Field data and calculations of the theoretically possible spore load suggest that low-level bunt infection represents a general danger. We conclude that treatment thresholds for control of common bunt must vary depending on the susceptibility of cultivar and the purpose of the harvested seed. When used for seed production, measures for prevention of disease accumulation are imperative. In those cases, we recommend a treatment threshold of one spore/seed for susceptible wheat cultivars and 20 spores/seed for less susceptible cultivars.

Key words: cultivar resistance, hot water treatment, inoculum level, organic farming, plant strengthening product, seed treatment, threshold value, *Tilletia tritici*

Zusammenfassung

Zur Bestimmung von Bekämpfungsschwellen für Weizensteinbrand (*Tilletia tritici*) und zur Untersuchung der Wirkung von alternativen Saatgutbehandlungen wurden in den Jahren 2002–2004 an drei Standorten Feldversuche mit jeweils drei Inokulumstufen (20, 100, 1000 Sporen/Korn), drei Sorten ('Naturastar', 'Aron', 'Batis') und den Behandlungen Tillecur® und Heißwasser angelegt. In allen Jahren und an allen Standorten waren deutliche Unterschiede zwischen den Inokulationsstufen und den Sorten vorhanden. Hochanfällige Sorten hatten in den unbehandelten Kontrollen nach Inokulation mit 20 Sporen/Korn Brandähren, die mittelanfällige Sorte zeigte im ersten Jahr bei 1000 Sporen/Korn, in den anderen Jahren bereits bei 20 Sporen/Korn Befall. Das Pflanzenstärkungsmittel Tillecur® erwies sich als wirkungsvollstes Behandlungsmittel gegen *T. tritici*. Nach Tillecur®-Behandlung wurden an allen Standorten und in allen Inokulationsstufen keine bis sehr wenige befallene Ähren gefunden. Die Wirkung der Heißwasserbehandlung war insgesamt geringer und eine signifikante Wirkung wurde nur in der höchsten Inokulationsstufe erreicht. Die Feldversuche und eine Berechnung der theoretisch möglichen Sporenbelastung des Ernteguts machten das Gefährdungspotenzial einer geringen Infektion deutlich. Befallstoleranzschwellen sind abhängig von der Sorte und dem Verwendungszweck des Ernteguts. Für die Saatgutproduktion, bei der eine Vermehrung des Erregers vermieden werden muss, müssen anfällige Sorten schon bei einer geringen Kontamination ab einer Spore/Korn behandelt werden. Weniger anfällige Sorten sind ab einem Befall von 20 Sporen/Korn zu behandeln.

Stichwörter: Befallstoleranzschwellen, Heißwasserbehandlung, Inokulationsstufe, ökologischer Landbau, Pflanzenstärkungsmittel, Saatgutbehandlung, Sortenresistenz, *Tilletia tritici*

1 Introduction

Common bunt of wheat is the most severe seed-borne disease in winter wheat in organic farming (Spiess 1999). The disease is caused by *Tilletia tritici* (Bjerk.) Wint. [syn. *Tilletia caries* D.C. Tul.]. In recent years, contamination of wheat with spores of *T. tritici* has become a serious problem, resulting in considerable losses of seed quality and yield (Weinhappel and Girsch 2003). Common bunt is easily controlled by chemical seed treatments, so it normally is not of economical importance. In organic farming, where the use of synthetic chemicals is not allowed, seed-borne diseases tend to accumulate and may become a problem after several multiplication cycles without adequate disease control (Kristensen and Borgen 2001). Due to legal regulations on the use of organic seed and to the general expansion of organic agriculture, the significance of healthy seed in organic farming is increasing. According to Commission Regulation (EC) No 1452/2003 of 14 August 2003 (Anonymous 2003), all plant materials used for organic agriculture since January 2004 must be produced under organic farming conditions. Therefore, the occurrence of *T. tritici* often cannot be avoided, even by preventive measures such as seed cleaning and sowing at higher soil temperatures. Control of bunt by a combination of indirect and direct sanitation methods is therefore a main area of research interest. Treatment thresholds based on the pre-determined seed contamination levels are useful tools for bunt control. The thresholds currently prescribed or recommended in different countries vary. The proposed and/or prescribed thresholds range from 20 spores/seed in Germany (Spiess and Dutschke 1991) and 10 spores/seed in Austria and Switzerland (Zwatz and Zederbauer 1997, Schachermayr et al. 2003) to only one spore/seed in Scotland (Cockerell and McNeil 2004). In Denmark, intervention is recommended at the first appearance of *Tilletia* spores (Nielsen et al. 1998). The use of partially or fully resistant wheat cultivars is another important bunt protection strategy. However, due to the availability of effective chemical treatments, resistance to bunt was not important for breeders and farmers in past decades. Since much information on the susceptibility of registered wheat cultivars to bunt is therefore lacking, several resistance screening studies have been performed in the last years (Zwatz and Zederbauer 1997; Pospisil et al. 1999; Koch and Spiess 2002; Bänziger et al. 2003; Wächter et al. 2007). Research has confirmed that susceptibility of different wheat cultivars to bunt varies and that no fully resistant wheat cultivars exist. Non-chemical seed treatment methods, such as warm or hot water treatment, electron treatment and the application of substances of natural origin like milk and whey powder, plant extracts or microorganisms, provide additional bunt control possibilities that may also be suitable for organic farming (Jahn 2005). Though interesting in theory, not all of these methods have been tested sufficiently in practice. The objective of the present study was therefore to establish treatment thresholds for bunt of wheat based on cultivar-specific resistance and to evaluate the efficacy and applicability of two alternative seed treatment methods that comply with the principles of organic farming. The first was a physical method (hot water treatment) and the second a plant strengthening product (Tillecur®). The ultimate goal was to achieve complete protection against bunt by using a combination of the different measures.

2 Materials and methods

2.1 Seed and inoculation method

Certified seed of three winter wheat cultivars with different levels of susceptibility to bunt were used: 'Batis': highly susceptible, 'Aron': moderately susceptible, 'Naturastar': new cultivar with unknown susceptibility. The seed was artificially inoculated with teliospores of *T. tritici*. Spores were applied to the walls of a 3-l-polyethylene Ziploc® bag. One kilogram of seed was then placed in the bag and the closed bag was shaken for 2 min. The three target inoculum levels were 20, 100 and 1000 spores/seed. According to the results of pre-tests, 0.75, 5 and 50 mg spores/kg grain were applied (Jahn et al. 2004). After inoculation, spores were counted according to the method of Piorr (1991), which is a modification of the International Seed Testing Association (ISTA) method described by Pirson (1977). In contrast to ISTA, the PIORR method uses a defined number of seeds and a small amount of water in the washing procedure to avoid time-consuming centrifugation. 150 seeds/10 ml of water were used for 100 and 1000 spores/seed and 200 seeds/12 ml of water were used for 20 spores/seed.

2.2 Treatments

Seed inoculated to a low degree was first treated to avoid contamination. Hot water treatment (52°C, 10 min) was carried out by the company Hild Samen GmbH of Marbach, Germany. The seeds were filled in cheesecloth bags and placed in a hot water bath. The water was kept at a uniform temperature using a thermostat. After treatment, the seed was dried in drying lofts. The plant strengthening product Tillecur® (yellow mustard powder) was applied at concentrations of 20%, 4 l per 100 kg grain for the two lower inoculum levels and of 22%, 5 l per 100 kg grain for the high inoculum level, according to the instructions of the manufacturer (Dr. Schaette AG, Bad Waldsee, Germany). The powder was suspended in water for one hour and was then evenly distributed over the seeds in a container, which was closed with a lid and rotated for 5 minutes. The seeds were dried after treatment and spores were counted (see 2.1). Each inoculated and treated seed lot was divided into three parts for planting at the three sites.

2.3 Germination tests

After treatment, germination tests according to ISTA rules (4 x 100 seeds between paper, 3 days at 4°C, 7 days at 20°C) were carried out to evaluate the effects of treatment on germinability. No negative effects were observed. Germination rates ranged from 93 to 99% in the untreated controls and from 92 to 99% after hot water treatment and from 92 to 98% after Tillecur treatment.

2.4 Field trials

Field trials were performed at three sites in different German states: Dahnsdorf (Brandenburg), Ahlum (Lower Saxony) and Bad Vilbel (Hesse). The trials were carried out using a randomised plot design with four replicates, plot sizes of 11 m^2 (eight rows), 8.25 m^2 (12 rows), and 6 m^2 (eight rows), and a density of 380–400 germinable seeds m^{-2}. At sowing, the same machine was used potentially allowing some re-contamination from untreated to treated seed or from higher to lower contaminated seed. Comparison of the results of the replicates showed that this procedure was not a problem. Depending on local conditions in the respective years 2002–2004, sowing was generally performed between October 13 and 22. Because of extreme weather conditions, sowing was performed later (October 31) in Ahlum and in Bad Vilbel (November 20) in 2002. Mid-October was chosen because the low soil temperatures (5–10°C) present then have been described as optimal for bunt infection (Johnsson 1992; Gaudet et al. 1994; Polisenska et al. 1998). The soil temperatures were measured at a depth of 5 cm within 14 days after sowing. Average temperatures were highest in 2004 at all sites (11.2°C, 12.1°C, and 12.2°C). Temperatures at Dahnsdorf and Bad Vilbel were higher in 2002 (8.4°C and 7.4°C) than in 2003 (4.8°C and 5.5°C). Soil temperatures in Ahlum in autumn 2002 and 2003 were low and nearly identical (4.9°C, 5.2°C) although sowing was much earlier in 2003. The yield per plot was determined only in the first year of the trial at the Dahnsdorf site.

2.5 Disease assessment

The total number of ears per one meter was determined in four rows of each plot. Bunted ears were counted between growth stage BBCH 69 and BBCH 75 and the percentage of infected ears was computed. Infected ears were counted in the whole plot. In plants produced from untreated seeds of the susceptible cultivars 'Batis' and 'Naturastar' inoculated with 1000 spores/seed, only half of the plot could be examined due to the high level of infection. At Bad Vilbel, the whole plots were analyzed for all inoculum sizes and treatment methods.

2.6 Statistical analysis

Statistical analyses were performed with using the Statistical Analysis System (SAS Institute, Cary, NC, USA). The analysis of variance was performed using ln-transformed data. Mean values were compared using the TUKEY test (alpha ≤ 0.05).

3 Results

3.1 Inoculum size (spores/seed) in untreated and treated seed

The recorded inoculum sizes (number of spores per seed) in 2002 and 2003 are shown in Table 1. In 2004 (data not

Table 1: Mean and range of actual inoculum (number of spores/seed) of untreated and treated winter wheat seed after inoculation with *Tilletia tritici* (target numbers: 1000, 100 and 20 spores/seed, respectively)

Cultivar	Treatment	2002		2003	
		Mean[1)]	Range	Mean[1)]	Range
'Naturastar'	Untreated	1435	1323–1542	911	771–1021
		148	115–188	101	63–134
		43	39–55	14	0–28
	Tillecur	1089	1021–1188	742	646–813
		162	63–271	44	21–83
		33	0–75	23	19–28
	Hot water	240	167–313	125	83–188
		31	21–63	26	10–31
		5	0–19	9	0–19
'Aron'	Untreated	1172	1010–1510	1143	1031–1219
		143	104–156	86	31–115
		35	31–39	26	10–55
	Tillecur	713	687–729	497	313–625
		130	104–188	52	21–104
		23	19–38	33	19–75
	Hot water	109	42–167	112	94–125
		5	0–21	18	0–31
		9	0–38	5	0–9
'Batis'	Untreated	948	865–1031	1058	939–1146
		107	73–146	89	52–135
		21	0–39	30	9–47
	Tillecur	792	667–896	719	656–781
		120	63–146	44	42–52
		23	0–56	23	19–28
	Hot water	99	63–188	141	94–177
		21	21	18	10–31
		5	0–19	7	0–19

[1)] Mean of four replicate counts.

shown), only a few sample tests were conducted, the results of which coincided with those of the other years. The differences between the target and actual numbers in the untreated controls were in an acceptable range; hence the target inoculum levels were achieved. In few cases, the range was very high, e.g. in the combination 'Aron'-'Untreated'-'1000 spores/seed' in 2002. Nevertheless, an adequate representation of the intended inoculum levels was assumed. Hot water treatment reduced the number of spores as expected. But, in no case the mean of nil was achieved. Tillecur-treated seeds had spore loads comparable to those in the untreated controls. A lower number of spores/seed was observed only at the highest inoculum level which means that the number of spores was probably reduced due to rubbing off during treatment. The figures and tables below use the target numbers of spores to provide certain degree of uniformity.

3.2 *Influence of inoculum size, cultivar, and site on bunt incidence*

Effects of cultivar and inoculum size on bunt incidence were studied at the Dahnsdorf, Ahlum, and Bad Vilbel sites for three years (Fig. 1 a-i). With 'Batis' and 'Naturastar', seed inoculated with 1000 spores/seed, there was a significant difference in bunt incidence between untreated and all other test combinations (e.g. 'Aron' – Untreated – 20 spores/seed') in almost all trials. The new cultivar 'Naturastar' was proved to be highly susceptible. The highest infection rates in the untreated controls were 9.1% for 'Batis' (39.3 infected ears m^{-2}), 13.4% for 'Naturastar' (50.5 infected ears m^{-2}), and 1.4% for 'Aron' (11.9 infected ears m^{-2}). The highly susceptible cultivars developed bunted ears starting at inoculation levels of 20 spores/seed. With the moderately susceptible cultivar, bunt developed only in plants produced with 1000 spores/seed in the first year (Fig. 1 a, d), but also in those produced with 20 spores/seed and 100 spores/seed in the next two years. In the untreated controls, infection levels in 'Aron' was significantly lower than in 'Batis' and 'Naturastar' cultivars (Fig. 1 b, c, f, h). Infection levels between the three sites also varied. At Bad Vilbel, counts in spring 2003 revealed a considerable to complete loss of 'Aron' and 'Batis' cultivars due to the harsh winter (Fig. 1 g); hence, evaluation of infection was possible only for 'Naturastar' that year. At Bad Vilbel, the lower inoculation levels led to higher infection levels than at the two other sites, particularly in 'Aron', the moderately susceptible cultivar. At the two sites located in the more eastern regions of Germany (Brandenburg and Lower Saxony), the three-year results showed better reproducibility than at the site in western Germany (Hesse). Infection levels at Bad Vilbel, where incomplete results were obtained in the first year, differed greatly from those at the two other sites. The infection level at Bad Vilbel was higher for all three cultivars at the lower inoculation levels than at the other two sites, especially in the first two years. A corre-

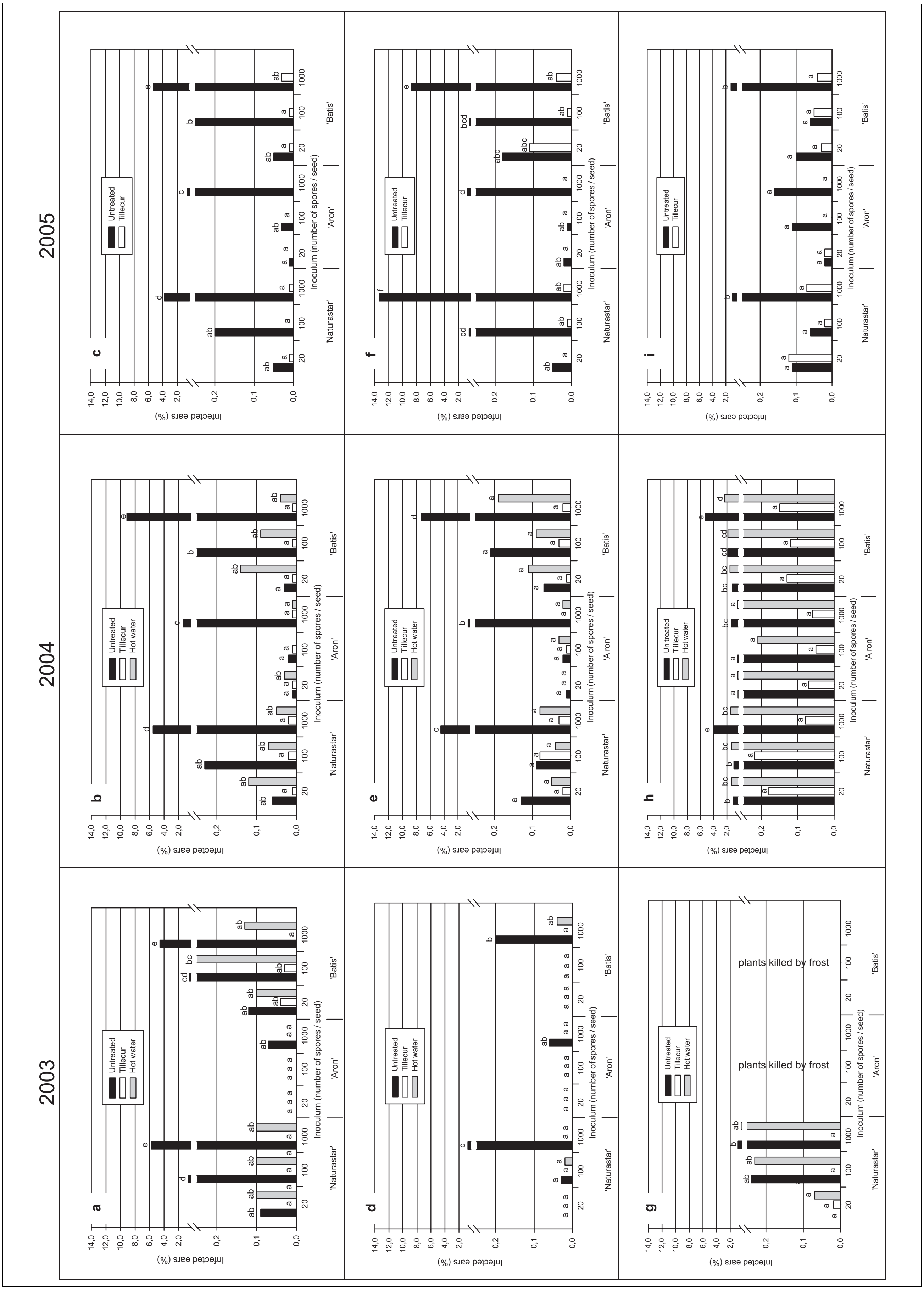

Fig. 1: Bunt (*Tilletia tritici*) infection of winter wheat according to cultivar, inoculum size and treatment method at three sites (a-c: Dahnsdorf, d-f: Ahlum, g-i: Bad Vilbel) during the years 2003 to 2005. Columns within in each graph with a letter in common are not significantly different from each other according to Tukey's test (alpha ≤ 0.05).

lation between soil temperature and infection level could not be confirmed in this trial. A high infection level was observed at all sites in 2005 following the warm autumn of 2004. At Dahnsdorf, temperatures in 2003 were clearly lower than in 2002, but the incidence of common bunt was nearly identical in all years.

3.3 *Effect of non-chemical protection measures*

The non-chemical protection measures generally had an effect on bunt infection. Significant differences between the treatment methods and controls were observed in plants produced from seed of the highly susceptible cultivars ('Naturastar' and 'Batis') inoculated with 1000 spores/seed in all cases (Fig. 1 a-i) and with 100 spores/seed in most cases (Fig. 1 a-c, f, h). In the case of the moderately susceptible cultivar ('Aron'), the difference was significant only at the highest inoculum level (1000 spores/seed) in 2004 and 2005. With Tillecur®, the number of infested ears was low to nil at all sites and inoculum levels. Although the plant-strengthening agent did not always suppress bunt infection completely, it did reduce disease development. Hot water treatment induced significant effects only at the highest inoculum level and was clearly inferior to Tillecur®. A summary of the field data is presented in Table 2. In all three cultivars, infection levels in wheat produced from seeds treated with hot water were higher than in the untreated controls in the 20 spores/seed groups in Dahnsdorf and Bad Vilbel, and in the 100 spores/seed groups in Bad Vilbel. At the 1000 spores/seed level, hot water distinctly reduced infection in all three cultivars and sites. After Tillecur® treatment, infection levels remained constant at approximately the same low level. On the whole, the treatments at Bad Vilbel were less effective than at the other sites. The analysis of variance for the three years showed that, at Dahnsdorf, all three test factors (cultivar type, treatment method and inoculum size) had a significant effect on the infection rate and that interactions between the factors were significant (Table 3). At Ahlum, there was clear evidence of an effect of inoculum size on infection rate, of interaction between treatment type and inoculum size, and of interactions between all three test factors. At Bad Vilbel, no significant effect of the test factors on infection for three years could be demonstrated due to the incomplete data; however, differences for the individual years could be demonstrated by statistical analysis (see Fig. 1). The effects of the test factors on infection were also influenced by environmental conditions (by site and year).

3.4 *Derivation of risk potential*

The risk of bunt infection was estimated based on theoretical spore load calculations. At Dahnsdorf, the infected ears in each plot were therefore collected, the spores extracted, and the spore weight determined. Based on the results of the preliminary tests in which the number of spores per seed was counted after application of defined amounts of spores to one kilogram of grain, the theoretically possible contamination of the harvested seed as related to plot yield was calculated for the year 2003 (Table 4). It was determined that 0.53 g of spores in 'Batis – untreated – 21 spores/seed' is sufficient to contaminate the harvested grains (about 4 kg/plot) with 338 spores/seed after deducting 90% loss of spores through harvesting (Paveley et al. 2004). Infection was 0.12% (Fig. 1a) or 2.2 infected ears/plot in this case (Table 3). Hot water treatment decreased the number of spores at all inoculum levels; for example, the initial load of 20 spores/seed was reduced to 5 to 9 spores/seed after treatment. In 'Batis – hot water' an inoculum of five spores/seed resulted in an infection of 2.5 bunted ears per plot from which 0.6 g of spores were extracted; this is sufficient to contaminate the harvested grains in the plot with 407 spores/seed. The same effect was observed with 'Naturastar'. Apparently, a low percentage of spores survived that treatment. The calculated spore load in the untreated controls at the highest inoculum level was only

Table 2: Average infection rates of *Tilletia tritici* according to inoculum size and treatment type at the three sites, calculated as the mean of four replicates for three winter wheat cultivars over three years (untreated and Tillecur®) and two years (hot water), respectively

	Dahnsdorf			Ahlum			Bad Vilbel		
	Inoculum level (number of spores/seed)								
	20	100	1000	20	100	1000	20	100	1000
	Infected ears/plot (%)								
Untreated	0.05	0.24	4.02	0.05	0.14	4.01	0.41	0.54	1.98
Tillecur	0.01	0.01	0.01	0.05	0.01	0.01	0.08	0.07	0.06
Hot water	0.08	0.09	0.06	0.03	0.03	0.06	0.86	0.94	1.23

Table 3: Table of variance (alpha ≤ 0.05) and F-tests of fixed effects over three years

	Dahnsdorf		Ahlum		Bad Vilbel	
Effect	F-Value	Prob > F	F-Value	Prob > F	F-Value	Prob > F
Cultivar	18.70	0.0081 *	3.36	0.1547	0.60	0.6164
Treatment	61.31	< .0001 *	3.98	0.1346	3.49	0.1726
Inoculum	43.38	0.0005 *	4.44	0.0421 *	7.14	0.0702
Cult*Treat	20.30	0.0003 *	3.36	0.0848	13.67	0.1308
Cult*Inoc	21.80	0.0002 *	3.29	0.0842	4.35	0.0981
Treat*Inoc	54.89	< .0001 *	4.71	0.0217 *	7.06	0.0547
Cult*Treat*Inoc	28.55	< .0001 *	3.60	0.0197 *	2.85	0.1659

Cult = cultivar type; Inoc = inoculum size; Treat = treatment method; Prob = probability.

Table 4: Inoculum size, infection rate and contamination potential of *Tilletia tritici* in winter wheat seed harvested at the Dahnsdorf site (2003)

Cultivar	Treatment method	Actual inoculum size (spores/seed)	Number of infected ears/plot[1,3]	Amount of spores (g)[1]	Yield (kg/plot)[1]	Contamination potential (spores/ seed) for plot[1,2]
'Naturastar'	Untreated	1435	106.8* c	17.94	1.93*	24670
		148	14.0 b	3.15	4.05	1972
		43	2.2 a	0.41	3.73	267
	Tillecur	1089	0 a	0.00	3.83	–
		162	0 a	0.00	4.03	–
		33	0 a	0.00	4.63	–
	Hot Water	240	2.5 a	0.48	3.70	315
		31	2.0 a	0.51	4.45	427
		5	2.2 a	0.38	3.75	226
'Aron'	Untreated	1172	1.0 a	0.16	3.65	102
		143	0 a	0.00	3.48	–
		35	0 a	0.00	3.58	–
	Tillecur	713	0 a	0.00	4.00	–
		130	0 a	0.00	4.45	–
		23	0 a	0.00	3.88	–
	Hot Water	109	0 a	0.00	4.75	–
		5	0 a	0.00	5.18	–
		9	0 a	0.00	5.08	–
'Batis'	Untreated	948	91.5* d	21.23	3.04*	21248
		107	10.2 c	2.06	4.58	1284
		21	2.2 ab	0.53	4.03	338
	Tillecur	792	0 a	0.00	4.20	–
		120	0.8 a	0.15	3.38	135
		23	0.8 a	0.17	4.33	126
	Hot Water	99	2.5 ab	0.76	4.93	357
		21	5.8 bc	1.35	5.23	646
		5	2.5 ab	0.60	3.90	407

[1] Mean of four replicate plots.
* 'Naturastar' and 'Batis', untreated, 1000 spores/seed: $^1/_2$ plot (5.5 m^{-2}) was counted and harvested, all others: whole plot (11 m^{-2}) was counted and harvested.
[2] Calculated after deducting 90% loss of spores through harvesting.
[3] Letters in common are not significantly different from each other according to Tukey's test (alpha ≤ 0.05).

one-tenth of the initial inoculum size in the moderately susceptible cultivar 'Aron'. In 'Batis' and 'Naturastar', however, it was around twenty times the initial inoculum size. At the lower inoculum levels in the untreated controls, the spore load increased roughly tenfold in the susceptible cultivars but decreased in the moderately susceptible cultivar. Tillecur® treatment led to a clear spore load reduction in almost all cultivars and inoculum levels. Results for the year 2004 were comparable to the year 2003 in 'Naturastar' and 'Batis'. With 'Aron', infection rate was higher in 2004; in plants produced from untreated seed inoculated with 1000 spores/seed, 11.9 g of spores were harvested from 67 bunted ears per plot (data not shown). The yield was not determined in 2004; assuming that it was around 5 kg/plot the harvested grains could be contaminated by roughly 5.600 spores/seed. That means a fivefold increase of the initial inoculum.

4 Discussion

For the determination of threshold values, the rates of common bunt in wheat cultivars of different resistance produced with seeds inoculated with variable quantities of *T. tritici* spores were investigated. Field data and theoretical spore load calculations confirmed that there is a risk potential for low-level infection. Independent of site and year, loads of 5 to 20 spores/seed were sufficient to produce a distinct infection. Moreover, the results of the spore counts confirm the restricted precision of the method used for numbers of ≤ 20 spores/seed. It might be questioned whether any of the described methods is able to provide more reliable results. A precise threshold value can not be given. The results show that a threshold of 20 spores/seed, as was proposed in Germany (Spiess and Dutschke 1991), is too high for higher susceptible cultivars. Many of the frequently grown cultivars in Germany belong to this category (Wächter et al. 2007). Cultivar-specific treatment thresholds and recommendations for the treatment of infected wheat can be derived from these results. Knowledge of cultivar resistance is an important prerequisite. Susceptible cultivars should be treated from a threshold of 1-5 spores/seed. Moderately susceptible cultivars should be treated at a contamination level of 20 spores. This is important for avoidance of disease accumulation, especially in seed production; in seed used for forage or consumption purposes, this level of contamination is unproblematic. In Germany, certified seed must not yield more than five bunted ears per 150 m^2, corresponding to a limit of 0.03 infected ears m^{-2} (ANONYMOUS 1999). In the present three-year trial, only 53 out of 198 test combinations met this requirement; 'Aron' and/or Tillecur® were used in most of these cases. Obviously, some sites are at a higher risk of bunt development than others. If and to what extent soil-borne *T. tritici* contributes to this has not been investigated. An increasing importance of this kind of inoculum has been discussed (BORGEN 2000). The spore content of the soils was not determined. At Bad Vilbel, field experiments with *T. tritici* had been performed for several years whereas at Dahnsdorf and Ahlum no such trials have been carried out until the start of the field trials in 2002. The build-up of soil-borne inoculum was possible only at Bad Vilbel. This might also explain that treatment with Tillecur® had a lower effectiveness in Bad Vilbel than in the two eastern sites. Sowing date and soil temperatures do not explain the differences in infection between the sites. In the present study, the efficacy of two seed treatment methods for bunt control was evaluated. Overall, the plant strengthening product Tillecur® produced good results, and hot water treatment had inferior effects. Especially at low inoculum levels, the hot water apparently stimulated the spores that were not washed off or killed off. The hot water treatment parameters (52°C, 10 min) were chosen according to the results of WINTER et al. (1998). Different parameter combinations were not studied. In the first two years, hot water treatment produced unsatisfactory results, but there was no opportunity to optimize the parameters 'temperature' and 'duration' of treatment. In the mean time, it has been shown that extension of the treatment time does not reduce the germination of wheat (Wolff, unpublished). Treatment at 52–53°C for 20 min would provide more successful bunt control. The results of the present study demonstrate that prevention of *T. tritici* under organic farming conditions is possible, provided that important factors, alone or in combination, are taken into consideration. This includes the spore load. In summary, good bunt control can be achieved in organic farming through the use of a cultivar-specific treatment threshold, a cultivar with low susceptibility to bunt, and a suitable seed treatment method.

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