Cereal Seed Health and Seed Treatment Strategies: Exploiting new seed testing technology to optimise seed health decisions for wheat.

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The effect of seed-borne *Tilletia tritici* contamination on the quality of winter wheat seed in the UK.

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INTRODUCTION

Common bunt (*Tilletia tritici*) of wheat causes a direct loss of yield. *T. tritici* can be seed-borne or soil-borne and contaminated seed or soil can infect the developing seedling; the fungus develops systemically in the plant and bunt balls containing millions of spores eventually replace the seed (Hoffman, 1982). Perhaps more importantly the disease can also reduce grain quality and in the UK prices as low as £15/tonne have been paid for grain contaminated with *T. tritici* spores (Yarham & Jones, 1992).

Seed is considered the most important source of infection in the UK is seed. Seed becomes contaminated at harvest time when many of the bunt balls within an infected ear are broken during combining. Spores are released and contaminate healthy seed in the combine, and during subsequent movement, storage and processing of the seed. These spores can also be transmitted between seed lots in contaminated harvesting and processing equipment. Soil infestation occurs as a result of wind-borne spores released at harvest being deposited on clean land and germinating to infect wheat sown in the autumn (Hoffman, 1982; Yarham & McKeown, 1989). The survival of *T. tritici* spores in the soil was usually dependant on a period of dry weather between harvesting and sowing (Hoffman, 1982; Yarham & Jones, 1992) and most workers agreed that the viability of *T. tritici* spores in moist soils is very short (Vandervalle & Detroux, 1957; Parlak, 1986; Williams, 987). More recently however, there have been reports from Denmark, France and the UK that suggest longer-term survival of *T. tritici* spores in moist soils (Paveley *et al.*, 1996; Borgen, 2000).

During the early 1920s bunt was common in the UK wheat crop. In 1921, 33 % of wheat samples examined at the Official Seed Testing Station (OSTS), Cambridge contained bunt balls. The occurrence of the disease then declined and in 1957 only 0.2 % of wheat samples contained bunt balls (Marshall, 1960). Cockerell and Rennie (1996), tested over 200 samples of winter wheat seed in each of the years 1992, 1993 and 1994 and recorded just one broken bunt ball in a single sample of English produced certified seed in 1992. However, their results suggested fairly wide spread low level

contamination and between 20 % and 60% of certified and farm-saved seed samples had spores of *T. tritici* in the same three year period. The maximum contamination recorded was 121 spores per seed in a sample of farm-saved seed in 1993.

It is thought that the decrease in bunt was a result of the widespread use of organomercury[1] from the 1930s. However, it must be noted that organomercury did not offer complete control. Yarham and Jones (1992) showed less than 90% control of bunt in trials with organomercury. Unfortunately there is no seed testing information that compares levels of *T. tritici* contamination on seed during the time organomercury was most widely used and Cockerell and Rennie's survey. There are several seed treatment products approved for seed-borne bunt control in the UK, but not all of these give control of soil-borne infections. In Sweden, trials with winter wheat seed artificially inoculated with a high level of *T. tritici* spores showed that organomercury was significantly better at controlling bunt infection than guazatine and there were significant differences in the effectiveness of organomercury compounds between sites (Johnsson, 1991). Gaudet *et al*., 1989 have shown similar site-to-site variation in the effectiveness of carboxin in controlling bunt and Oxley and Cockerell (1996) found differences in the efficacy of seed treatments where seed was contaminated at a level of 1000 spores per seed.

In some European countries certification standards exist for *T. tritici* contamination and in an increasing number of countries, particularly in Scandinavia, threshold levels are set and seed health testing is carried out on a voluntary basis to decide whether seed treatment is required.

The relationship between *T. tritici* and field infection, at low seed contamination levels as described by Cockerell and Rennie (1996), is not known in the UK. The work reported in this paper investigates the relationship between the seed test used at the OSTS, Edinburgh and disease expression and the subsequent multiplication of the disease within the crop.

MATERIALS AND METHODS

Seed

A healthy certified seed stock of winter wheat variety cv Consort was purchased in each of the two years 2001 and 2002.

[1] Organomercury was withdrawn in March 1992 for environmental reasons

***T. tritici* chlamydospores**

Chlamydospores (spores) were obtained by harvesting ears from a bunt-infected crop sown at SASA, East Craigs, Edinburgh, from certified wheat seed artificially contaminated with *T. tritici* spores. Bunt balls (sori) were manually removed from ears and crushed through a series of sieves to remove debris and allow spores to be collected. These spores were then used to artificially inoculate seed used in field experiments.

Treatment

The seed stock was divided into 7 portions. Six were artificially inoculated with *T. tritici* spores to give the following approximate loadings of <1, 1, 5, 10, 100, and 1000 spores per seed and one portion was left un-inoculated as a control.

Field experiments

Six field experiments were sown; three at ADAS Boxworth, Cambridge and three at SASA's Gogarbank Farm, Edinburgh. Plots of 10 m^2 were drilled at a seed rate of 450 seeds per square metre using cleaned Oyjord seed drills at both sites. Sowing dates at the two sites are given in Table 1. In both years experiments were drilled in randomised complete block designs with three replicates of each treatment. Plots were drilled in order of increasing *T. tritici* contamination level to minimise risks of cross contamination.

Table 1 Field experiments: Sowing dates at the two sites used to assess the relationship between spore loading on seed and disease expression in the resultant crop.

Site	Sowing Dates
ADAS Boxworth, Cambridge	30 November 2001
	17 January 2002
	16 November 2002
SASA Gogarbank Farm, Edinburgh	17 October 2001
	20 February 2002
	29 October 2002

Plant assessments

Plots were intensively sampled to enable the detection of very low levels of infection. When the plants were beyond GS75, the number of ears in $5m^2$ was counted. The grains from each ear were rubbed out to expose any bunt balls present. Whole and partially bunted ears were assessed as infected. Sequential assessments were made in the SASA trials sown in October 2001 and February 2002. Initially 5 x 1m rows were counted and assessed, where no bunt infected ears were found a further 5 x 1m rows were examined, this was repeated until bunt infection was found or until the full $5m^2$ was examined.

Harvesting

The remaining ears from the plots were harvested using a mini combine to simulate commercial harvesting. Plots were harvested in order of initial spore loading beginning with the lowest and ending with the highest to minimise the risk of carry over between plots. At harvest 2003, in an attempt to further reduce potential contamination from one plot to another, the combine drum was cleaned by running the combine through a commercial barley (or wheat crop sown with a seed treatment effective against bunt) crop between each set of plots representing one spore loading. The combine drum was filled and emptied three times between harvesting plots from each spore loading (Borgen, 2000). Samples of the harvested seed were examined for bunt balls and tested for *T. tritici* spore contamination.

Bunt testing

Tests were carried out following the method described by Cockerell & Rennie (1996). A search for bunt balls was made on a working sample of approximately 120g from seed harvested in 2002 and approximately 2000g in 2003.

Statistical analysis

Analysis of percentage ears infected: the percentages were transformed using an angular transformation. Within-experiment analyses were conducted using ANOVA and the least significant difference (5% level) was calculated for each experiment.

Analysis of contamination of harvested seed: the number of spores per seed were transformed using a logarithm (base 10) transformation after adding 1. Within-experiment analyses were conducted using ANOVA and the Least Significant Difference (LSD) (5% level) was calculated for each experiment.

RESULTS

Disease expression

For each experiment the mean percentage of infected ears, per treatment, is given in table 2. The degree to which low seed contamination, produced tiller infections varied from experiment to experiment. In three of the six experiments no infection was recorded in plots where the spore loadings were 10 spores/seed or below. Where infection was found in plots with an initial spore loading of 10 spores per seed or below there was no statistical difference in the level of ears infected within each experiment. Levels of infection were consistently higher at SASA, Gogarbank than at ADAS, Boxworth.

Multiplication

The levels of *T. tritici* spores obtained on seed harvested from the plots are given in Table 3. Contamination in seed harvested from plots sown from seed with a low initial spore loadings varied from experiment to experiment. All initial seed loadings of 10 spores/seed or lower (including the control) gave harvested seed samples with bunt contamination's of between 0 – 21 spores/seed. In the SASA experiments, for spore loadings above 10 spores/seed the level of seed contamination was generally higher than the original spore loading. This was true in only one of the ADAS experiments.

Table 2 The relationship between original spore loading on seed and percentage ears infected in the resultant crop.

	Field infection (% ears infected)						
Original spore loading (spores/seed)	ADAS 2001 1st	ADAS 2001 2nd	ADAS 2002	SASA 2001 1st	SASA 2001 2nd	SASA 2002	Mean
Control	0.00[a*]	0.00[a]	0.02[a]	0.00[a]	0.00[a]	0.00[a]	0.003
<1	0.00[a]	0.00[a]	0.00[a]	0.03[a]	0.00[a]	0.00[a]	0.005
1	0.00[a]	0.00[a]	0.00[a]	0.07[a]	0.07[a]	0.00[a]	0.023
5	0.00[a]	0.00[a]	0.00[a]	0.06[a]	0.00[a]	0.00[a]	0.010
10	0.00[a]	0.00[a]	0.00[a]	0.10[a]	0.00[a]	0.00[a]	0.017
100	0.00[a]	0.03[a]	0.14[b]	1.60[b]	0.45[b]	0.26[b]	0.413
1000	0.27[b]	0.61[b]	3.33[c]	10.40[c]	4.54[c]	5.27[c]	4.070

Treatments within individual experiments with different letters are significantly different from one another at p = 5% (calculated after arcsin transformation of data)

Table 3 The relationship between original spore loading and the spore loading obtained on harvested seed at ADAS Boxworth, Cambridge and SASA, Gogarbank, Edinburgh.

	Final spore loading on harvested seed (spores/seed)						
Original spore loading (spores/seed)	ADAS 2001 1st	ADAS 2001 2nd	ADAS 2002	SASA 2001 1st	SASA 2001 2nd	SASA 2002	Mean
Control	0.25[a]	0.14[a]	20.97[bc]	3.62[ab]	7.40[a]	0.86[a]	5.54
<1	0.03[a]	0.00[a]	1.22[a]	2.51[a]	4.92[a]	0.50[a]	1.53
1	0.05[a]	0.00[a]	8.89[ab]	9.34[b]	4.86[a]	0.36[a]	3.92
5	0.00[a]	0.86[a]	7.05[a]	6.99[ab]	6.88[a]	0.80[a]	3.76
10	19.86[a b]	1.35[a]	1.11[a]	5.80[ab]	3.78[a]	8.04[a]	6.65
100	0.47[a]	34.61[b]	100.03[c]	218.97[c]	136.24[b]	116.88[b]	101.2
1000	29.75[b]	659.33[c]	1885.16[d]	1136.81[d]	1049.29[c]	960.65[c]	953.50

Treatments within individual experiments with different letters are significantly different from one another at p = 5% (calculated after logarithm (base 10) transformation)

Table 4 The percentage by weight of bunt balls found in the harvested samples at each spore loading at ADAS Boxworth, Cambridge and SASA, Gogarbank, Edinburgh.

	Percentage by weight of bunt balls found in harvested seed						
Original spore loading (spores/seed)	ADAS 2001 1st	ADAS 2001 2nd	ADAS 2002	SASA 2001 1st	SASA 2001 2nd	SASA 2002	Mean
Control	0.0000	0.0000	0.0004	0.0000	0.0000	0.0000	0.0001
<1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1	0.0000	0.0000	0.0001	0.0092	0.0000	0.0000	0.0015
5	0.0000	0.0000	0.0000	0.0050	0.0000	0.0004	0.0009
10	0.0000	0.0000	0.0000	0.0069	0.0000	0.0024	0.0016
100	0.0000	0.0000	0.0042	0.0919	0.0328	0.0230	0.0260
1000	0.0000	0.0206	0.0639	0.7436	0.2042	0.2334	0.2170

Bunt balls were found in seven of the 30 treatments with initial spore loadings of 10 spores/seed or less. Where original loading was 100 spores per seed or above, bunt balls were found in all of the SASA experiments but in only 50% of the ADAS experiments.

DISCUSSION

Since the 1930's bunt has been controlled using seed treatments. However there are differences in efficacy between seed treatments and even with blanket seed treatment there was a low background level of bunt in UK wheat samples (Cockerell & Rennie, (1996)). The significance of this low background level is unknown and this study was designed to investigate the significance of low bunt contamination levels on seed quality.

Many factors affect the ability of *T. tritici* spores to infect wheat seedlings including soil temperature, moisture and presence of antagonistic organisms (Hungerford, 1922; Benada & Pospisil, 1999). The experiment encompassed two different sites and three different sowing dates over two years. The results obtained from the two sites varied within site and between experiments. Overall, as spore loading increased there was an increase in the percentage of ears infected. With spore loadings of 10 spores/seed or less giving infection levels lower than 0.1%. For spore loadings of 100 spores/seed the mean infection level was 0.4% and a spore loading of 1000 spores/seed gave a mean infection of approximately 4%. Highest levels of tiller infection were found in the 2001 SASA experiment where an initial spore loading of 1000 spores per seed gave rise to 10% ears infected. In contrast the lowest ear infection was in the 2001 ADAS experiment where no field infection was found in loadings below 1000 spores/seed and 1000 spores/seed gave rise to only 0.27% ear infection.

Although the level of ears infected was very low where initial spore loadings were 10 spores/seed or below, there are significant levels of spores present in the harvested seed. The relationship between spore levels on harvested seed and initial spore loading varies from site to site and within experiments. There is no consistent relationship between original spore loading and contamination levels in the harvested seed. In the ADAS 2002 experiment the highest contamination (20.97) was found in the control sample, whereas the highest overall mean contamination over all sowings for treatments of 10 spores/seed or less was 6.65 spore/seed

Measuring the true multiplication potential of low levels of *T. tritici* contamination proved difficult in plots. The harvesting of these experiments was carried out in a systematic way, in order to minimise

cross contamination of bunt spores from one plot to the next. Despite this it was not possible to eliminate the possibility of contamination.

At spore loadings of 10 spore/seed or below there is no dramatic increase in seed contamination. Whereas, although initial spore loadings of 100 and 1000 spores/seed gave rise to 0.4% and 4% infected ears respectively the overall mean seed contamination (spores/seed) in the harvested seed did not increase above the initial contamination level (101.2 spores/seed for an initial loading of 100 and 935.5 spores/seed for an initial loading of 1000).

CONCLUSIONS

- Low levels of seed contamination can produce low levels of infected ears in the field.
- Multiplication through seed-borne contamination may be slower than thought previously.

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