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

Technical Paper No. 8

Modelling and threshold setting for bunt (Tilletia tritici).

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1. Executive Summary

The objective of this project was the identification of a threshold for *treatment according to need*, based on measures of the spore count per sown seed. The threshold should result in a continued downward pressure on infection levels, and minimise economic losses resulting from any reduction in grain quality.

A conceptual model of plant infection and spore release was developed, drawing upon existing literature and parameterised from field experiments undertaken during this project. Central to the model are the assumptions that a Poisson function describes the distribution of spores per seed, and that any successful infection by a single spore will result in the whole of the plant being infected and all of the grain on the tillers being replaced by sori filled with teliospores. Further, there is no reduction in tillering or shoot survival with increasing disease incidence.

The proportion of plants infected is calculated as a function of the probability of a spore infecting the seed, and a weighted sum of the mean number of spores per seed and the number of spores per unit area of seedbed, reduced in proportion to the efficacy of any seed treatment (Equation 1.1). The infection probability is known to vary with environment conditions, especially temperature, and with wheat variety and timing of exposure.

Individual sori typically contain 10^7 spores, and combined with high shoot densities and grains per head, result in a massive multiplication potential. At harvest, the majority of spores are released from the sori by the threshing process, and a small proportion of these adhere to the healthy grain and are returned to the seed bulk (Equation 1.2). The remainder are dispersed and fall to the ground over moderate distances (Equation 1.3). This spore shower may infect clean seed that is sown downwind of the combine. However, the spores have no means of preventing germination and will germinate in a cool and moist soil whether the host plant is present or not. The likelihood of spores germinating prior to sowing of the following crop can be represented by an exponential decay with time of the aerial spore load, in proportion to the frequency of significant rainfall events.

Analyses of the results of field trials, both historical and carried out under this project, have led to the identification of recommended mean values for each of the model parameters required by Equations 1.1 to 1.3.

The probability $\boldsymbol{b}$ of a single spore infecting a plant potentially varies with site, timing and variety, though insufficient data are currently available to establish predictive relationships. Figure 1.1 illustrates the range of calculated infection probabilities for *cv*. Consort at five sites for a spore loading of 1,000 spores per seed. The results are for single trials without replication for crops sown in October-November 1994 (Gogarbank, Bush Estates, Manor Farm and Rosemaund) and October-November 2001 (Boxworth). The calculated mean value of $\boldsymbol{b}$ is 6.2×10^{-5}. The observed data are zero bounded. Assuming that the data conform to a gamma distribution, the 95% confidence interval for $\boldsymbol{b}$ is 2.2×10^{-4} to 2.2×10^{-6}.

The proportion $\boldsymbol{d}$ of infected grain that are harvested and returned to the grain store whole, ie. without releasing their spore contents during threshing, has mean value of 0.18. The observed data are zero bounded. Assuming that the data conform to a gamma distribution, the 95% confidence interval for $\boldsymbol{d}$ is 0.04 to 0.39.

The proportion $\boldsymbol{c}$ of released spores that adhere to healthy grain and are returned to the grain store has a mean value of 0.0055. The observed data are zero bounded. Assuming that the data conform to a gamma distribution, the 95% confidence interval for $\boldsymbol{c}$ is 0.0003 to 0.0174.

Equation 1.1. Proportion of plants infected (*p*)

$$p = 1 - e^{-b \cdot m}$$

b Probability of a single spore infecting a plant
m Mean number of viable spores adhered to seed

Equation 1.2. Mean number of spores adhered to harvest grain (*s*)

$$s = \frac{p \cdot c \cdot k \cdot d}{1 - p \cdot d}$$

d Proportion of sori that release their spore contents
k Mean number of spores per sori
c Proportion of spores that adhere to healthy grain

Equation 1.3. Mean number of spores dispersed per hectare (*a*)

$$a = p \cdot (1 - c) \cdot k \cdot d \cdot g \cdot t$$

t Number of surviving shoots per hectare
g Number of grains per shoot

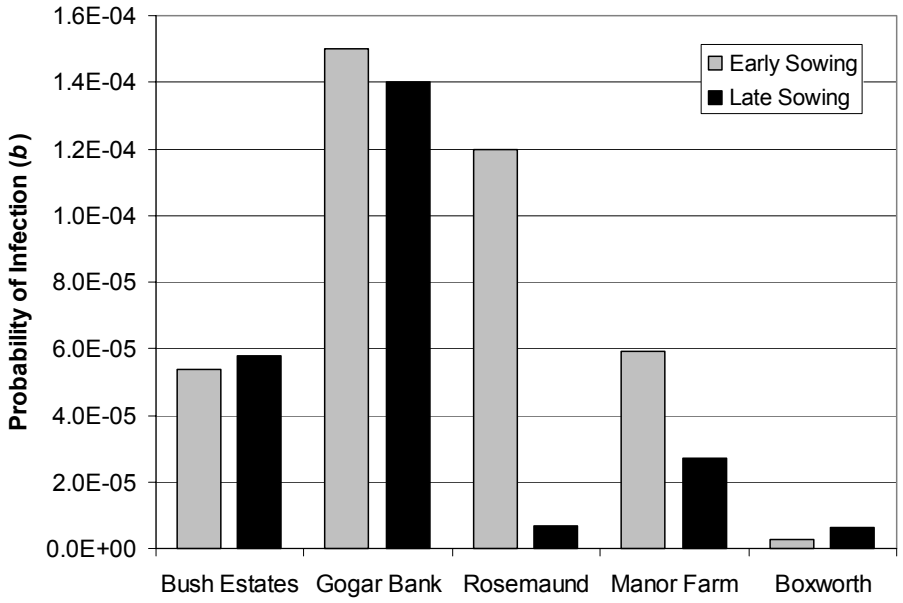


Figure 1.1. Calculated spore infection probabilties for *cv*. Consort at a spore loading of 1,000 spores per seed at UK research sites (1994 and 2001).

The number ***k*** of spores per bunt ball has a calculated mean value of 9.8×10^6. Assuming that the data conform to a normal distribution, the 95% confidence interval for ***k*** is 8.8×10^6 to 10.8×10^6.

The minimum expected efficacy ***e*** of seed treatment is 0.98, as required by the Pesticides Safety Directorate to support a claim for control on a product label (Anon., 2002).

The spores per seed equivalent ***h*** of 1 g m^{-2} of spores deposited on the soil immediately prior to sowing has a calculated mean value of 19,950 sp sd^{-1}. The 95% confidence interval for ***h*** is 14,375 sp sd^{-1} to 25,525 sp sd^{-1}.

The standard number of grains ***g*** on a wheat ear is 36 and each has a mean mass of 46 mg at 85% dry matter. The mean number of surviving tillers ***t*** at harvest is 600 m^{-2} (HGCA, 1997).

The model has been successfully applied to predict disease expression at a wide range of spore loadings (Figure 1.2), and to predict the number of spores adhered to grain harvested from trap crops sown immediately downwind of infected plots. The calibrated model predicts that a large percentage (>99%) of the spores held in infected grain are released into the air at harvest. These are deposited locally and may infect a following crop. The multiplication potential of the disease due to spores adhered to harvested grain, which may then be used as seed, is adequately controlled by seed treatment. However, under worse case conditions of immediate sowing of the following crop after harvest, the soil borne spores result in a multiplication potential that cannot be adequately controlled. Experimentation with the model, together with the results of soil inoculation trials, suggest that the spore dispersal may be responsible for maintaining the recorded small counts of spores per seed in surveys of certified bulks, despite year-on-year treatment of seed.

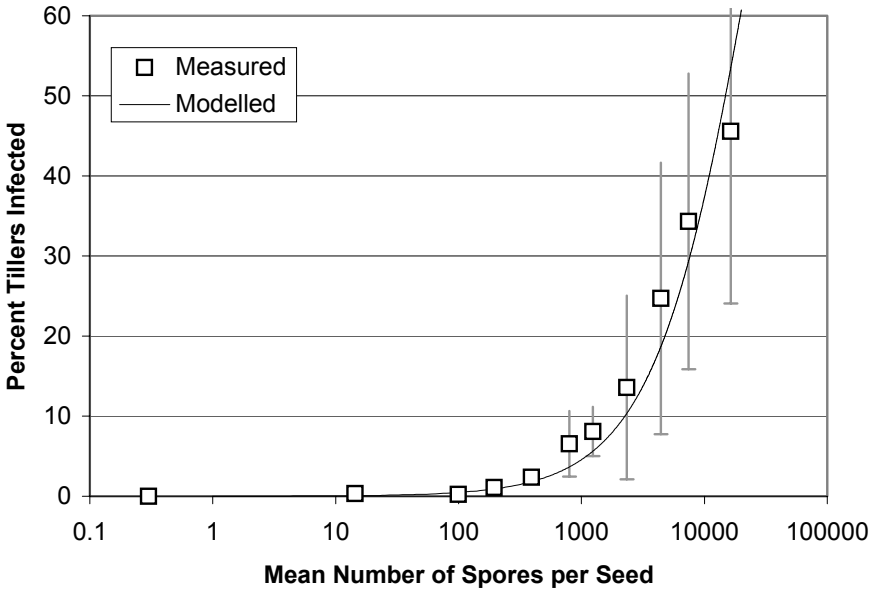


Figure 1.2. Measured and modelled percentage of tillers infected as a function of increasing spores per seed, for a winter wheat sown on 3rd November at Cammo Field, Edinburgh. The bars show the 95% confidence interval of the mean of 4 replicates (SASA, 1997).

To assess the potential outcome of *treatment according to need*, the model can be run in a stochastic framework to simulate the year on year levels of infection for a large number of fields growing wheat for seed, milling and animal feed. Critical to this simulation is an accurate function describing likelihood of a soil borne spore, released at harvest, surviving to infect the following crop. The distribution of time delays between harvest and sowing of following wheat crops can be obtained from surveys of farm practice. However, the survival of soil borne spores with respect to rainfall and temperature is currently very poorly quantified. Spore survival data are required before the model can be used as the basis of setting a seed treatment threshold.

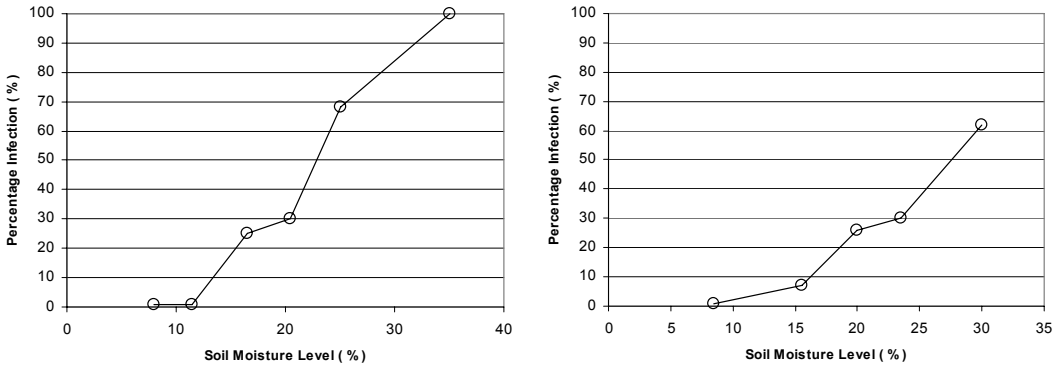
Cursory analyses of historical disease data indicate that the net multiplication potential due to soil borne spores, averaged across the farm landscape, is less than one, i.e. disease levels should naturally decrease. A treatment threshold is therefore necessary only to maintain the quality of the harvest grain, and not to prevent a soil borne epidemic. However, it is recommended to take account of the worst-case scenario of local spore deposition and immediate sowing by setting a treatment threshold that ensures the quality of adjacent crops.

2. Common Bunt of Wheat

The physiology, infection and life-cycle of bunt and related diseases have been comprehensively reviewed by Wilcoxson and Saari (1996). The disease is spread through contaminated seed and soil and infects the developing seedling. The fungus develops systemically in the plant and bunt balls containing spore masses eventually replace the kernal. Benada *et al*. (1995) have shown that it is common for all tillers of an infected plant to be diseased. Bunt spores are released at harvest by the threshing of the grain. Some spores are returned to the store, adhered to the grain, and may infect the following crop if used for seed. Others are released to the air and may travel long distances. This is a concern as the released spores may directly contaminate seed sown at a later date.

Seed is vulnerable to infection only for a period of *c*. 10 days during germination (Sartoris, 1924) and only a small percentage of the UK wheat crop is sown and harvested concurrently. Most spores deposited on moist soil in advance of drilling would therefore be expected to rapidly germinate and die to no effect. Nevertheless, field reports of bunt disease indicate that soil borne infection does occasionally occur. Yarham (1993), for example, reported 50% infection in a crop of winter wheat, grown from seed that had been home saved without fungicidal treatment for two years. Yarham and McKeown (1989) also reported a case of 10-50% infection in a crop that had been sown from organo-mercury treated seed, and was known to have been sown on the day a neighbouring bunt infected crop was harvested upwind of it. Dry soils may also prolong the life of the bunt spores, enabling infection of wheat sown many weeks after spore dispersal.

In addition to the direct loss of grain yields, the disease reduces grain quality at levels exceeding 0.05% (bunt balls by weight). The bunt spores are believed to present no health risk to consumers of infected grain or grain products, but infected milled wheat is discoloured and wheat containing more than 3% bunted kernels is considered unfit for human consumption. The bunt balls also have a fishy odour that can be imparted to processed food products. T he commercial value of a crop may be reduced at low levels of plant infection, due to the tainting of flour by the black teliospores or the tri-methylamine evaporate that gives the grain the odour of rotten fish. Millers will reject grain on the basis of finding one bunted grain in a sample of 500g. In a Swedish experiment, 50% of persons could smell the presence of *c*. 50 spores per seed, associated with plant infection of 0.1% (Johnsson, 1991). More



(a) (b)

Figure 2.1. Percentage of bunt infected plants as a function of soil moisture levels during the period of germination for experimental field sites at Moscow (a) and Nez Perce (b), United States (after Hungerford, 1922).

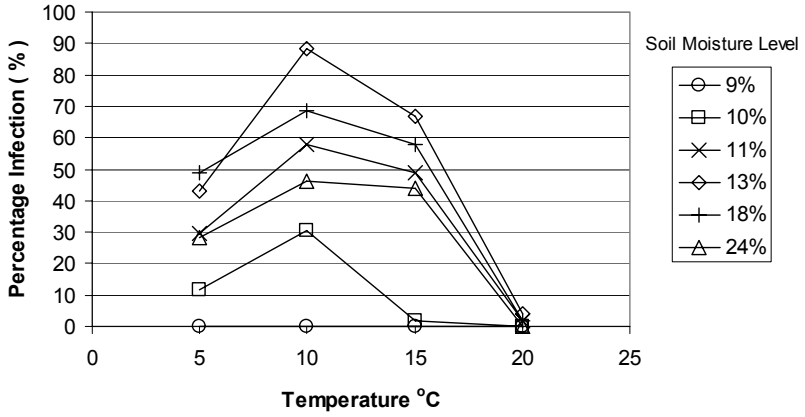


Figure 2.2. Percentage of bunt infected plants as a function of soil moisture levels and soil temperatures during the period of germination for experimental sites in the Pacific north-west, United States (after Kendrick and Purdy, 1959).

significantly, infected wheat may be unsuitable for seed due to the rapid multiplication of disease infection, even though there is only a single life cycle each year.

Bunt spores were recorded in between 20% and 60% of certified and farm-saved seed samples over a three year (1992-1994) survey of winter wheat seed in the UK. Between 2% and 8% of the samples recorded more than one spore per seed (Cockerell and Rennie, 1995). The variation in the residual contamination of seed in the UK is likely to be associated with variations in environmental conditions. Johnsson (1991) reported that the residual infection rate in samples of winter wheat taken from commercial farms in Sweden, the majority of which are using treated seed, were significantly correlated with the levels of infection in untreated experimental plots that had been inoculated with bunt spores over the period 1967-1987.

Bunt of wheat has been controlled by the application of fungicides to seed. Within the United Kingdom (UK) the advisory limit for seed treatment is one spore per seed, equivalent to 20 spores per gram of seed. Spore contamination of around 100 spores per seed is considered high and advice may be to discard the seed. The use of seed treatments could be limited using principles of integrated plant protection, taking advantage of emerging technologies for the rapid measurement of spore loading. However, it is necessary to take into consideration that seed dressing may also control other seed borne diseases (Polisenka *et al*., 1998), and that the efficacy of seed treatment and disease expression is controlled by a range of environmental factors (Gaudet *et al*., 1989).

The severity of bunt infection has been repeatedly correlated with environmental variability in soil moisture and temperature during the period of germination of the winter wheat. Hungerford (1922) demonstrated a strong positive relationship between the percentage of bunt infected winter wheat plants and the mean soil moisture during the period of germination at two field sites in the United States (Figure 2.1). There was no infection at soil moisture levels of less than 10%. Kendrick and Purdy (1959) also demonstrated the significance of soil moisture under experimental conditions when soil moisture and temperature conditions were held stable during the period of germination (Figure 2.2). At soil moisture levels of 9% there was no infection. Infection rates peaked at a soil moisture level of 13%, regardless of soil temperature. However, in contrast to the results of Hungerford (1922), infection rates declined at moisture levels in excess of 13%. From these results, it is suggested that the optimum soil moisture level for germination of bunt spores is *c*. 13-14 % for germination, whilst winter wheat grains may germinate at soil moisture levels of *c*. 10 %. Hence, the sowing of wheat in a relatively dry soil is likely to reduce the rate of bunt infection.

Soil moisture may also impact on the rate of infection by influencing the temperature regime of a soil. The heavier the soil, the higher the soil moisture level, and the higher the soil temperature, all other factors being held constant. The depth of sowing and the soil albedo will also control the temperature regime experienced by the seed (Arafa, 1981). Johnsson (1992) in a review of the climate factors influencing the attack of common bunt in winter wheat in Sweden (1940-1988) demonstrated a strong negative correlation between soil temperatures during days 1-11 after sowing (the germination period) and the severity of attack. The attack was strongest when the mean temperature was in the range 6-7^{o}C. Polisenka *et al*. (1998) also reported a strong negative correlation between soil temperature and the percentage infection, regardless of sowing date. The lower the temperature, the higher the rate of infection. Thus, winter cereals sown late and spring cereals sown early are particularly at risk of infection.

Kendrick and Purdy (1959) also demonstrated the significance of soil temperature under experimental conditions when soil moisture and temperature conditions were held stable during the period of germination (Figure 2.2). Peak rates of infection occurred at soil temperatures of 10^{o}C. At temperatures between 10^{o}C and 20^{o}C there was a strong negative

correlation between infection and soil temperature. Below 10°C, percentage infection decreased with decreasing temperature. Other authors have confirmed that infection is negligible at soil temperatures in excess of 25°C (Hungerford, 1922).

The sensitivity to temperatures may explain a large percentage of the between year variance of infection at constant levels of inoculum under experimental conditions. For example, Gaudet *et al*. (1989) reported percentage infestation of winter wheat spikes of 18% to 84% at a site receiving untreated seed inoculated with 0.83 g of bunt spores per 100 g of seed.

Disease expression is dependent upon both the germination of the bunt spores and the infection of the wheat plant. Purdy and Kendrick (1957) showed that the rate and percentage germination of spores of *Tilletia tritici* were controlled by soil temperature and moisture content. At moisture contents less than the permanent wilting point, percentage germination was zero. Increasing moisture content (to field capacity) increased the rate of germination and hence maximum germination within 10 days. Rates of germination were greatest for temperatures in the range 10-15 °C.

Kendrick and Purdy (1959) showed that percentage infection of seed was also controlled by soil temperature and moisture content. The results generally reflected the data on percentage germination of spores. However, at temperatures of 20°C and above, seed infection was reduced to zero even though spore germination was high. It is believed that this was due to the rapid emergence of the wheat plant, and slow rate of spore germination, at high temperatures. Sartoris (1924) demonstrated that the proportion of wheat plants infected declines with the age of the seed at which it is exposed to chlamydospores or sporidia of *Tilletia tritici*. Maximum infection occurs on the day of germination, and declines to zero on the day of emergence of the coleoptile above the soil surface. Kendrick and Purdy (1959) also showed that maximum seed infection occurred at moderate soil moisture contents, between wilting point and field capacity, whilst rates of spore germination increased with moisture content.

Increased depth of planting is believed to lengthen the period in which the seed is most vulnerable to infection. This has been demonstrated in a number of plot experiments (see, for example, Jones & Seif-el-Nasr, 1940). Polisenka *et al*. (1998) demonstrated significant variation in bunt infection between 28 winter wheat cultivars, regardless of sowing date and soil temperatures. The most infected cultivars (inoculated with 0.2 g of spores per kg of seed) were Ina and Simona, with mean infection rates across two years and two sowing dates of 19.2% and 13.5% infected spikes, respectively. The least infected cultivars were Viginta and Rexia with mean infection rates of 1.1% and 0.3% infected spikes, respectively.

The objective of this project was the identification of a threshold for *treatment according to need*, based on measures of the spore count per sown seed. The threshold should result in a continued downward pressure on infection levels, and minimise economic losses resulting from any reduction in grain quality. This required the development of a model framework predicting infection as a function of seed and soil borne spore loadings. Given the environmental sensitivities identified above, this would have to be parameterised for sites representative of the range of environmental conditions in the UK. In this project, results from a wide range of field trials held at ADAS Boxworth, Cambridge and SASA, East Craigs, Edinburgh have been used to provide model parameters.

3. Model Structure

A conceptual model structure has been developed that predicts the proportion of wheat plants infected as a function of the probability of a single bunt spore infecting a seed grain (itself a function of physical environment conditions) and the seed quality. At harvest, a proportion of the bunt spores released by the threshing process adhere to the healthy grain and are returned to the seed bulk. Iterative application of the model, with and without seed treatment, allows simulation of the build up of disease in the wheat crop and identification of infection levels that result in significant economic losses.

Infection generally refers to the establishment of a pathogen within a host, and disease does not necessarily follow. Infection by Common Bunt occurs by penetration at the root node or coleoptile. In resistant wheat cultivars, growth of the fungus within the plant is retarded and it may fail to establish in the growing points before they develop beyond reach (Bruehl, 2003). Despite this knowledge, infection is here referred to as disease development following inoculation.

Literature indicates that, unlike Dwarf Bunt (*Tilletia controversa*), Common Bunt (*Tilletia tritici*) reduces yield in proportion to the percentage of plants infected as it does not affect emergence or tillering (Goates, 1996). When a plant is infected, it is normal for every grain on each tiller to be replaced with bunt sori filled with teliospores. This has been confirmed under UK conditions by experimentation carried out by SASA (East Craigs, Midlothian) and SAC (Bush Estate, Midlothian). In 1994 (harvest), a stock of cv. Hunter seed was artificially inoculated with 100 to 18,000 *T. tritici* spores per seed. Counts of infected and partially infected tillers confirmed that tiller infection is rarely incomplete (Table 3.1). In 1998 (harvest), a stock of Hunter seed was artificially inoculated with 1 to 17,000 *T. tritici* spores per seed. Counts of tillers confirmed that there was no reduction at high levels of infection (Table 3.2). The assumptions that there is no yield reduction and that tiller infection is complete are fundamental to the model structure described below.

Table 3.1. Counts of wheat ears wholly and partially infected in five one meter plot lengths, following inoculation of cv. Hunter seed with *Tilletia tritici* at Gogarbank Farm (SASA, 1994).

Plot	Sowing Date	Spores per Seed	Healthy Ears	Ears Wholly Infected	Ears Partially Infected
1	Early	100	271	1	0
2	Early	119	252	3	0
3	Early	119	242	4	0
4	Early	119	273	5	0
5	Early	158	295	1	0
6	Early	158	238	1	0
7	Early	158	232	9	0
8	Early	1000	236	10	2
9	Early	1000	291	8	0
10	Early	1000	240	28	0
11	Early	1000	282	27	0
12	Early	18000	202	42	0
13	Early	18000	124	172	7
14	Early	18000	114	177	0
15	Early	18000	63	198	0
16	Late	100	220	2	0
17	Late	100	236	1	0
18	Late	119	236	4	0
19	Late	119	231	3	0
20	Late	119	227	4	0
21	Late	119	211	3	1
22	Late	158	203	1	0
23	Late	1000	196	12	0
24	Late	1000	227	18	0
25	Late	1000	240	20	0
26	Late	18000	131	83	0
27	Late	18000	109	147	0
28	Late	18000	109	88	0
29	Late	18000	101	94	0

Table 3.2. Counts of wheat tillers in five one meter plot lengths, following inoculation of cv.Hunter seed with spores of *Tilletia tritici* at Gogarbank Farm (SASA, 1999).

Spores per Seed	Percent Plant Infection	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average Count
0.3	0.0	244	216	234	250	236
14.4	0.3	222	229	187	219	214
99.2	0.2	209	238	208	227	221
194.0	1.1	268	250	234	237	247
391.6	2.3	215	248	221	262	237
794.6	8.1	219	243	233	241	234
1234.4	7.5	233	195	246	245	230
2340.2	11.4	215	232	250	247	236
4423.2	20.2	241	271	228	215	239
7432.0	25.5	223	267	242	241	243
16355.6	30.9	212	230	247	236	231

3.1. Seed Quality

Seed quality is described here by the mean number of bunt spores per seed. It is assumed that the spores are distributed randomly and described by the Poisson distribution, so that the proportion of all seeds having ***X*** spores on them is calculated according to Equation 3.1 as:

$$P_X = \frac{e^{-m} \cdot m^X}{X!} \qquad (3.1.)$$

Where ***m*** is the mean number of effective spores per seed, also referred to as the disease *pressure* parameter. From this, it can be shown that the proportion of all seed grains that are free from spores is calculated according to Equation 3.2 as:

$$P_0 = e^{-m} \qquad (3.2.)$$

Substantial literature indicates that both seed and soil borne spores can result in infection. The mean number of effective spores per seed is therefore a sum of the seed borne count and a weighted density of spores in the soil, according to Equation 3.3:

$$m = m_g + w \cdot m_s \qquad (3.3.)$$

Where $\boldsymbol{m_g}$ is the mean number of spores per seed, $\boldsymbol{m_s}$ is the density of spores in the soil and ***w*** measures the likelihood of a soil borne spore making contact with a seed or the coleoptile. When a treatment is applied, it is assumed that the effective number of spores is reduced in proportion to the efficacy of the treatment, according to Equation 3.4:

$$m' = m \cdot \left(1 - \frac{E}{100}\right) \qquad (3.4.)$$

Where ***E*** is the efficiency of the treatment, expressed as a percentage of spores killed. Treatment efficiency may differ for seed and soil borne spores.

3.2. Plant Infection

If it is assumed that each spore on a seed grain has a defined probability ***b*** of infecting the seed, resulting in *at least* one bunted grain on the plant at harvest, then the proportion of all wheat plants that have one or more bunted grain on any of the tillers is calculated according to Equation 3.5 as:

$$\sum_{n=1}^{n=G} P_n = 1 - \left(e^{-m} + \sum_{X=1}^{X=\infty} \frac{e^{-M} \cdot M^X}{X!} \cdot (1-b)^X \right) \qquad (3.5.)$$

Where ***g*** is the total number of grains on the wheat plant. This equation has an analytical solution, Equation 3.6:

$$\sum_{n=1}^{n=G} P_n = 1 - e^{-b \cdot m'} \qquad (3.6.)$$

In field trials, bunt infection is measured as the proportion of tillers infected. It is assumed that this is equal to the proportion of wheat plants infected. This is justified given that there is

no evidence in the literature that high infection will result in a reduced number of surviving tillers.

The probability of infection parameter ***b*** is dependent upon soil moisture and temperature conditions, the relative rates of spore and wheat germination, depth of sowing and genetic resistance factors. The form of Equation 3.6 has been proven with historical data. Heald (1921) measured the plant infection resulting from spores per seed in the range 100 to 165,000 for two winter wheat varieties sown on disinfected soils. Using a value of ***b*** of 5×10^{-5} derived from UK field experimentation, the model is able to explain 95% of the observed variance (Tables 3.3 and 3.4) and adequately describes the shape of the infection response to spore pressure (Figure 3.1).

3.3. Spore Release

At harvest, spores are released by the rupture of the bunt balls during threshing. Released spores may adhere to healthy grain or be blown from the rear of the combine. The number of spores retained adhered to the grain at harvest can be calculated according to Equation 3.7 as:

$$S_p = p \cdot c \cdot k \cdot (1-d) \quad (3.7.)$$

where ***p*** is the proportion of plants infected, ***c*** is the proportion of spores released retained by the combine, ***k*** is the mean number of spores per bunt ball and ***d*** is the proportion of bunt balls retained whole by the combine. This equation is appropriate for low levels (p<0.01) of infection when the number of healthy grain are not significantly reduced. The bunt balls are of a lower mass than the grain and are preferentially filtered by the combine mechanism. At high levels of infection, the spores released adhere to a progressively reduced number of healthy grain. In these circumstances, the number of spores adhered to harvested grain is calculated according to Equation 3.8 as:

$$S_p = p \cdot c \cdot k \cdot (1-d) \cdot \frac{1}{1-p \cdot (1-d)} \quad (3.8.)$$

By calculation of a simple mass balance, the number of spores released to the wind can be calculated according to Equation 3.9 as:

$$S_c = p \cdot (1-c) \cdot k \cdot (1-d) \cdot g \cdot t \quad (3.9.)$$

where $\boldsymbol{S_c}$ is the spore count per m^2, ***g*** is the number of grains per shoot and ***t*** is the number of surviving shoots per m^2 of field area.

Table 3.3. Bunt infection resulting from spore pressure on cv. Jones Winter Fife winter wheat (after Heald, 1921).

Spores per Seed	Percent of Plants Infected	
	Observed	Modelled
937	10.4	4.6
833	12.9	4.1
937	17.0	4.6
3,437	23.2	15.8
4,583	31.6	20.5
40,104	70.5	86.5

Table 3.4. Bunt infection resulting from spore pressure on cv. Jenkin's Club winter wheat (after Heald, 1921).

Spores per Seed	Percent of Plants Infected	
	Observed	Modelled
104	1.9	0.5
458	2.5	2.3
533	5.9	2.6
5,333	32.8	23.4
20,687	43.7	64.5
36,770	84.6	84.1
65,229	92.9	96.2
166,971	92.5	100.0
164,208	94.5	100.0

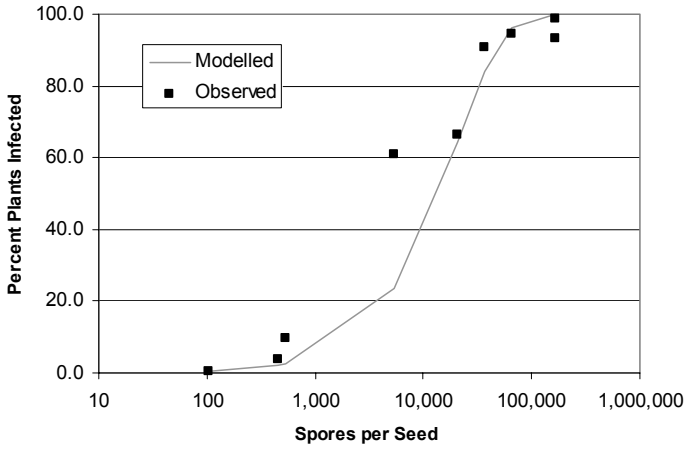


Figure 3.1. Observed and modelled bunt infection resulting from spore pressure on cv. Jenkin's Club winter wheat (after Heald, 1921).

4. Model Parameterisation

4.1. Spores per Bunt Ball

The number of spores per infected grain or bunt ball (***k***, Equation 3.7) dictate the multiplication potential of the disease. NIAB Cambridge (2002) analysed the spore contents of 20 bunt balls. The bunt balls had a mass in the range 7 to 23 mg. The mean mass was 15.45 mg compared to the expected mean grain mass of 46 mg at 85% dry matter (HGCA, 1997). The number of spores was correlated with the mass of the bunt ball (Figure 4.1) and was in the range 0.27×10^6 to 1.07×10^6 per mg. The distribution of spores per bunt ball, regardless of size, corresponded to a normal distribution. The mean number of spores per bunt ball was 9.80×10^6 and the standard deviation was 0.49×10^6. It was estimated that the shell of the bunt ball accounted for *c*. 17% of the total mass.

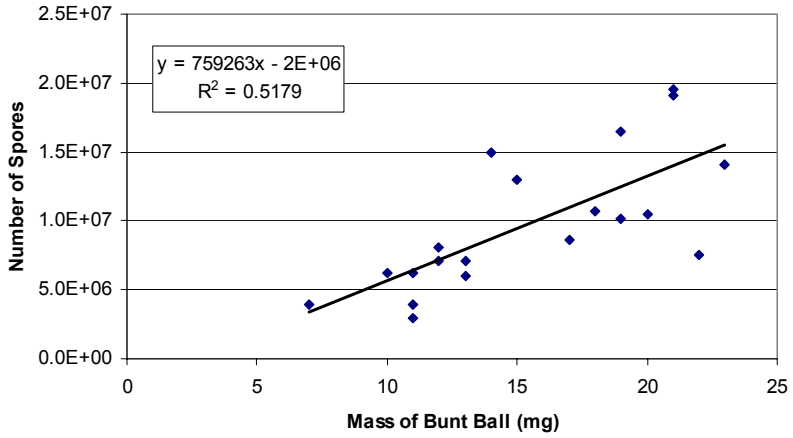


Figure 4.1. Correlation between number of spores and mass of bunt ball (NIAB, 2002)

SASA Edinburgh (2002) measured the number of spores in 10 bunt balls with a mean mass of 17.3 mg, by weighing of the bunt ball contents and assuming a single spore mass of 3.15×10^{-6} mg. The mean number of spores per bunt ball was 5.49×10^6. It was estimated that the shell of the bunt ball accounted for *c*. 25% of the total mass.

Pirson (1978) reported the mass of 111 bunt balls to be in the range 2.7 to 22.5 mg with a mean of 11.17 mg. The spore count was reported as 0.2×10^6 per mg, compared to the 0.6×10^6 per mg reported by NIAB (2002). Polisenka *et al.* (1998) reported a mean of 176 spores per seed when samples of grain were inoculated with 0.2 g of spores per kg of seed. This indicates a mean spore mass of 52×10^{-6} mg. Oxley and Cockerell (1996) reported a mean of 18,000 spores per seed when samples of grain were inoculated with 1 g of spores per kg of seed. This indicates a mean spore mass of 2.5×10^{-6} mg.

The number of spores per bunt ball is a critical variable in estimating the number of spores per harvested grain, and the number of spores deposited on the soil that may infect the following crop. At low spore loading, the model is approximately linear with respect to ***k*** so that a doubling will result in a doubling of the spores per harvested grain.

Based on the above measurements, ***k*** has been assumed equal to 10×10^6 spores per bunt ball. To minimise the risk of infection by spores settled on the soil, it is best to over-estimate the number of spores per bunt ball when using the model to set the threshold for seed treatment.

4.2. Infection Probability

The probability of a single spore successfully germinating, infecting a plant and showing disease (***b***, Equation 3.6) potentially varies with site, timing with respect to plant development, prevailing weather conditions and plant variety, though insufficient data are currently available to develop predictive relationships. The infection probability can be calculated from field trial data where a known spore loading (spores per seed, ***m***, Equation 3.6) is varied and the percent of plants infected is measured, by rearrangement of the model equation. Figure 4.2 illustrates the range of calculated infection probabilities for *cv*. Consort at five sites, in England and Scotland, for a spore loading of 1,000 spores per seed. The results are for single trials without replication for crops sown in October-November 1994 (Gogarbank, Bush Estates, Manor Farm and Rosemaund) and October-November 2001 (Boxworth). The average calculated value of ***b*** is 6.2×10^{-5}. The observed data are zero bounded. Assuming that the data conform to a gamma distribution, the 95% confidence interval for ***b*** is 2.2×10^{-4} to 2.2×10^{-6}.

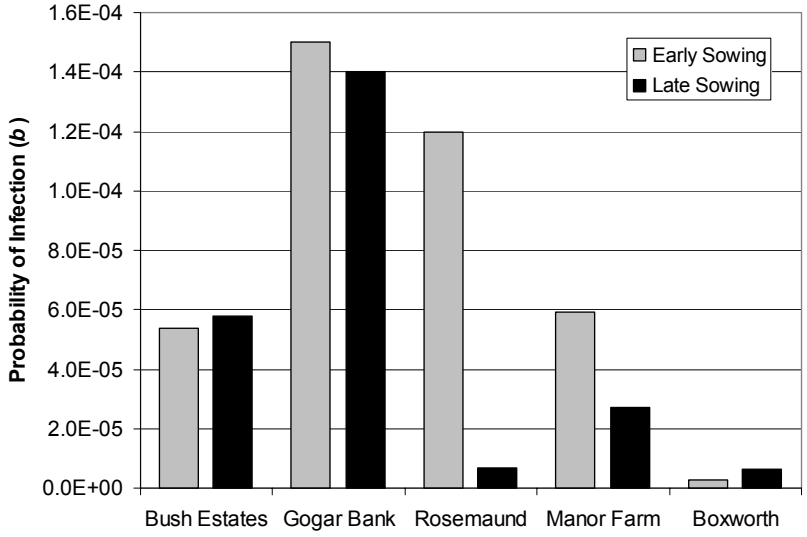


Figure 4.2. Calculated spore infection probabilties for *cv*. Consort at a spore loading of 1,000 spores per seed at UK research sites (1994 and 2001).

4.2.1 Effect of Spore Loading

Validation of the dose response equation, required demonstration that the probability of disease (***b***, Equation 3.6) was independent of the spore loading, i.e. there is no spore density dependence or competition for infection sites. The exponential form of the model equation predicts a decreasing rate of additional disease expression with increasing spore loading. It was therefore also necessary to demonstrate that the exponential model was a better fit to the observed data than a simple linear relationship. The infection resulting from spore loadings of up to 16,400 spores per seed was investigated at SASA in 1997. Wheat with a known number of spores per seed was sown in early November on experimental plots and the numbers of tillers infected recorded at harvest. This extreme level of spore loading enabled us to test the structure of the infection model. Table 4.1 summarises the observed levels of infection with spore loading for four replicates.

Table 4.1. Summary of percentage of tillers infected by bunt for extreme spore loadings at Cammo field, Edinburgh, for a crop sown on 3rd November (SASA, 1997).

Spores per Seed	Rep. 1	Rep. 2	Rep. 3	Rep. 4
0.3	0.0	0.0	0.0	0.0
14.4	0.5	0.9	0.0	0.0
99.2	0.5	0.0	0.0	0.4
194.0	2.2	1.2	0.9	0.0
391.6	3.3	1.6	2.7	1.9
794.6	13.2	5.8	5.2	2.0
1234.4	12.9	6.7	4.5	8.2
2340.2	33.0	10.8	6.0	4.5
4423.2	51.9	25.8	12.3	8.8
7432.0	57.0	47.2	21.5	11.6
16355.6	69.3	61.3	37.3	14.4

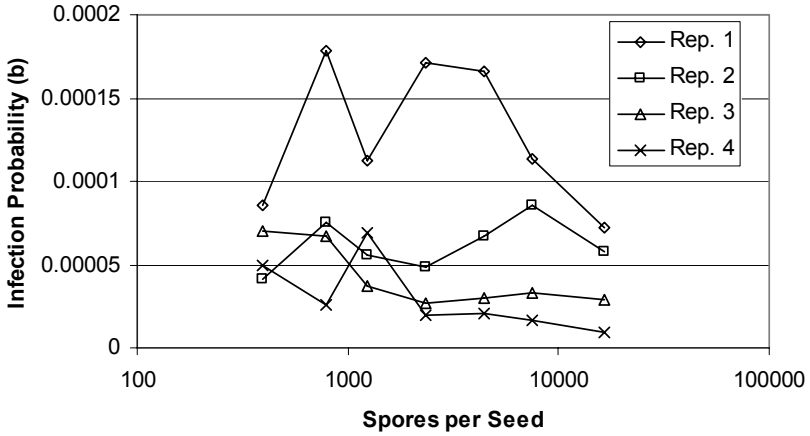


Figure 4.3. Calculated infection probability as a function of spore loading at Cammo Field, Edinburgh, for a crop sown on 3rd November (SASA, 1997).

The infection probability was calculated for each non-zero disease response by rearrangement of Equation 3.6. Linear regression analysis failed to identify a significant increase or decrease of the infection probability with increasing spore loading (P>0.05), supporting the assumption of a constant probability for a given site (Figure 4.3).

Figure 4.4 shows that for each replicate, the rate of additional infection decreased with increasing number of spores per seed. In each case, the proposed model (Equation 3.6) explained a higher percentage (2-13%) of the total variance than a simple linear relationship between spore loading and percentage infection. The observed data were log-transformed to estimate the infection probability ***b*** by linear regression analysis. For each replicate, linear regression analysis was applied to the transformed data to estimate the probability ***b*** of a single spore infecting a wheat plant (Table 4.2). The intercept was not significant (P>0.05) for any replicate. There was a ten-fold variation in infection probability between replicates. The mean probability of infection was 4.2×10^{-5}.

Table 4.2. Summary of spore infection probability estimates for each replicate of the extreme spore loading experiment at Cammo field, Edinburgh (SASA, 1997).

Replicate	r^2	P	Spore Infection Probability	
			Estimate	Standard Error
1	84	< 0.001	8.6×10^{-5}	8.63×10^{-6}
2	96	< 0.001	6.3×10^{-5}	3.27×10^{-6}
3	99	< 0.001	2.9×10^{-5}	0.76×10^{-6}
4	67	< 0.001	1.2×10^{-5}	1.69×10^{-6}
Mean	92	< 0.001	4.2×10^{-5}	3.02×10^{-6}

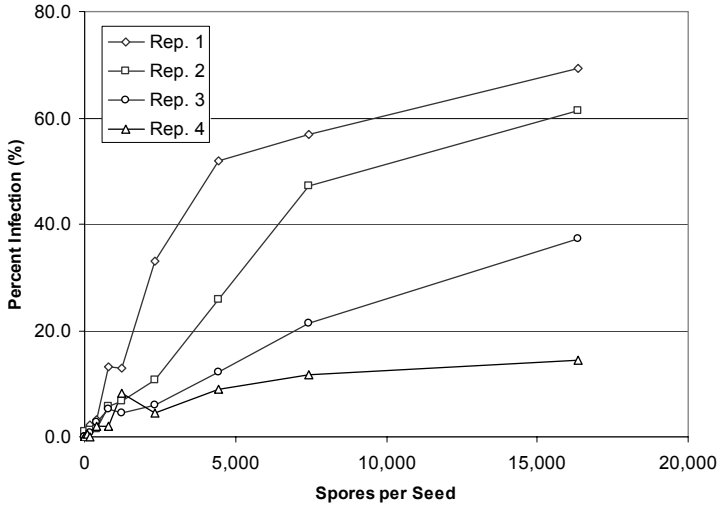


Figure 4.4. Summary of percentage of tillers infected by bunt for extreme spore loadings at Cammo field, Edinburgh, for a crop sown on 3rd November (SASA, 1997).

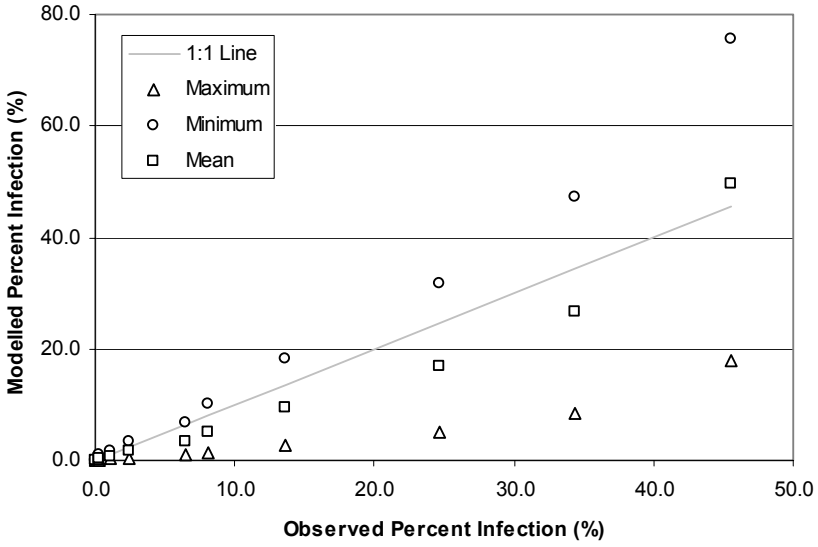


Figure 4.5. Observed and modelled mean percentage of tillers infected by bunt for extreme spore loadings at Cammo field, Edinburgh, for a crop sown on 3rd November (SASA, 1997).

Mean percentage infection was modelled and compared to the observations for the minimum, mean and maximum infection probabilities (Figure 4.5). It was observed that modelled infection was highly sensitive to the calculated infection probability. The replicates were all located in one field. However, known significant variations in topography and soil moisture conditions between replicate plots may explain the wide range of infection probabilities.

In addition to the analysis of the historical experimental data, wheat with known levels of spores per seed were sown in 2001 and 2002 at Boxworth (England) and Gogarbank (Scotland) as part of this project. Figure 4.6 illustrates how infection varied with the spore loading. The infection model was fitted to each dataset to estimate the mean probability of infection $\boldsymbol{b}$ for a single spore (Tables 4.3 and 4.4). For each sowing date and site, the infection probability $\boldsymbol{b}$ was held constant across the range of spore loadings. There was evidence that the probability of infection was greater at Gogarbank than at Boxworth. Figure 4.7 compares observed and predicted percent infection. Modelled and observed infection compare well except at low spore loadings when uncertainty in the observations would be expected.

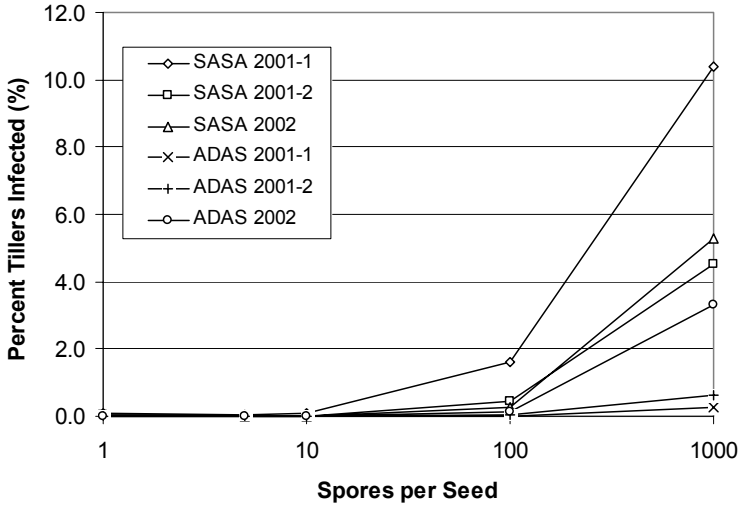


Figure 4.6. Mean observed percentage of tillers infected for Field trials at Gogarbank (Scotland) and Boxworth (England).

Table 4.3. Mean percentage of tillers infected for trials at Gogarbank, SASA.

Spores per Seed	SASA 2001-1	SASA 2001-2	SASA 2002
0	0.00	0.00	0.00
0.1	0.03	0.00	0.00
1	0.07	0.07	0.00
5	0.06	0.00	0.02
10	0.10	0.00	0.00
100	1.60	0.45	0.26
1000	10.40	4.54	5.27
Infection Probability:	11.0×10^{-5}	4.6×10^{-5}	5.4×10^{-5}

Table 4.4. Mean percentage of tillers infected for trials at Boxworth.

Spores per Seed	ADAS 2001-1	ADAS 2001-2	ADAS 2002
0	0.00	0.00	0.02
0.1	0.00	0.00	0.00
1	0.00	0.00	0.00
5	0.00	0.00	0.00
10	0.00	0.00	0.00
100	0.00	0.03	0.14
1000	0.27	0.61	3.33
Infection Probability:	2.7×10^{-6}	6.1×10^{-6}	3.4×10^{-5}

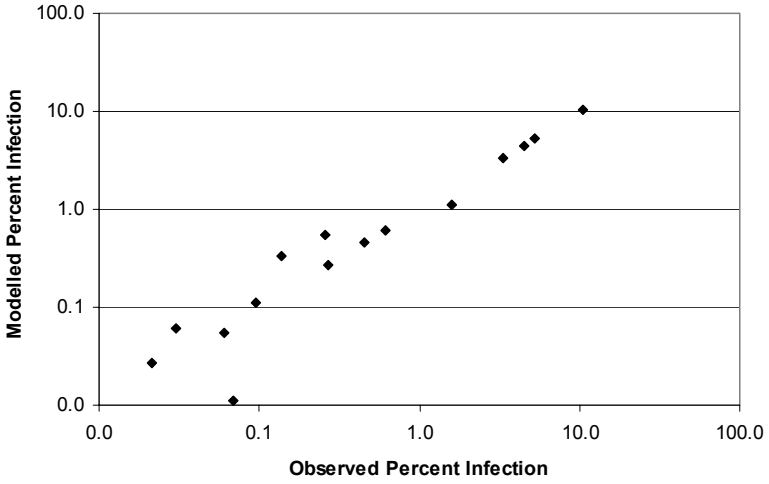


Figure 4.7. Observed and modelled percentage of tillers infected for spore loadings in the range 0 to 1,000 spores per seed for field trials at Boxworth and Gogarbank.

4.2.2 Effect of Variety and Site

Some varieties of wheat may be naturally resistance to bunt infection. To investigate this, five varieties of winter wheat were inoculated with 1,000 spores per seed at four sites in 1995 (SASA, 1996). The sites were Gogarbank, Bush Estates, Rosemaund and Manor Farm. The varieties were Riband, Hunter, Encore, Consort and Hereward. The wheat was sown both early (October) and late (November). The probability ***b*** of a single spore infecting a plant was calculated from the observed percentage infection of tillers at harvest (Table 4.5). The probability varied from 0 to 0.000187 with a mean value of 0.000064.

There was no significant effect ($P>0.05$) of sowing date on the probability of infection. There was evidence of a significant varietal effect with Hereward having significantly less ($P<0.05$) infection that the other varieties (Table 4.6). There was also evidence of a significant site effect with Gogarbank having a significantly higher ($P<0.05$) probability of infection than the other sites (Table 4.7)

Table 4.5. Summary of observed percentage tillers infected and calculated infection probabilities for all sites, varieties and sowing dates (SASA, 1996).

Variety	Site	Percentage Tillers Infected (%)	Probability of Infection (*b*)	Sowing Date	Sowing Class
Riband	Bush	4.2	4.3E-05	10/16/95	Early
Hunter	Bush	8.2	8.6E-05		Early
Encore	Bush	9.3	9.8E-05		Early
Consort	Bush	5.3	5.4E-05		Early
Hereward	Bush	0.4	4.0E-06		Early
Riband	Bush	0	0.0E+00	11/09/95	Late
Hunter	Bush	10.7	1.1E-04		Late
Encore	Bush	6.3	6.5E-05		Late
Consort	Bush	5.6	5.8E-05		Late
Hereward	Bush	0	0.0E+00		Late
Riband	Gogar	13.01	1.4E-04	10/28/95	Early
Hunter	Gogar	13.91	1.5E-04		Early
Encore	Gogar	17.09	1.9E-04		Early
Consort	Gogar	14.33	1.5E-04		Early
Hereward	Gogar	0	0.0E+00		Early
Riband	Gogar	12.26	1.3E-04	11/25/95	Late
Hunter	Gogar	13.28	1.4E-04		Late
Encore	Gogar	15.63	1.7E-04		Late
Consort	Gogar	12.98	1.4E-04		Late
Hereward	Gogar	0.52	5.2E-06		Late
Riband	Rosemaund	10	1.1E-04	10/11/95	Early
Hunter	Rosemaund	9.5	1.0E-04		Early
Encore	Rosemaund	15.1	1.6E-04		Early
Consort	Rosemaund	10.9	1.2E-04		Early
Hereward	Rosemaund	0	0.0E+00		Early
Riband	Rosemaund	0.7	7.0E-06	11/01/95	Late
Hunter	Rosemaund	0.4	4.0E-06		Late
Encore	Rosemaund	1.2	1.2E-05		Late
Consort	Rosemaund	0.7	7.0E-06		Late
Hereward	Rosemaund	0.2	2.0E-06		Late
Riband	Manor Farm	1.5	1.5E-05	10/03/95	Early
Hunter	Manor Farm	2.5	2.5E-05		Early
Encore	Manor Farm	3.3	3.4E-05		Early
Consort	Manor Farm	5.7	5.9E-05		Early
Hereward	Manor Farm	0	0.0E+00		Early
Riband	Manor Farm	4.4	4.5E-05	11/03/95	Late
Hunter	Manor Farm	3.5	3.6E-05		Late
Encore	Manor Farm	7.2	7.5E-05		Late
Consort	Manor Farm	2.7	2.7E-05		Late
Hereward	Manor Farm	0	0.0E+00		Late

Table 4.6. Calculated mean and range of infection probabilities for each variety (SASA, 1996)

Variety	Count	Probability of Infection (*b*)	
		Mean	Std Deviation
Riband	8	7.1×10^{-5}	5.6×10^{-5}
Hunter	8	8.2×10^{-5}	5.5×10^{-5}
Encore	8	10.1×10^{-5}	6.6×10^{-5}
Consort	8	7.7×10^{-5}	5.3×10^{-5}
Hereward	8	0.1×10^{-5}	0.2×10^{-5}

Table 4.7. Calculated mean and range of infection probabilities for each site (SASA, 1996)

Site	Count	Probability of Infection (*b*)	
		Mean	Std Deviation
Bush Estates	10	5.2×10^{-5}	4.1×10^{-5}
Gogarbank	10	12.2×10^{-5}	6.5×10^{-5}
Rosemaund	10	5.2×10^{-5}	6.2×10^{-5}
Manor Farm	10	3.2×10^{-5}	2.4×10^{-5}

4.3. Spore Release and Retention

The threshing of the grain at harvest releases a large number of spores from infected grain. The spores may be retained adhered to the grain that is returned to the store, or be dispersed and deposited over neighbouring fields. The proportion of bunt balls retained whole by the combine (***d***, Equation 3.8) and the proportion of spores retained (***c***, Equation 3.8) control the number of spores per grain returned to the store, and hence the disease multiplication potential due to infected seed. To investigate the retention of spores under this project, plots of infected wheat were combined and the numbers of spores per harvest grain and whole bunt balls returned to the store sampled.

4.3.1 Bunt Ball Retention

Wheat with known levels of spores per seed were sown in 2001 and 2002 at Boxworth and Gogarbank as part of this project. Spore loadings were in the range 0 to 1,000 per seed. In addition to percentage tiller infection, the number of spores per grain harvested and the weight of bunt balls in the harvest were measured.

For each spore loading, the observed percentage tiller infection was used to predict the expected number of bunt balls in the grain samples, assuming that all bunt balls were retained whole by the combine. This was compared with the observed number of retained bunt balls to calculate the parameter ***d*** that is the proportion of bunt balls retained whole by the combine. The observed number of bunt balls was calculated from the observed weight by assuming a mean bunt ball weight of 15.45 mg. The calculation was performed only for those trials with a non-zero weight of bunt balls in the grain sample. The numbers of bunt balls observed at Boxworth was less than at Gogarbank, presumably because of the lower probability of infection (Table 4.8).

Table 4.8. Summary of observed and expected numbers of bunt balls in samples taken from the harvested grain following spore loading trials at Boxworth and Gogarbank.

Site	Spores per Seed	Weight of Bunt Balls (g)	Weight of Grain Sample (g)	Percent Tiller Infection	Expected Bunt Ball Count	Observed Bunt Ball Count	Bunt Ball Retention
SASA 2001-1	1	0.011	120	0.1	1.8	0.7	0.40
	5	0.006	120	0.1	1.6	0.4	0.24
	10	0.008	120	0.1	2.5	0.5	0.22
	100	0.110	120	1.6	41.7	7.1	0.17
	1000	0.892	120	10.4	271.2	57.8	0.21
SASA 2001-2	100	0.039	120	0.5	11.7	2.5	0.22
	1000	0.245	120	4.5	118.4	15.9	0.13
SASA-2002	5	0.010	2446	0.0	0.6	11.3	0.06
	100	0.548	2379	0.3	35.4	134.4	0.26
	1000	5.460	2340	5.3	353.4	2678.5	0.13
ADAS 2001-2	1000	0.025	120	0.6	1.6	15.9	0.10
ADAS 2002	100	0.084	1990	0.1	5.4	60.1	0.09
	1000	1.271	1990	3.4	82.3	1466.6	0.06

Retention of bunt balls was calculated to be in the range 0.09 to 0.4 with a mean value of 0.18 (Table 4.8). The estimates of retention were not correlated with the spore loading, nor the observed weight of bunt balls ($P>0.05$). It was assumed that the mean retention ***d*** of bunt balls

is equal to 0.2 in all further calculations. It was further assumed in the calculations that all bunt balls that are not retained are cracked and release their spore contents.

4.3.2 Spore Retention

For each spore loading, the numbers of spores per grain harvested were also sampled. For each trial where the spore loading was 100 or 1,000 spores per seed, resulting in a significant infection percentage, the proportion ***c*** of spores released retained adhered to the grain by the combine was calculated, assuming ***d*** equal to 0.2, by rearrangement of Equation 3.8.

The calculated spore retention parameter was in the range 0.001 to 0.014 with a mean value of 0.006, indicating that less than 1% of spores released from bunt balls are retained adhered to harvested grain (Table 4.9).

Table 4.9. Summary of observed spores per harvested grain and calculated spore retention for trials at Boxworth and Gogarbank.

Site	Spores per Seed	Rep. 1	Rep. 2	Rep. 3	Mean Spores per Harvested Grain	Spore Retention
SASA 2001-1	100	332	155	170	219	0.002
	1000	966	1183	1262	1137	0.001
SASA 2001-2	100	195	59	155	136	0.004
	1000	1238	835	1074	1049	0.003
SASA 2002	100	171	164	16	117	0.006
	1000	439	1047	1396	961	0.002
ADAS 2001-1	100	1	0	0	0	-
	1000	50	37	2	30	0.001
ADAS 2001-2	100	20	25	59	35	0.014
	1000	678	849	450	659	0.013
ADAS 2002	100	15	45	239	100	0.009
	1000	2964	1217	1474	1885	0.007

Multiplication of ***c*** and *(1-**d**)* gives the combine retention efficiency at low levels of infection, i.e. the proportion of all spores in infected grain that is returned to the store. This was calculated to have a mean value of 0.005, indicating that a very high proportion of spores are released into the atmosphere, and potentially result in soil borne infection.

To establish an independent validation of the estimated ***c*** and ***d*** parameters, in a separate trial whole infected ears were placed in clean experimental plots prior to combining to provide an estimate of the proportion of spores released retained adhered to the grain. The experiment was carried out at SASA in 2002, and at ADAS in 2001 and 2002. The count of infected ears varied in the range 1 to 100 per 100,000 tillers. Three replicates were sampled for each count.

It had been proposed that the number of spores retained adhered to the grain can be calculated according to Equation 3.1 as:

$$S_p = p \cdot c \cdot k \cdot (1 - d) \quad (3.1)$$

where ***p*** is the proportion of plants infected, ***c*** is the proportion of spores released retained by the combine, ***k*** is the mean number of spores per bunt ball and ***d*** is the proportion of bunt balls retained whole by the combine. For this experiment, ***p*** was known from the number of infected ears and ***k*** was assumed to be 10×10^6. The experimental data were used to estimate the product of $c \cdot (1 - d)$ which is the retention efficiency of the combine process (Table 4.10).

Table 4.10. Summary of spores per seed on grain harvested from clean plots in which a known number of bunt infected ears had been placed.

Site and Year		Spores per Seed				Combine Efficiency
SASA 2002	Infected Ears	Rep.1	Rep. 2	Rep. 3	Mean	$c \cdot (1 - d)$
	0	0.5	0.08	0.33	0.3	Control
	1	0.58	0.17	1.41	0.7	0.0041
	10	3.31	3.4	2.74	3.2	0.0028
	100	58.09	56.68	149.25	88.0	0.0087
ADAS 2001	0	0.25	0	0	0.1	Control
	1	0.25	0	1.2	0.5	0.0040
	10	4.47	10.8	6.08	7.1	0.0070
	100	0.91	68.66	45.95	38.5	0.0038
ADAS 2002	0	1.49	3.15	0.91	1.9	-0.0127
	1	0.91	0.83	0	0.6	0.0151
	10	11.69	16.33	22.89	16.9	0.0078
	100	82.79	65.14	94.23	80.7	0.0038

Excluding the one observation when the number of spores per seed was less than for the control plot, the efficiency of the combine process was in the range 0.0028 to 0.0151. The mean value was 0.0070, indicating that less than 1% of spores in bunt balls were released and adhered to the grain to return to the store. This value compares very well with the independent estimate of 0.005.

In 2002, the number of whole bunt balls returned to the grain store were also estimated for the plots which had been prepared with infected wheat ears. Bunt balls were detected only in the grain samples from plots receiving 10 and 100 infected ears at ADAS.

Bunt balls accounted for 5×10^{-6} and 35×10^{-6} of the total weight of sampled grain. Taking account of the three-fold difference in mass of bunted and whole grain, this indicated that 10-15% of bunted grain were retained whole by the combining process. Therefore, the proportion of spores released that were retained by the combine ***c*** had a mean value of 0.008, again comparable to the previous estimate of 0.006.

4.4. Soil Borne Infection

Identification of the combine retention parameters (Section 4.3) has demonstrated that a large proportion of bunt spores are released to the atmosphere and may be re-deposited on fields due to be drilled. The number of spores released depends on the number of grains per shoot (***g***, Equation 3.9) and the number of surviving shoots per unit area of field (***t***, Equation 3.9). The significance of the soil borne load depends upon their survival and the likelihood of a soil borne spore making contact with a seed or the coleoptile (***w***, Equation 3.3).

To quantify the risk of infection due to spore dispersal, ADAS and SASA carried out plot trials to establish the mass of soil borne spores necessary to result in infection under worse case conditions (ADAS, 2002). Seed was sown at a depth of 5 cm in plots which received a bunt spore loading of 0.00005 to 1 g m^{-2} mixed with fine sand. Infection was measured by counting infected tillers at harvest. The experiment was carried out at ADAS Boxworth. A parallel experiment investigated the effect of seed loadings on percent infection. At a spore loading of 1,000 spores per seed, the resulting infection was in the range 0.4 to 1.3% with a mean value of 0.9%. The estimated mean probability of infection ***b*** was 0.9×10^{-5}.

Observed infection increased with the loading of soil borne spores (Table 4.11). There was some evidence of cross contamination between plots, as an infection rate of 3.3% was observed for a plot receiving no inoculum. Assuming a constant mean probability of infection ***b*** of 0.9×10^{-5} the effective number of spores per seed *m'* was calculated (Figure 4.8). Simple linear regression was used to calculate that 1 g m^{-2} of spores spread to the soil on the day of drilling was equivalent to *c*. 20,000 spores per seed.

An inoculum rate of 1 g m^{-2} represents 760×10^{6} spores m^{-2} (Section 4.1). Seed was spread at a rate of 450 m^{-2}. Assuming the dimensions of a plump milling wheat grain are 3 mm by 8 mm, the surface area of spore capture is 0.28 cm^2 per seed. This equates to 21,300 spores per seed that is remarkably similar to the estimated equivalent.

Further investigation of the soil borne infection potential considered spores released by the combine and deposited naturally on the soil surface rather than manually mixed with the topsoil. At ADAS Boxworth in 2001, an infected plot was combined allowing spores to be released and deposited within the field downwind. Spore traps set a fixed distances downwind of the crop measured the deposition of spores. Trap crops were sown the day after harvesting at fixed distances from the source crop. When the trap crops were harvested, the numbers of spores per harvested grain were calculated.

From the seed inoculum trials at Boxworth in 2001, the mean probability of infection *b* was estimated as 4.4×10^{-6}. Combined with the spore trap loadings and the effective weight of 1 g m^{-2} of soil borne spores of 20,000 the expected mean percent tiller infection was calculated. From this, by application of Equation 3.8 and assuming *c* equal to 0.006 and *d* equal to 0.2, the spores per harvested grain were estimated for each trap crop at distances from the source crop.

Table 4.11. Summary of percentage tiller infection resulting from soil inoculum (ADAS, 2002).

Spore Inoculum ($g\ m^2$)	Percentage of Tillers Infected (%)
0	0.0
0	0.0
0	3.3
0.00005	2.5
0.00005	0.0
0.00005	0.0
0.0005	0.0
0.0005	1.6
0.0005	1.0
0.005	1.0
0.005	0.0
0.005	0.5
0.05	3.2
0.05	2.2
0.05	5.4
0.5	19.5
0.5	16.8
0.5	2.7
1	12.9
1	9.4
1	19.6

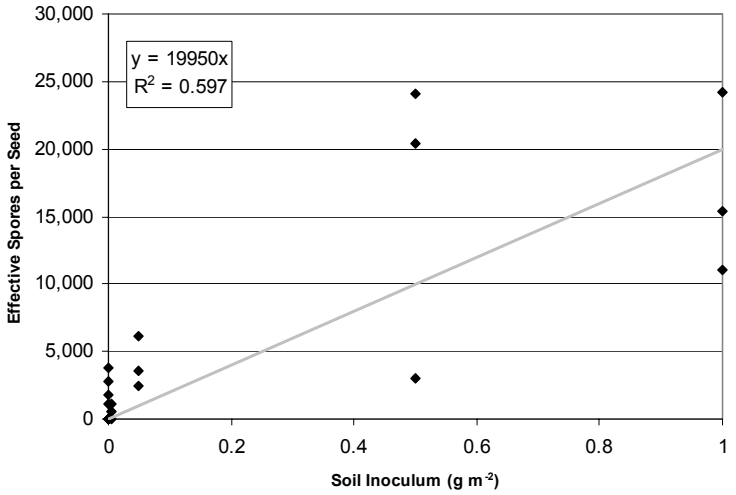


Figure 4.8. Relationship between mass of spores used to inoculate soil and effective number of spores per seed that results in the same level of infection observed (ADAS, 2002).

The model predictions and observed levels of plant infection were very low, with less than 0.1% of tillers infected. However, these low levels of infection still resulted in positive counts of spores per harvest grain that were comparable with the observations (Table 4.12). Although it was necessary to combine several model parameters in making these predictions, varying parameters ***c*** and ***d*** in the observed ranges caused the peak spores per harvest grain at 1m from the source crop to vary from only 1.4 to 28.5 spores per grain. Sensitivity analyses demonstrated that the seed borne equivalent of 1 g m^{-2} soil borne spores was required to be at least 2,000 to reproduce the observed integer counts of spores per harvest grain. This experiment, together with the previously described soil inoculum trial, was strong evidence that under worst case conditions of an immediately following crop, spore dispersal presents a significant risk.

Table 4.12. Summary of mean soil spore loadings resulting from spore dispersal trial and modelled and observed spores per harvest grain in the following crop.

	Deposited Spores per m^2		Model Predictions		Observations
Distance from Infected Crop (m)	Average	Std.Dev	Percent Tillers Infected	Spores per Harvest Grain	Spores per Harvest Grain
1	1,952,820	782,082	0.023	10.9	1.7
2	1,287,034	665,596	0.015	7.2	1.0
4	401,984	210,338	0.005	2.2	28.7
8	262,888	171,745	0.003	1.5	1.2
16	86,404	96,487	0.001	0.5	0.5
64	15,189	20,539	0.000	0.1	1.3
256	493	862	0.000	0.0	0.7
1024	473	801	0.000	0.0	0.0

4.5. Efficacy of Seed Treatment

It was proposed that the effect of seed treatments in the infection model can be represented by an effective spore loading that is the product of the treatment efficacy E and the true spore loading ***m*** according to Equation 3.4:

$$m' = m \cdot \left(1 - \frac{E}{100}\right) \qquad (2.4.)$$

The infection response to seed treatment at a spore loading of 1,000 spores per seed was investigated at SASA in 1994. A wheat crop sown on 25th November, was variously treated with full and half rates of Panoctine, Beret, Baytan and Sibutol. The data from this experiment were used to define the expected efficacy of the available seed treatments. The percentage of infected tillers was measured for four replicates for each of the available seed treatments and for one untreated control (Table 4.13). With the exception of the half-rate Panoctine treatment, no infected tillers were detected in the plots. This indicated a high, but unquantifiable efficacy. However, positive counts of spores adhered to the harvested grain indicated that there were low levels of infection in the treated plots (Table 4.14).

Table 4.13. Percentage of tillers infected by bunt following various seed treatments, for a spore loading of 1,000 spores per seed (SASA, 1995)

Treatment	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean
Control	2.7	1.6	1.4	10.8	3.5
Beret	0.0	0.0	0.0	0.0	0.0
Beret Half-Rate	0.0	0.0	0.0	0.0	0.0
Baytan	0.0	0.0	0.0	0.0	0.0
Baytan Half-Rate	0.0	0.0	0.0	0.0	0.0
Panoctine	0.0	0.0	0.0	0.0	0.0
Panoctine Half-Rate	0.5	0.0	0.0	1.6	0.5
Sibutol	0.0	0.0	0.0	0.0	0.0
Sibutol Half-Rate	0.0	0.0	0.0	0.0	0.0

Table 4.14. Count of bunt spores per harvested grain following various seed treatments (SASA, 1995).

Treatment	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean	Effective Spore Loading
Control	2,069.5	352.2	795.6	1,385.9	1,150.8	1,000.00
Beret	0.2	0.3	0.1	0.0	0.2	0.05
Beret Half-Rate	0.2	0.4	0.8	0.3	0.4	0.39
Baytan	0.9	3.7	0.6	0.1	1.3	0.44
Baytan Half-Rate	0.1	0.1	0.4	0.1	0.2	0.10
Panoctine	169.5	1.2	0.5	0.1	42.8	18.90
Panoctine Half-Rate	148.0	1.0	3.7	343.1	124.0	177.47
Sibutol	0.1	0.3	0.0	0.0	0.1	0.03
Sibutol Half-Rate	0.1	0.2	0.5	0.3	0.3	0.17

The spore count data were used to estimate the efficacy of the seed treatments. Firstly, for the control plot, the retention efficiency of the combine process was calculated, assuming 10×10^6 spores per bunt ball. The mean retention efficiency was 0.0017 and was within a factor 3 of the efficiency measured at ADAS and SASA in 2001 and 2002 (Section 4.3). It is possible that the difference is an effect of different combine machinery and weather conditions on the harvest day. Re-arrangement of the infection model then allowed calculation of the effective number of spores per seed for each of the treatments, and hence calculation of the efficacy of the treatments according to Equation 4.2:

$$m' = \frac{\log_e\left(1 - \frac{S_p}{c \cdot k \cdot (1-d)}\right)}{-b} \quad (4.2)$$

where S_p is the observed spores per harvested grain. The probability $\boldsymbol{b}$ of a single spore infecting a plant was calculated from the control replicates to be in the range 1.4×10^{-5} to 11.4×10^{-5} with a mean value of 3.6×10^{-5}. With the exception of the Panoctine treatments, the efficacy was greater than 99.9% (Table 4.15).

Table 4.15. Estimated seed treatment efficacy, calculated by comparison of relative numbers of spores adhered to harvested grain for the treated and control plots (SASA, 1995).

Treatment	Efficacy
Baytan	0.99956
Baytan Half-Rate	0.99990
Beret	0.99995
Beret Half-Rate	0.99979
Panoctine	0.98110
Panoctine Half-Rate	0.82253
Sibutol	0.99997
Sibutol Half-Rate	0.99983

5. Seed Treatment Threshold

According to the HGCA Cereal Disease Survey, 98% of certified and 92% of farm saved seed are treated with fungicides (HGCA, 1995). The objective of this project was the identification of a threshold for *treatment according to need*. The threshold should result in a continued downward pressure on infection levels, and minimise economic losses resulting from any reduction in grain quality.

5.1. Model Based Multiplication Threshold

Iterative, year on year, application of the infection model described by Equations 3.1 to 3.9 with the derived mean parameter values (Section 4), allows calculation of levels of disease in a harvested crop and the numbers of bunt spores on grain that may be used for seed. Application of the model to a sequence of following crops in a single field is mathematically trivial. However, it requires information on the time delay between harvest and sowing, and a measure of the likelihood of soil borne spores dying in this period and not being effective against the following crop. The field trials carried out under this project have established that soil borne spores represent a high risk to the following crop if the time delay is equal to 1 day. No data have been generated on how this risk decays with increasing delay. It was anticipated early in this project that this might be related to the frequency of rainfall and the wetting of the soil, causing the spores to germinate before the following crop is sown, which would vary geographically. To represent this, an exponential decay was introduced that reduced the inherited soil borne spore load as a function of time since harvest. The rate of decay $\boldsymbol{h}$ was a parameter that required calibration.

Equation 3.3 was modified to give the mean number of effective spores per seed as a sum of the seed borne count and a time weighted density of spores in the soil, according to Equation 5.1:

$$m = m_g + e^{-h \cdot t} \cdot w \cdot m_s \quad (5.1.)$$

Where $\boldsymbol{m_g}$ is the mean number of spores per seed, $\boldsymbol{m_s}$ is the density of spores in the soil, $\boldsymbol{w}$ measures the likelihood of a soil borne spore making contact with a seed or the coleoptile, $\boldsymbol{t}$ is the time delay between harvest and sowing, and $\boldsymbol{h}$ is a rate parameter.

To further assess the risk of an epidemic of bunt at the regional or national scale, due to local treatment decisions, required the construction of a model framework that described the exchange of potentially contaminated seed from a large number of fields, growing wheat for

ware and seed, from certified and home save bulks. This was achieved via a stochastic framework in which a population of fields were randomly assigned to seed and ware producing farms based on sector information. Infection in the harvested crop is calculated for each field at the end of each year and local decisions on whether to treat grain intended for seed are made by comparing infection levels against a threshold.

5.1.1. Model Calculation Stages

Model Initialisation

Each field in the stochastic framework is represented by two stores that hold counts of mean number of spores per seed. The first **bulk** store holds the count of spores adhered to the seed to be sown. The count is equivalent to the spores per seed adhered to the grain from the previous harvest. The second **soil** store holds the additional effective count of spores per seed due to spores in the soil that may infect the seed as effectively as the spores in the bulk. The soil borne spores are due to the release and deposition of spores by the harvest of a previously infected crop in the field (Figure 5.1).

The total number of fields is divided into those producing **seed** and those producing **ware**. The ware fields are further sub-divided into those grown from **certified** seed and those grown from **home saved** seed.

The relative numbers of each field type were taken from sector data. For 1,000 fields, the default number of **seed** fields is 30, the number of **certified ware** fields is 650 and the number of **home saved ware** fields is 320.

The number of spores per seed in the **bulk** store for each **seed** field is initialised to one at the beginning of a simulation. All other stores are initialised to zero.

Ware Promotion

At the beginning of each year in the model simulation, the counts of spores per seed in the **certified ware bulk** stores are copied into the **home saved bulk** stores to represent the sowing of home saved seed (Figure 5.1). This is a deterministic process.

Seed Promotion

To represent the purchase and sowing of certified seed, the numbers of spores per seed in the **seed bulk** stores are copied into the **certified ware bulk** stores (Figure 5.1). This is a deterministic process.

Seed Treatment

For each **seed bulk** store, treatment is applied and the count of number of spores per seed reduced in proportion to the efficacy of the treatment. The assumption is that commercially sown seed crops will always be treated.

If a **seed bulk** store count is greater than 100 spores per seed, then it is replaced rather than treated. It is replaced with the lowest value of spores per seed in any of the **seed bulk** stores (Figure 5.2).

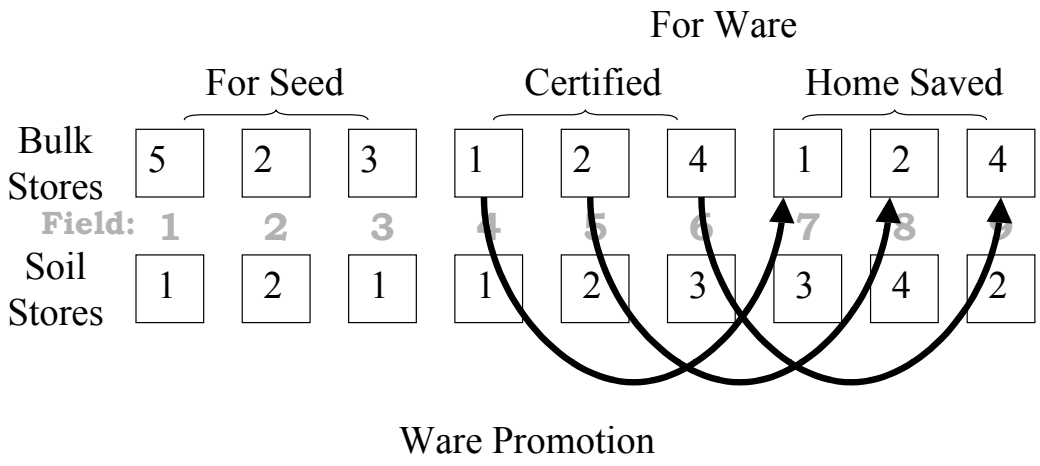


Figure 5.1. Schematic of bulk and soil stores for each field, holding counts of effective spores per seed, and showing the process of copying certified ware bulk counts into the home saved bulk counts to represent home saving of seed from the previous harvest.

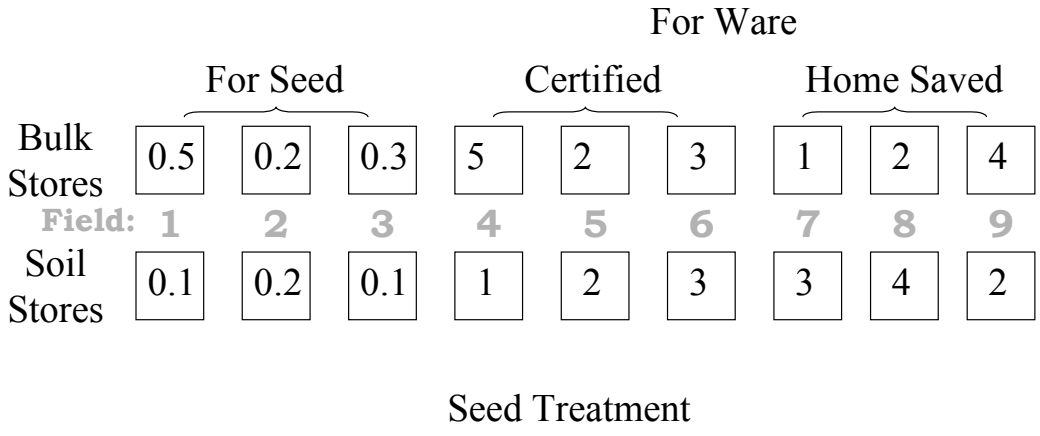


Figure 5.2. Schematic of bulk and soil stores for each field, following application of treatment to each bulk intended for producing seed. In this schematic, the treatment has an efficacy of 90% and is equally effective against soil spores.

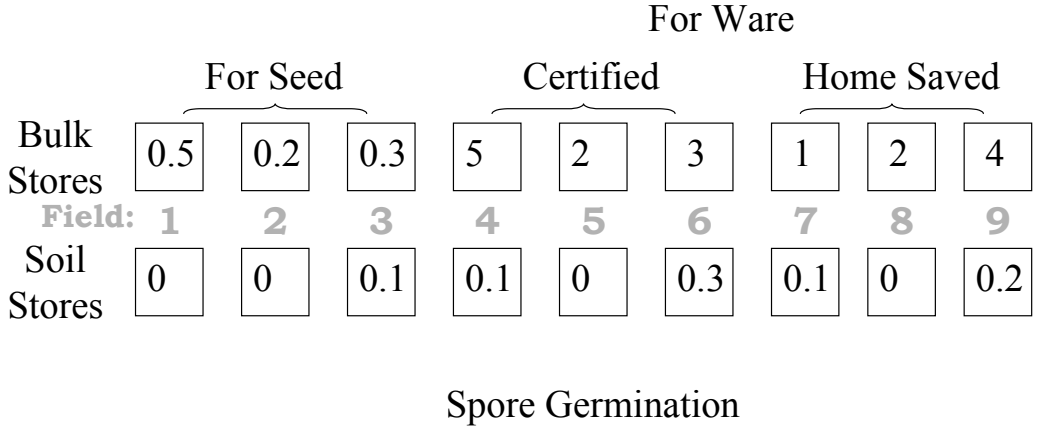


Figure 5.3. Schematic of bulk and soil stores for each field, following reduction of soil store counts to represent germination of spores prior to sowing of following crop.

For each **certified ware bulk** store, the count of spores per seed is compared against a seed treatment threshold. If the count is greater than the threshold, then treatment is applied and the count reduced in proportion to the efficacy of the treatment. A random 1% of the bulks requiring treatment are not treated to represent the small number of bulks that would not be tested. This assumption can be changed in the model.

For each **home saved ware bulk** store, the count of spores per seed is compared against a seed treatment threshold. If the count is greater than the threshold, then treatment is applied and the count reduced in proportion to the efficacy of the treatment. A random 10% of the bulks requiring treatment are not treated. This assumption can be changed in the model.

If the treatment applied to any bulk is also effective against soil borne spores, then the count of spores per seed in the **soil** store for a field is also reduced in proportion to the efficacy of the treatment. By default, a random 75% of current treatments are effective against soil borne spores, based on current usage lists. This assumption can be changed in the model.

By default, the efficacy of any seed treatment is 99.9%. The efficacy of any treatment against soil borne spores is fixed at 99%. These assumptions can be changed in the model.

Soil Spore Germination

The effectiveness of the soil borne spores is reduced by spore germination prior to the sowing of the following crop. In the absence of a host, the spores die to no effect. The proportion of spores that have not germinated by the time of sowing is modelled using the exponential function described above (Section 5.1). The rate constant $\boldsymbol{h}$ has a default value of 0.1, so that only 5% of spores remain viable after 30 days. The time delay $\boldsymbol{t}$ for each **soil** store is randomly sampled from a statistical distribution of observed delays for continuous winter wheat cropping within Nitrate Sensitive Areas, recorded by ADAS. The count of spores per seed in each store is multiplied by the calculated proportion of effective spores (Figure 5.3).

Plant Infection

The proportion of plants infected in each field is calculated by application of Equation 3.6 where the mean number of effective spores per seed $\boldsymbol{m'}$ is calculated as the sum of the **bulk** and **soil** store counts. By default, the probability of infection $\boldsymbol{b}$ is 5×10^{-5} and may vary in the range 10^{-4} to 10^{-6} under UK conditions. This assumption can be changed in the model.

Harvested Grain

The mean number of spores per harvested grain from each field is calculated by application of Equation 3.8 where the mean number of spores per bunt ball $\boldsymbol{k}$ is 10^7 and the proportion $\boldsymbol{d}$ of bunt balls that are not ruptured by the threshing process is 0.2. The proportion $\boldsymbol{c}$ of released spores adhered to the healthy grain is 0.005. These assumptions can be changed in the model.

The count of spores per harvest grain are used to replace the **bulk** store counts for each field.

Soil Deposition

The mean number of spores released and deposited per unit area within each field is calculated by application of Equation 3.9 where the standard number of grains $\boldsymbol{g}$ on a wheat ear is 36 and each has a mean mass of 46 mg at 85% dry matter. The number of surviving tillers $\boldsymbol{t}$ at harvest is 600 m^{-2} (HGCA, 1997). The mean number is converted to a mean mass of spores deposited per unit area, assuming that 750×10^6 spores have a mass of 1 g.

If the following crop were to be sown immediately after harvest, field experiments have shown that the soil borne spores would result in infection comparable to seed borne spores. The number of spores per seed resulting in an equivalent level of infection is calculated by multiplication of the spore mass by an empirical scalar of 20,000 (Section 4.1).

The **soil** store counts are replaced by the equivalent number of spores per seed for each field.

Landscape Factor

A proportion of the spores released during harvest will deposit on non-agricultural land or non-wheat crops, reducing the pressure due to the soil borne phase. To represent this, the **soil** store counts are each multiplied by a scalar ***a*** equal to the proportion of the landscape under wheat cropping. By default, the value of ***a*** is equal to 0.2, based upon agricultural statistics for the Anglian region. This assumption can be changed in the model.

Randomise Soil Stores

The **soil** stores represent the potential carry-over effect of the crop previously harvested from each field. There is no reason to believe that any one field will be sown for seed or ware *ad infinitum*. Grain sown for seed may inherit the soil borne spore pressure due to a previous crop sown for ware from certified or home saved seed. To represent this, the contents of the **soil** stores are randomly shuffled.

Reporting

The model records the levels of infection and counts of spores per seed for each **bulk** and **seed** store. The proportion of seed that has to be discarded is also recorded. The proportions of the ware crop that are treated and rejected by millers (due to a spore count threshold) are also recorded.

Iteration

The model iterates over the previous steps, each iteration representing a crop year. Due to the stochastic nature of the model, it may require a number of years before the results show a stable equilibrium.

5.1.2. Model Application

The stochastic framework for regional scale disease simulation was developed as a computer program using Microsoft Visual Basic 6, which provided a simple user interface for changing the model parameters and viewing time series of model results (Figure 5.4).

The software was used to generate time series of the spore loading and levels of bunt infection in seed and ware crops (Figure 5.5). The parameters of the model controlling the threshold for seed treatment and the rate of decay ***h*** of the effectiveness of soil borne spores were repeatedly adjusted in an attempt to reproduce the levels of infection that have been reported in recent surveys (Cockerell and Rennie, 1996). It was found that the output from the model was extremely sensitive to the soil borne decay rate parameter. Disease levels would rapidly multiply to catastrophic levels or decline to zero within a small parameter range. It was not possible to simulate the observed continuous low levels of bunt infection in seed bulks without extreme fine adjustments of the rate parameter. The extreme sensitivity, and the unsupported assumption that the decay was exponential, led to the conclusion that the model could not safely be used to define a threshold for seed treatment.

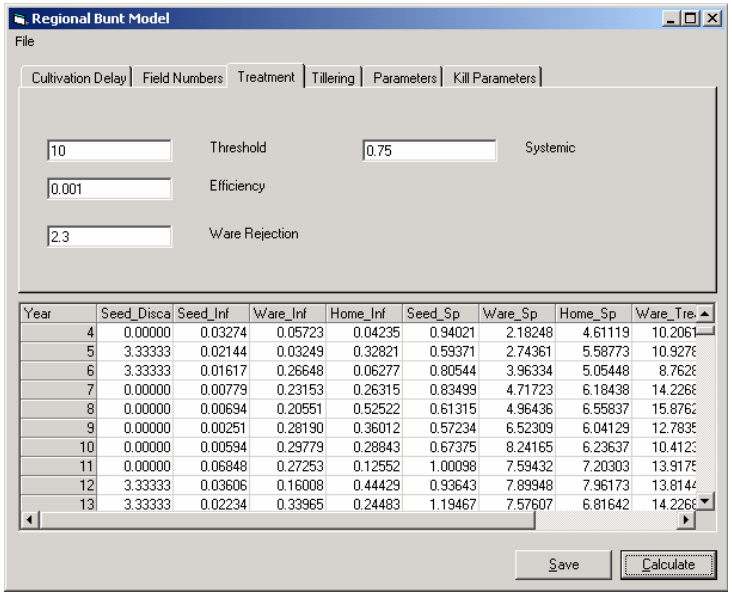


Figure 5.4. Interface to software developed for running the regional scale model of bunt infection and response to changing seed treatment thresholds.

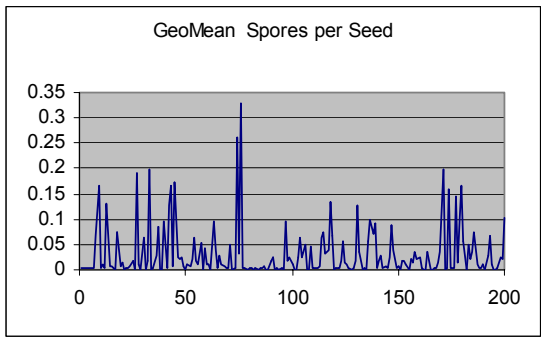
5.2. Data Based Multiplication Threshold

A treatment threshold is required that results in a continuous downward pressure on infection levels. Bunt spore infection probabilities in the range 1 to10×10^{-5} have been estimated from the field experimental data. If seed borne spores were the only source of infection, then the calculated annual multiplication potential would be in the range 0.8-8 with a mean value of 4, giving rise to a 100-fold increase of disease levels within 4 years if untreated. The multiplication potential is sensitive to winter wheat variety and weather conditions, and is lower than some that have been reported. Borgen *et al*. (1991), for example, reported a 100-fold increase in a single year at field sites in Denmark.

If seed borne spores were the only source of infection, and given current seed treatment efficiencies of *c*. 99%, treatments when applied would always result in a downward pressure on the disease. Therefore, the threshold for seed treatment could be defined simply in terms of the acceptable level of infection for quality and price. In years between treatments, the mean level of infection would rise to the treatment threshold. The mean interval between treatments will be dictated by the ratio of the treatment efficiency and the annual multiplication potential. For a spore infection probability of 10×10^{-5}, the interval would be 2 years, giving a 66% saving of treatment costs.

It has, however, been demonstrated that soil borne spores, from the dispersal and deposition of spores released at harvest, can result in high levels of infection if the delay between harvest and sowing is short (see, for example, Yarham and McKeown, 1989; Weston, 1932). Under the worst-case scenario of immediate sowing and local re-deposition of spores, experimental data from this project suggest an annual multiplication potential of 45-450, in proportion to the spore infection probability. However, it is known that historic use of seed dressings that were not effective against soil borne spores still resulted in a downward pressure on the disease. Figure 5.6 graphs the percentage incidence of macroscopic portions of bunt balls in wheat samples analysed by the Cambridge Official Seed Testing Station (OSTS) (Marshall, 1960). The percentage declines from a peak of 33% in 1921 to only 0.2% in 1957 following the widespread adoption of organo-mercury seed dressings. Marshall (1960) reported that of 122 samples examined microscopically for bunt spores in 1957, none showed the presence of

(a)



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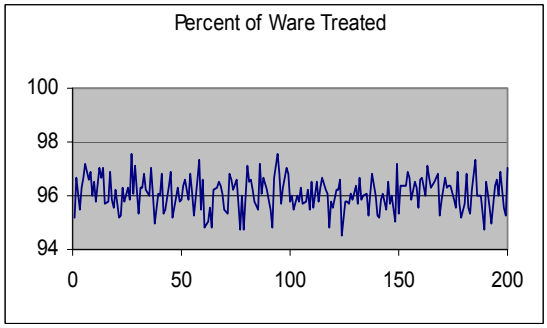

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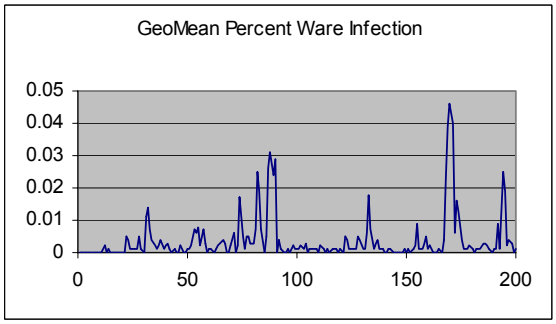

(d)

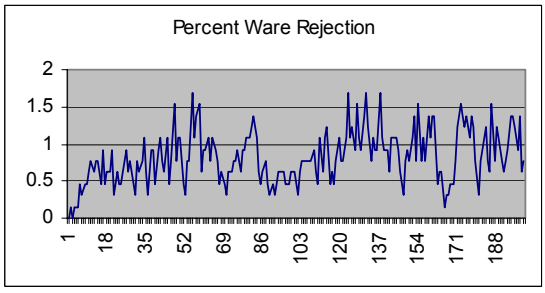

Figure 5.5. Sample output from the regional scale model of bunt disease and seed treatment, showing the a) mean seed quality, b) percent of ware seed treated, c) percent of the ware crop spores. Only 18% of samples were recorded as free of bunt spores

in the 1920s (Eastham, 1927). More recently, Cockerell and Rennie (1996) recorded only one broken bunt ball in 1,000 samples of winter wheat seed from certified and farm saved seed bulks in England and Scotland. However, only 60% of samples were free of bunt spores. infected and d) the percent of harvest rejected by processors. The results are for a continuous simulation of 200 years.

The observed decline could not have taken place if the average soil borne spore multiplication potential were not less than one. This could occur if a proportion of the spores released at harvest were deposited on break-crops or non-agricultural land, and if a large proportion of the soil spores died, failed to germinate or germinated and then died before sowing of the following crop. The fact that disease levels declined and were maintained at low levels in the early 1990s, despite the increase in the area of wheat and the proportion that was winter sown (Figure 5.7), is strong evidence for an effective soil borne spore multiplication potential of less than one when averaged across the farmed area and over a number seasons. Therefore, it is tentatively concluded that bunt can be effectively controlled at a national scale by seed treatment only, and the significance of the soil borne spores will be further reduced by the use of systemic fungicide treatments. It is estimated that only 25% of seed dressings were effective against soil borne spores in 1995, compared to 75% in 2001 (DEFRA Cereal Disease Survey).

In conclusion, a treatment threshold is necessary only to maintain the quality of the harvest grain, and not to prevent a soil borne epidemic. However, it is recommended to take account of the worst-case scenario of local spore deposition and immediate sowing by setting a treatment threshold that ensures the quality of the second or third wheat crops.

5.3. Quality Threshold

The quality threshold for wheat grain is dependent upon end use. The quality issues are odour and colour imparted to the flour. In a Swedish experiment, 50% of persons could smell the presence of 1,000 spores per gram of seeds (Johnsson, 1991), equivalent to 50 spores per seed. Although the odour would decrease in storage, this would most likely result in rejection by millers. Shine (*pers. comm*.) reported that Rank Hovis requires milling wheat to be *free of bunt*. A single bunt ball in a test sample will result in rejection because there is the probability (near certainty) that other bunt balls will have broken, releasing spores over the healthy grain.

Assuming a sample size of *c*. 1 kg for a trailer and a standard grain size of 46 mg at 85% dry matter (21,740 grains per kg), the probability of detecting at least one bunt ball in the sample can be calculated using the normal approximation (Wonnacott and Wonnacott, 1977) according to Equation 5.2 as:

$$\Pr(P > \frac{1}{21{,}740}) = \Pr\left(\frac{P-\pi}{\sqrt{\frac{\pi(1-\pi)}{n}}} \geq \frac{\frac{1}{21{,}740} - N}{\sqrt{\frac{N(1-N)}{21{,}740}}} \right) = \Pr\left(Z \geq \frac{\frac{1}{21{,}740} - N}{\sqrt{\frac{N(1-N)}{21{,}740}}} \right) \quad (5.2)$$

Where $\boldsymbol{N}$ is the proportion of grain that is bunted and $\boldsymbol{Z}$ is the standard normal cumulative distribution. This calculation assumes that the grain are perfectly mixed in the trailer. For a 95% probability of detecting at least one bunt ball in the sample, at least 0.02% of the trailer grain must be bunted. Application of the deterministic infection model (Section 3.2) allows calculation of the threshold mean number of spores per seed that will result in this level of contamination in the harvested grain, in the absence of seed treatment. For spore infection

probabilities $\boldsymbol{b}$ in the range $1\text{-}10\times10^{-5}$, the calculated threshold varies in the range 10-100 spores per seed (Figure 5.8).

An empirical threshold can be calculated from current treatment practice. Current advice for seed treatment given by the OSTS is to apply a fungicide dressing if the number of spores per seed grain is greater than one (Cockerell, *pers. comm*.). Bunt spore infection probabilities in the range $1\text{-}10\times10^{-5}$ have been estimated from the field experimental data, with the largest values for sites in Scotland. Application of the infection model at the threshold for treatment results in 0.01-0.001% of tillers infected and 0.8-8.0 spores adhered to harvested grain. This is the empirical threshold for acceptable grain quality based on current practice, i.e. we should expect to find these levels of spores per seed and associated counts of bunt balls grain for ware. We can assume that this level of contamination is acceptable as there has been no general concern from millers concerning bunt. This assumption is supported by the fact that the empirical threshold is lower (more stringent) by a factor of ten than the theoretical threshold calculated from the likelihood of finding a bunt ball in a sample from a grain trailer. This might be expected, as it is the consequence of a risk adverse approach to selecting a treatment threshold, taking account of the uncertainties in sampling seed bulks to determine the need for treatment.

Thus far we have concerned ourselves only with the quality of the grain from crops knowingly sown untreated from contaminated seed. To minimise the risk due to soil borne spores, it is also necessary to set a treatment threshold that prevents a following crop from reaching infection levels of 0.01-0.001% tiller infection. The following crop may be sown in a neighbouring or same field as the crop grown from contaminated seed and be infected by spores released during harvest. Under the worst case situation of immediate sowing after harvest, assuming no treatment of the seed effective against soil borne spores, and assuming a quality threshold of 0.01% tiller infection, and that all spores are deposited locally, a new seed treatment threshold was calculated to be in the range 0.002-0.2 spores per seed and was inversely related to the spore infection probability (Figure 5.9). The threshold is 10-100 fold less than the current advisory threshold for seed treatment.

However, in practice, the risk from soil-borne bunt is reduced by: (i) death of spores between harvest and drilling of the following crop, (ii) loss of spores by deposition on non-wheat land, (iii) infection probabilities which are lower than the worst recorded case, and (iv) widespread use of seed treatments effective against the soil-borne phase.

The need for the last of these could be better estimated if the rate of decline of viable spores in soil was quantified under UK conditions.

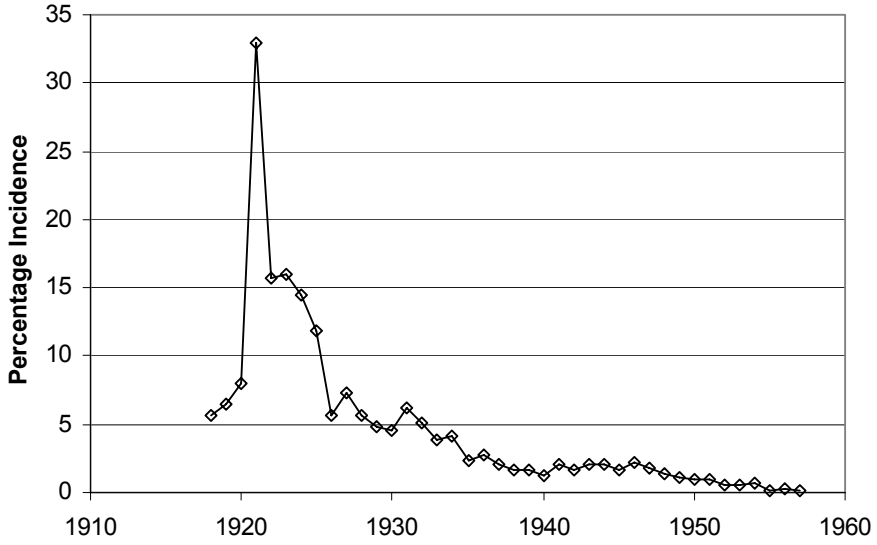

Figure 5.6. Percentage incidence of macroscopic portions of bunt balls in winter wheat samples analysed by the Cambridge OSTS, 1918-1957 (Marshall, 1960).

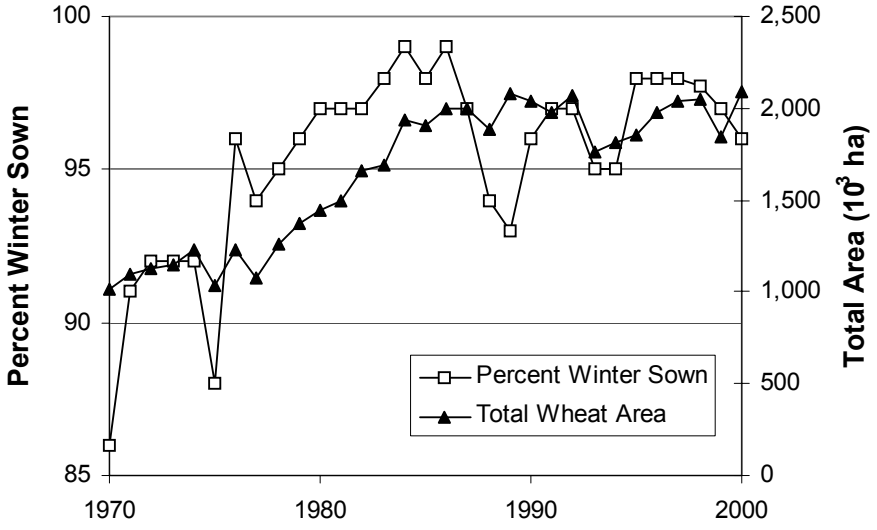

Figure 5.7. Total area of wheat and the percentage winter sown in the United Kingdom, 1970-2000 (DEFRA, 2002).

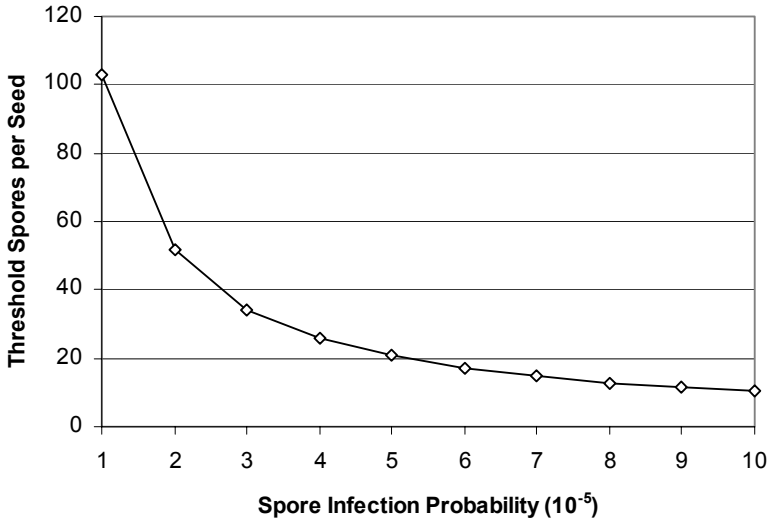

Figure 5.8. Calculated seed threshold treatment level, as a function of spore infection probability, to minimise the risk of rejection of harvested grain for milling.

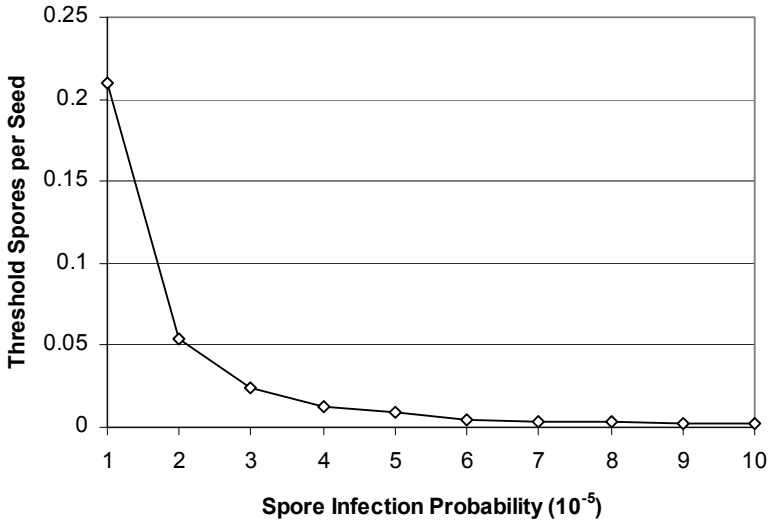

Figure 5.9. Calculated seed threshold treatment level, as a function of spore infection probability, to minimise the risk of poor grain quality in the following crop.

6. References

Anonymous (2002) Seed Treatments – Efficacy and Physical / Mechanical Data Requirements. Efficacy Guideline No. 208, pp.7, Pesticides Safety Directorate.

Arafa, M. A. (1981) Studies on factors affecting infection with bunt diseases, grain yield and some agronomic characteristics. *Agricultural Research Review*, **59** (2), pp. 97-111.

Benada, J., J. Milotov, og A. Pospíšil 1995: OdrÁdová citlivost pšenice vÁÖi snçti mazlavé a jeÖmene vÁÖi pruhovitosti a prašné snçti. (Varietal sensitivity to common bunt in wheat and to stripe disease and loose smut in barley). *Rostinná Výroba (Plant production)*. **41**(4):185-188.

Borgen, A. and Davanlou, M. (2001) Biological Control of Common Bunt (*Tilletia tritici*) in Organic Agriculture. *Journal of Crop Production* **3** (5), pp.159-174.

Cockerell, V. (2001) Personal Communication.

Eastham, A. (1927) Official seed testing station for England and Wales: ninth annual report (1925-26). *Journal of the National Insitute of Agricultural and Botany*, **1**, 6, pp. 78.

Gaudet, D. A., Puchalski, B. J., and Entz, T. (1989) Effect of environment on efficacy of seed-treatment fungicides for control of common bunt in spring and winter wheat. *Pestic. Sci*., **26**, pp. 241-252.

Heald, F.D. (1921) The relation of spore load to the per cent of stinking smut appearing in the crop. *Phytopathology* **11**:269-278.

HGCA (1998) The Wheat Growth Guide – to Improve Husbandry Decisions. HGCA, London.

Hungerford, C. W. (1922) The relation of soil moisture and soil temperature to bunt infection in wheat. *Phytopathology*, **12**, pp. 337-352.

Johnsson, L. (1991) Survey of common bunt (*Tilletia caries* (DC) Tul.) in winter wheat during the period 1967-1987 in Sweden. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, **98** (1), pp. 67-72.

Johnsson, L. (1992) Climate factors influencing attack of common bunt (*Tilletia caries* (DC) Tul.) in winter wheat in 1940-1988 in Sweden. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, **99** (1), pp. 21-28.

Jones, G. H., and Seif-el-nasr, A. G. (1940) The influence of sowing depth and moisture on smut diseases, and the prospects of a new method of control. *Annals of Applied Biology*, **1**, pp. 35-57.

Kendrick, E. L., and Purdy, L. H. (1959) Influence of environmental factors on the development of wheat bunt in the Pacific north-west. II. Effect of soil temperature and soil moisture on infection by seed-borne spores. *Phytopathology*, **49**, pp. 433-434.

Marshall, G. M. (1960) The incidence of certain seed-borne diseases in commercial seed samples. *Annals of Applied Biology*, **48** (1), pp. 34-38.

Oxley, S. J. P. and Cockrell, V. (1996) Transmission of Bunt (*Tilletia caries*) in winter wheat with reference to seed borne inoculum, site and climate. In *Proceedings Crop Protection in Northern Britain*, pp. 97-102.

Pirson, H. (1978) A simple method for the detection of Bunt and Covered Smut spores as contaminants on cereal seeds. *In Report on the Sixteenth International Workshop on Seed Pathology*, ISTA-PDC, Karlsruhe, pp. 54-57.

Polisenka, I., Pospisil, A. and Benada, J. (1998) Effects of sowing date on common Bunt (*Tilletia caries*) infection in winter wheat at lower inoculum rates. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, **105**, pp. 295-305.

Purdy, L. H., and Kendrick, E. L. (1957) Influence of environmental factors on the development of wheat bunt in the Pacific north-west. I. Effect of soil moisture and soil temperature on spore germination. *Phytopathology*, **47**, pp. 591-594.

Sartoris, G. B. (1924) Studies in the life history of certain smuts. *American Journal of Botany*, **11**, pp. 617-646.

Weston, D. (1932) The relative resistance of some wheat varieties to *Tilletia caries* (DC). *The Annals of Applied Biology*, **19**, pp. 35-54.

Wilcoxson, R. D. and Saari, E. E. (1996) Bunt and smut diseases of wheat: concepts and methods of disease management. Mexico, CIMMYT, pp.67.

Wonnacott, T.H. and Wonnacott, R. J. (1977) Statistics for Business and Economics. John Wiley & Sons, New York, pp. 753.

Yarham, D. and McKeown, B. (1989) Airborne spores of *Tilletia caries* as a source of wheat bunt through soil contamination. *Plant Pathology* **38**:612-614.

Yarham, D. (1993) Soil-borne spores as a source of inoculum for wheat bunt (*Tilletia caries*). *Plant Pathology* **42**:654-656.