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Consequences of intensive fungicide use or integrated disease management for fungicide resistance and sustainable control

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1. Abstract

Fungicide use on winter wheat in the UK has intensified over recent decades. Intensification is likely to be both a cause and an effect of fungicide resistance, because (i) the rate of selection for fungicide resistant strains is related to the number and dose of treatments, and (ii) as fungicides have become less effective, growers have responded by applying them more frequently to maintain control. The aim of this project was to produce evidence on the consequences – for fungicide resistance evolution and for gross margin - of intensive fungicide use compared to an integrated disease management approach, combining variety disease resistance and fungicides. Field experiments with winter wheat varieties rated as resistant, intermediate or susceptible to septoria (*Zymoseptoria tritici*) infection, compared the effect on selection for fungicide insensitive strains of *Z. tritici* from the combined effect of host resistance and a reduction in foliar azole fungicides enabled by host resistance and/or using disease forecasting. Results showed a clear increase in selection for less sensitive isolates of septoria with increased total fungicide dose, increased number of sprays and a more susceptible variety.

The effect of integrated disease control on gross margins (GM) was analysed for trials contributed by industry partners (at least 4 trials/year for 3 years) all using the same fungicide products to provide low, moderate and high intensity programmes applied to varieties rated as resistant, intermediate or susceptible to *Z. tritici*. In 2014, septoria pressure was high and all programmes gave an increase in GM over untreated. With no treatment, the resistant variety gave the highest GM. Across all treatments in 2014, on average the susceptible variety had the highest GM. In contrast, 2015 was a low-septoria year, and the untreated resistant variety had the highest GM. Of the three treatment programmes, for all varieties, the highest GM was with the lowest fungicide inputs. Responses to disease control were moderate in 2016, and GM were similar between fungicide programmes.

Modelling work showed that the effective life of a fungicide active ingredient can be prolonged by using resistant varieties and disease forecasting. A forecasting model, developed previously, was coupled to an economic model accounting for risk aversion in spray decisions and extended to account for selection for insensitive pathogen strains due to fungicide applications. Simulations using this model showed that the use of a septoria resistant variety, or combining a septoria resistant variety with using disease forecasting to guide treatments, could substantially slow the development of fungicide resistance.

The key messages from the project are [a] development of resistance to fungicides is driven by the number of sprays and the dose rate, [b] uncertainty about future disease encourages risk-averse intensive fungicide programmes, [c] current UK fungicide programmes are appropriate when disease is high, on susceptible varieties, [d] variety resistance makes the intensity of spray programmes less critical, and forecasting economically viable, and [e] use of strategies which integrate variety resistance will slow selection for fungicide resistance.

2. Introduction

Fungicide use on the major UK arable crops continues to intensify, as measured by the number of spray applications and the total dose, For example, on wheat the average number of fungicide applications per crop was similar in 2014 and 2015 (3.8 and 3.7, respectively), yet 2015 was a low septoria year compared with 2014 (Crop Monitor, 2016). Intensive use of fungicides has arisen through a combination of: (i) disease susceptible varieties, (ii) large variation in disease pressure between seasons, with very high disease pressure in some years, (iii) uncertainty about predictions of disease forecasting schemes, and (iv) a feedback loop, whereby high fungicide inputs produce high selection pressure for fungicide resistance, resulting in fungicide inputs rising to maintain control.

Loss of effective fungicides due to the development of pathogen resistance is a well-documented problem, exemplified by the loss of the strobilurin (quinone outside inhibitor) fungicides in the early 2000s for the control of septoria tritici blotch (caused by the fungus *Zymoseptoria tritici*) due to fungicide resistance. Now the efficacy of the triazoles has also declined after the loss of strobilurins caused control of septoria leaf blotch to become more dependent on triazoles and chlorothalonil (a multi-site fungicide introduced in the 1960s). The effectiveness of azole fungicides is eroding due to the development of pathogen insensitivity (AHDB Fungicide Performance data). The future availability of some azole active substances and chlorothalonil remains uncertain under recent pesticide legislation. A new generation of SDHI (succinate dehydrogenase) fungicides was introduced just in time to help reduce losses in 2012. This fungicide group is highly effective, but has been shown to be at high risk of fungicide resistance (Avenot & Michailides, 2010). SDHI insensitive *Z. tritici* strains are now starting to be seen in cereal crops in the UK (AHDB Agronomists Conference, 2016; Young *et al,* 2017).

The current balance between chemical and genetic disease control has arisen due to a set of economic, social and regulatory drivers acting on different parts of the industry. These drivers are summarised below, because they determine the extent to which re-balancing towards a more integrated approach is feasible.

Crop protection industry: For the past 40 years the crop protection industry has developed new fungicide modes of action and a stream of new active substances to control major cereal diseases. This innovation requires huge investment (Leadbeater, 2010). 'Patent busting' (whereby companies develop active substances which differ in chemical structure, but not MOA, from active substances patented by other companies) means that several fungicides within the same MOA often reach the market concurrently. Hence, the companies are exploiting a common MOA resource, as in the SDHI case. To recover its investment, each company has to maximise sales before efficacy is lost to resistance or the patent expires. For any individual company, reducing the

intensity of use in order to slow fungicide resistance evolution would not markedly prolong product life (unless all companies acted likewise) but would reduce revenue.

Regulators: In response to consumer and environmental concerns, regulatory constraints on the approval of pesticides are increasing with the introduction of hazard-based criteria. A hazard-based regulatory regime is not scientifically logical and these constraints and their associated uncertainty are likely to impact on investment in the development of new products. The combination of these pressures could reduce the diversity of fungicides reaching the market and restrict fungicide development to single-site modes of action which can be overcome by a single pathogen mutation. It is difficult to judge whether sufficient new modes of action are likely to be available in future because estimating pipelines from published patents is not a reliable indicator of the likelihood of new mode of action products reaching the market. Hence, there is a combination of unpredictability of loss of effective modes of action (to pathogen evolution and regulation) combined with unpredictability of new introductions. This represents a risk to crop production, particularly in countries, such as the UK, which are particularly prone to certain high resistance risk pathogens (such as *Z. tritici*).

Plant breeders: Breeding crop varieties with improved disease resistance is particularly challenging, because: (i) unlike other traits (such as yield or grain quality) pathogens evolve new virulences, so that progress is often reversed, (ii) variety resistance can be deleterious to yield due either to genetic linkage between resistance genes and poor alleles from the donor parent, or pleiotropic effects of resistance genes on yield, and (iii) heavy selection for disease resistance in the early generations of breeding programmes reduces the size of the breeding population for yield selection in later generations. Some of these limitations were addressed in Defra-LINK project LK0913 (Breeding for Improved Resistance to *Septoria tritici*), but with current breeding methods and knowledge, improving disease resistance is likely to slow progress with increasing yield. Since effective fungicides became available in the 1970s, plant breeders have been able to focus less on disease resistance and more on yield and grain quality. Much of the breeding effort on disease resistance has focussed on single major genes, which are simply inherited, highly effective and usually last long enough to get varieties onto the Recommended List (RL) and establish market share before the resistance breaks down. Polygenic, quantitative resistance tends to be more durable, but each individual gene is less effective and therefore overall it is more difficult to select for high levels of resistance in breeding programmes. After 40 years of effective chemical control, there has been a natural tendency for plant breeders to believe that chemical control will continue to cover for host resistance weaknesses, and to prioritise traits accordingly. In recent years the emphasis on chemical control has begun to shift, with a recognition that resistance rating is important to growers, as well as untreated yield. However, fungicide use statistics (Garthwaite *et*

al, 2014) suggest that growers are applying as much fungicide to new, more resistant varieties as to susceptible varieties.

AHDB: Recommended List (RL) trials use high fungicide inputs to quantify the 'genetic potential' of varieties in the near absence of biotic stress. Within each market sector (e.g. bread, biscuit or feed wheat varieties) the primary criterion for selection onto the list is fungicide treated yield. Hence, the first priority for breeders is a high treated yield, to ensure their varieties are selected for the RL in order to establish a place in the market. The characteristics for including a variety in the RL list give high priority to treated yield but medium priority to untreated yield. There has been limited enthusiasm for raising minimum standards of disease resistance for varieties to be listed, because varieties with good disease resistance historically have tended to have lower treated yields. Resistance rating is given high importance for *Septoria tritici* (which has a minimum rating of 4) but medium importance for other diseases. Except in extreme cases, it may take some time to agree the removal of popular varieties from the RL if their disease resistance breaks down, as growers have learnt how to grow them and value their other good agronomic traits. This reduces the commercial 'down side' to breeders of relying on ephemeral single major genes for resistance. The RL system operates by consent of the plant breeders (who submit varieties for testing) and growers (who fund it). Hence, the committees which regulate the RL focus on the economic interests of their stakeholders, and growers in particular. Currently, growers can choose between high-yielding susceptible varieties and lower yielding more resistant varieties. Short term economics may favour the former, if a relatively small yield penalty costs more than the savings from reducing fungicide inputs. Despite a move towards more disease resistant varieties, susceptible varieties are still grown across a substantial proportion of the wheat area, and multiple spray programmes are applied to achieve high yields.

The drivers described above are a consequence of a healthy, competitive market for commodities, fungicides and varieties. But this may not produce the optimum balance between short-term economics against the medium and long-term consequences for maintaining effective disease control. Unless the future supply of new fungicide modes of action can be guaranteed, the current approach is unlikely to be sustainable and is therefore not in the best interests of the UK arable industry. There are longer-term and collective issues which arise from:

(i) The effect of intensive fungicide inputs on fungicide resistance.

(ii) The 'feedback loop' effect of fungicide resistance driving higher inputs, resulting in more selection for resistance.

(iii) The effect of poor disease control on a proportion of UK crops on the disease inoculum burden experienced by all growers.

(iv) The resulting risk to our ability to maintain disease control, and hence productivity, in the medium and long term.

There is a strong mechanistic rationale that growing varieties with better disease resistance would substantially slow the development of fungicide resistance (Paveley & van den Bosch, 2012), because variety disease resistance: (i) decreases the difference in relative epidemic growth rates between fungicide sensitive and fungicide insensitive strains (the difference in growth rates determines the speed at which insensitive strains become dominant in the pathogen population), (ii) allows effective control to be obtained with a lower total fungicide dose (fewer treatments per crop or lower dose per treatment), thus further reducing selection pressure (van den Bosch *et al* 2011), and (iii) makes the economics of disease prediction schemes more favourable, because 'false negative' predictions become less frequent and have less impact on yield and because the range of possible future disease severity is limited by variety resistance (te Beest *et al* 2009). Hence, the feedback loop of more fungicide use driving more fungicide resistance, driving more fungicide use, should be alleviated. However, these hypotheses have not been tested. Hard evidence proving the hypotheses would provide a compelling case for a more integrated approach. The aim of this work was to produce such evidence, to demonstrate the effect on selection for fungicide insensitive strains from the combined effect of host resistance and a reduction in foliar fungicides enabled by host resistance and/or using disease forecasting. There were three main objectives: [1] Quantify the effect of integrated disease management on selection for fungicide insensitive pathogen strains, using field experiments which measure developments over one season, [2] Quantify the effect of intensification of fungicide use or integrated disease management strategies on the effective life of fungicide modes of action, using a modelling approach which enables calculations across multiple seasons, and [3] Develop guidance for growers, to interpret the implications for (i) the likelihood of the rate of introduction of new fungicide modes of action remaining ahead of the rate of loss, (ii) disease resistance requirements for crop varieties to be included on Recommended Lists for growers, and (iii) guidance on the integrated use of fungicides, resistant varieties and disease forecasting.

3. Materials and methods

3.1. Modelling the effect of variety resistance on the evolution of fungicide resistance

3.1.1. Model description

The model represents a winter wheat (*Triticum aestivum*) crop affected by *Zymoseptoria tritici* (septoria leaf blotch; previously known as *Mycosphaerella graminicola*) with the timing of crop and pathogen summarised in Figure 1. The model tracks the seasonal dynamics of healthy, latently infected, infectious and removed/senesced leaf material (Figure 2). The model also tracks the development of the pathogen's sub-populations which are sensitive and insensitive to the fungicide

applied. The example fungicide used for the analysis was the evolution of strobilurin resistance, exemplified for the active substance pyraclostrobin.

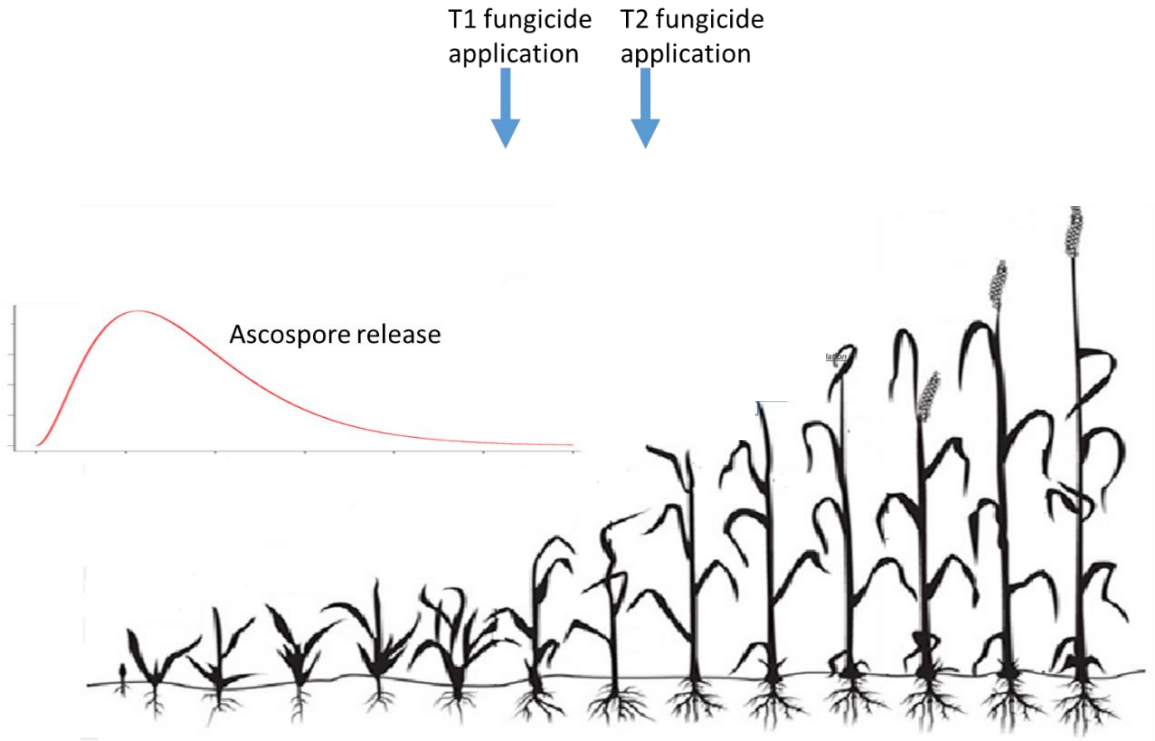

Figure 1. Summary of the timing of typical wheat crop growth stages, pathogen spore release and fungicide applications.

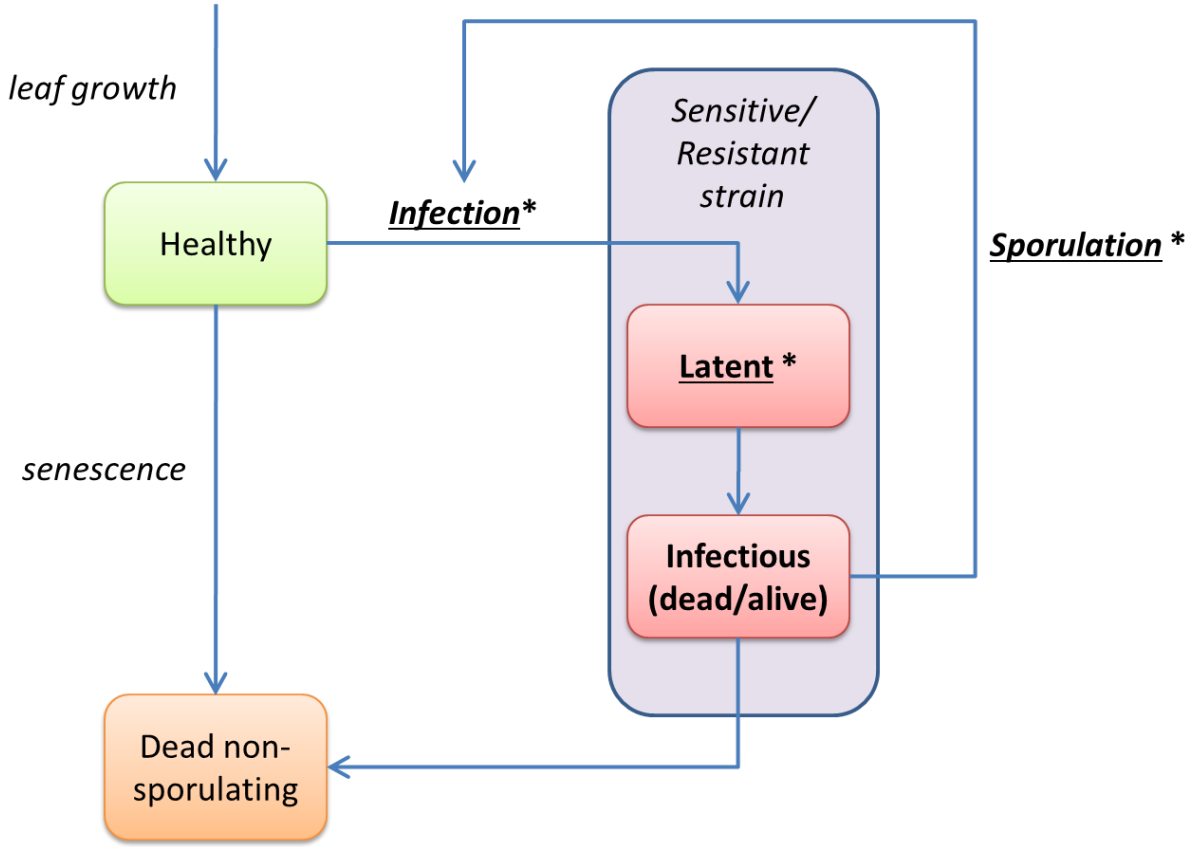

Figure 2. Cycles of asexual spore production and infection...

Healthy area dynamics

The wheat crop canopy growth model developed is an extension of the model described by Van den Berg *et al*. (2013) and simulates the growth and senescence of 11 leaf layers. The flag leaf is numbered leaf 1 and the lowest leaf is leaf 11. The leaf area of each leaf layer is measured in units of area index, which is defined as the ratio of the total planar leaf area to ground area. The life cycle of each leaf layer contains three phases: a growth phase, where leaf area increases according to a monomolecular function to a maximum; a lag phase, where leaf area remains constant; and a senescent phase, where healthy leaf area decreases from necrosis. Dead leaf layers are removed from the simulation. The growth rate, gk(t), and the senescence rate, sk(t), determine the leaf area dynamics of leaf Layer k.

Lesion area dynamics

Infection by ascospores: In autumn and early spring ascospores produce the initial infections in the canopy. The ascospore density, *A(t)*, released at time *t* in season x from pseudothecia formed at the end of season $x-1$, is described by

$$A(t) = \varepsilon t^2 e^{-\eta t}, \qquad \text{(C1)}$$

where ε and η are shape parameters (Table 1). Each ascospore has a probability, α_{asc}, to land on a leaf instead of the ground, a probability, $P_k(t)$, to land on healthy host tissue of leaf layer *k* and causes an infection with a probability defined by the infection efficiency, $\mathrm{IE}_{\mathrm{asc}}$. Probability $P_k(t)$ is given by

the proportion of healthy, latent, and infectious host tissue density within the crop that is healthy, such that

$$P_k(t) = \frac{H_k(t)}{\sum_{k=1}^{11} H_k(t) + \sum_{k=1}^{11} L_k(t) + \sum_{k=1}^{11} I_k(t)}, \quad \text{(C2)}$$

Where H_k , L_k and I_k are the healthy, latent and infectious tissue densities in leaf layer k, respectively. The fraction of the ascospores resistant to the fungicide under consideration is equal to θ_x.

Infection by conidiospores: Infections by conidiospores produced within sporulating lesions occur throughout the growing season. In line with the ascospore infection process each conidiospore has a probability, α_{con}, to land back into the crop, a probability, *P(t)*, to land on healthy host tissue and has infection efficiency, $\mathrm{IE}_{\mathrm{con}}$. The rate of change in latent tissues in leaf layer *k* is then dependent on the conidiospore production of leaf layer n and the probability that a conidiospore from leaf layer n reaches leaf layer k, which is given by

$$\sum_{n=1}^{11} k \quad I_n M_{nk}$$

, (C3)

where K is the spore production rate and I_n the infectious lesion density of on leaf layer *n* and M_{nk} the probability that spores produced on leaf layer n reaches leaf layer k.
Lesions remain latent for a period of

$$1/v$$

days before they become infectious and start sporulating. These lesions subsequently sporulate for a period of $1/\rho$ days. Leaf senescence leads to a reduction in latent tissue density, but it is assumed to not contribute to lesion death of hemibiotrophic pathogens such as *Z. tritici*.

The full model for leaf layer k is then given by

$$\frac{dH_k}{dt} = \big(g_k(t) - s_k(t)\big)H_k(t) - A(t)\alpha_{\mathrm{asc}}P_k(t)\mathrm{IE}_{\mathrm{asc}} - \alpha_{\mathrm{con}}\mathrm{IE}_{\mathrm{con}}\textstyle\sum_{n=1}^{11}[k \quad I_n M_{nk}] \quad \text{(C4)}$$

$$\frac{dL_k}{dt} = A(t)\alpha_{\mathrm{asc}}P_k(t)\mathrm{IE}_{\mathrm{asc}} + \alpha_{\mathrm{con}}\mathrm{IE}_{\mathrm{con}}\textstyle\sum_{n=1}^{11}[k \quad I_n M_{nk}] - v \quad L_k - s_k(t)L_k \quad \text{(C5)}$$

$$\frac{dI_k}{dt} = v \quad L_k - \rho I_k \quad \text{(C6)}$$

This set of equations is repeated for each strain in the pathogen population. In the cases simulated here there is a fungicide sensitive and a fungicide insensitive strain.

Foliar spray fungicide model

At each spray time a dose of the fungicide is sprayed (Figure 1). The dose intercepted by the leaf layer *k*, D_k, at $t = t_{spray,}$ is calculated using Beer's law. The dose intercepted by leaf layer *k* increases with its area index (AI) and is reduced according to the product of the AI of each leaf layer *j* above that intercepts the sprayed fungicide:

$$D_k = D_0 \left(1 - e^{-\tau Q_k(t_{spray})}\right) \prod_{j=1}^{k-1} e^{-\tau Q_j(t_{spray})} \quad \text{(C7)}$$

where τ is the angle of leaf layer k, measured as a projection onto a horizontal surface, which ranges from zero when fully vertical to 1 at a fully horizontal projection. D_0 is the total dose that is sprayed onto the field at $t = t_{spray}$. $Q_k = H_k + L_k + I_k + R_k$.

Dose response functions

Our dose response equations are of exponential type and not traditional probit-log curves, as the exponential curve fits to the observed data (Lockley & Clarke 2005). The modelled fungicide has both protectant activity, which reduces the ascospore and pycnidiospore infection efficiency, *IEas* and *IEcon*, and eradicant activity, which increases the length of the latent period, as given by $1/\upsilon$. The dose response functions have two parameters: *RD* which we define as the maximum proportional reduction in the target pathogen parameter, and *g* which serves as the dose response function shape parameter.

For the susceptible strain we have,

$$IE_{..S}(D) = IE_0 \left(1 - RD(1 - e^{-gD})\right) \quad \text{(C8)}$$

And since we only consider absolute resistance, we have for the resistant strain

$$IE_{....R}(D) = IE_0 \quad \text{(C9)}$$

Where IE_0 is the infection efficiency unaffected by the fungicide. For the latent period we have for the sensitive strain

$$\vartheta_S(F_1) = \vartheta_0 \left(1 - RD(1 - e^{-gD})\right) \quad \text{(C10)}$$

And $\vartheta_S(F_1) = \vartheta_0$ for the resistant strain

Two types of variety resistance

Variety resistance is modelled through the reduction of the infection efficiency of both asco- and conidio-spores. The infection efficiency of the susceptible variety was parameterised such that without fungicide applications there was a HAD loss of 20-25%, which reflects the current susceptible varieties (te Beest *et al* 2009a). By decreasing the infection efficiency, we change the level of variety resistance. The level of resistance will be shown as the HAD loss in the absence of fungicides. We modelled two types of variety resistance; seedling resistance, where all leaves express the host resistance equally, and adult resistance where only the top 4 leaves express host resistance.

3.1.2. Parameter values

We parameterised the model for *Z. tritici* on winter wheat treated with a two spray program (T1 and T2) of the strobilurin (QoI) fungicide pyraclostrobin (Table 1).

Table 1. Parameter values of the model for effect of variety resistance on the evolution of fungicide resistance (values are all from Kitchen *et al* (2016)

Parameter	Description	value	Units
ν	$1/\nu$ latent period		Degree days
ν_0	$1/\nu_0$ latent period in the absence of fungicides	244	Degree days
ρ	$1/\rho$ infectious period	456	Degree days
ε	Ascospore production rate	1.0	Degree day^{-3}
η	Shape parameter of ascospore production curve	0.0035	Degree day^{-1}
$IE_0\ \kappa\varepsilon_{con}$	Infection efficiency in the absence of fungicide x conidiospore production rate x the probability that a spore lands on a leaf	0.007	Degree days^{-1}
$IE_0\ \gamma\varepsilon_{asc}$	Infection efficiency x ascospore production rate x the probability that an ascospore lands on a leaf	0.0007	Degree days^{-1}
IE_{con}, IE_{asc}	Infection efficiency of conidiospores and of ascospores	No data	No data
RD	Asymptote of the fungicide dose response curve	0.5	Dimensionless
g	Curvature parameter of the dose response curve	0.003	Dose^{-1}

3.2. Modelling the effect of disease forecasting on the evolution of fungicide resistance

3.2.1. Model description

We developed a method to quantify the effect of disease forecasting on the build-up of fungicide resistance in a pathogen population and the associated economic gains from using such a forecast.

Disease forecasting model

Te Beest *et al* (2009b) developed an early warning forecasting system for *Zymoseptoria tritici*. The model predicts the presence or absence of a damaging epidemic. A damaging epidemic is defined as an epidemic that develops a severity larger than 5% at GS75. This threshold has been used previously as the economic injury threshold for *Z. tritici* on winter wheat (Gladders *et al* 2001, Pietravalle *et al* 2003). The model produces a prediction at GS31, just before the first fungicide application. This could be used to help growers decide on the fungicide application programme to implement. The model is developed using the window-pane analysis as developed by Pietravalle *et al* (2003). This method resulted in the following model:

$$F(rain, MinT) = 0.046*Rain + 0.042* MinT - 6.69. \quad (F1)$$

Where Rain is the daily rainfall above 3mm accumulated in the 80 days before GS31, MinT is the daily minimum temperature above 0 ^{O}C accumulated in the window starting 120 days before GS31 and ending 70 days before GS31. A damaging epidemic is predicted to develop if F is larger than 0, and no damaging epidemic is predicted to develop if F is smaller than 0.

Economic evaluation

For *Zymoseptoria tritici* it is well known that impact on yield is closely correlated with the disease severity at GS75 (Pietravalle *et al* 2003). We therefore consider the severity at this growth stage. Due to differences in weather etc. each year, a different severity, S, will develop at GS75. We will characterize this variability in untreated severity with the probability density P(S). Te Beest *et al* (2009) have found that P(S) is well described by an exponential probability distribution.

Te Beest *et al* (2009a) introduced a simple model to calculate the economically optimal fungicide application dose. They reasoned that the cost of disease, CD, is the sum of (i) the costs of buying and applying the fungicide, CF, and (ii) the cost due to the yield loss caused by the disease, CY. The cost CF of the fungicide increases linearly with the dose applied (

Figure **3**). The model assumes a fixed number of applications (two in Figure 3) and each application has the same dose.

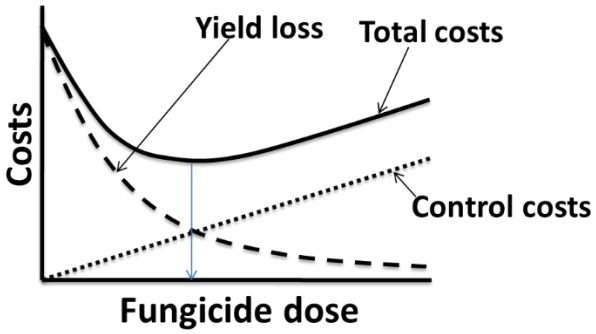

Figure 3. Cost due to disease as a function of fungicide application dose. The costs of control (dotted line), buying and applying the fungicide, increase with increasing dose. The costs due to yield loss (dashed line) decrease with increasing application dose. The total cost due to disease (solid line) is the sum of the two costs. The arrow marks the minimum cost due to disease.

The cost due to disease, CY, decreases non-linearly with dose leveling off at higher dose because the dose response curve levels off at higher dose (

Figure **3**). The sum of the costs, CD=CF+CY, therefore will have a minimum, which is the economically optimal dose.

To further specify the model, C_D denotes the cost of one full dose of the fungicide (in £ per ha), and so:

$$CF=C_D*D. \quad \text{(F2)}$$

The cost due to yield loss induced by the disease is the product of what the yield in the absence of disease would be sold for, PY, times the fraction yield loss due to the disease. P is the price of the yield (£ per tonne). Y is the yield in the absence of disease (tonnes per ha).

The fraction yield loss depends on the disease severity, X, in the crop after the fungicide applications, g(X), is assumed to be linear in the disease severity, g(X) = α X, and we have L_Y=PY g(X). The disease severity in the crop, X, is given by

$$X= S_S(D)+ S_R(D) \quad \text{(F3)}$$

where S_S(D) is the severity at GS75 due to the sensitive strain and fungicide application dose D, and S_R(D) is the severity at GS75 due to the resistant strain and fungicide application dose D

The severities of the two strains are given by:

$$Ss(D) = (1-\theta)\, S\ e^{-(r - r_S(D))(\Delta)} \quad \text{(F4)}$$

$$S_r(D) = \theta\, S\ e^{-(r - r_R(D))(\Delta)} \quad \text{(F5)}$$

where *S* is the severity of the epidemic when no fungicide treatment programme is in place, θ is the fraction of the pathogen population resistant to the fungicide, and $r_S(D)$ and $r_R(D)$ are the growth rates of the sensitive and the resistant strain when dose D is applied. Clearly when the insensitive strain is absolutely resistant to the fungicide $S_R^*=\theta S$.

We use a H-L-I-R model (Madden *et al* 2007) to model the dynamics of the strains. Where *H* is the density of healthy/uninfected host tissue, L is the density of exposed/latently infected host tissue, *I* is the density of infectious/sporulating host tissue and *R* is the density of removed/post-sporulating host tissue. The pathogen has a transmission rate, β, $p = 1/\gamma$ is the mean length of the latent period and $i = 1/\alpha$ is the mean length of the infectious period. From this model we can derive that the rate of growth of the epidemic, *r*, is given by:

$$r = \sqrt{m^2 + \frac{1}{p\,i}\,(\beta\,H - 1)} - m \qquad \text{(F6)} \qquad \text{where}$$

$$m = \frac{p+i}{2\,p\,i} \qquad \text{(F7)}$$

Each strain of the pathogen has its own growth rate, r_S and r_R, and depends on the fungicide applied.

The transmission rate, β:

A dose *D* of the fungicide results in a transmission rate

$$\beta_S(D) = \beta_0\left(1 - \beta_{mS}\left(1 - e^{-k_S D}\right)\right) \qquad \text{(F8)}$$

for the sensitive strain and

$$\beta_R(D) = \beta_0\left(1 - \beta_{mR}\left(1 - e^{-k_R D}\right)\right) \qquad \text{(F9)}$$

for the insensitive strain, where β_0 is the maximum transmission rate when no fungicide is used, β_{mSF1} and β_{mRF1} are the maximum fractional reduction of the transmission rate (i.e. at infinite dose) of the sensitive and the insensitive strain respectively under applications of fungicide F_1. When the insensitive strain is completely resistant we have $\beta_R(F_1)=\beta_0$ (by taking $k_{RF1}=0$).

The latent period, p:

In the HLIR models, as used by Hobbelen *et al*. (2011a & b) the latent period is given by $p=1/\gamma$, where γ is the probability per time unit that a lesion in the latent stage becomes an infectious lesion. The latent period is only affected by the systemic fungicide.

A results in a transmission rate

$$\gamma_S(D) = \gamma_0\left(1 - \beta_{mS}\left(1 - e^{-k_S D}\right)\right) \qquad \text{(F10)}$$

for the sensitive strain and

$$\gamma_R(D) = \gamma_0\left(1 - \beta_{mR}\left(1 - e^{-k_R D}\right)\right) \qquad \text{(F11)}$$

Where $1/\gamma_0$ is the latent period when no fungicide is used. We have assumed that the shape parameters, k_{SF1} and k_{RF1}, and the maximum reduction parameters, β_{SF1} and β_{RF1}, have the same values as for the effect of the fungicide on transmission rate.

Optimal dose calculations, mean costs of disease and risk aversion

The expected cost of control, i.e. the long term average cost, when applying a fungicide dose D, is calculated from

$$E(CD) = \int_0^1 P(S)CDdS \qquad \text{(F12)}$$

The optimal application dose is defined as the application program that minimises the long term mean cost due to disease.

As discussed by te Beest *et al* (2009a) growers may not aim to optimise the mean economic gain. They may be aiming to protect their crop against the 1 in 10 year biggest epidemics that will develop. This behaviour is called risk averseness, and is an important factor to take into account, as most growers will be risk averse. Growers may be willing to pay the additional cost of a higher fungicide dose each year as a risk-reducing investment (i.e., "insurance") in order to be protected from occasional large costs. We phrase grower's aim as: *A grower aims to minimise the expected costs incurred from the once each N years where a large epidemic develops during the growing season.* From the probability density, *P*(*S*), we find that once each N years the epidemic severity, *S,* is $S \geq S_N$, and S_N is found from

$$\frac{1}{N} = \int_{S_N}^1 P(S)dS \qquad \text{(F13)}$$

The expected costs incurred given that the epidemic develops a severity equal to or larger than S_N is calculated from

$$E(C|S \geq S_N) = \int_{S_N}^1 \frac{P(S)}{(1/N)} C(S,A)\, dS \qquad \text{(F14)}$$

the factor (1/N) is to scale P(S) such that for $S>S_N$ it is a probability density.

Combining the forecast and optimal dose calculation

Using the data from te Beest *at al* (2009a) we constructed the probability density of untreated yields (Figure 4), and using the economic evaluation model plotted the cost due to disease as a function of dose (Figure 5), which gives the optimal dose

i. When no disease prediction is available
ii. When the forecasting system says that no damaging epidemic will develop
iii. When the forecasting system predicts that a damaging epidemic will develop.

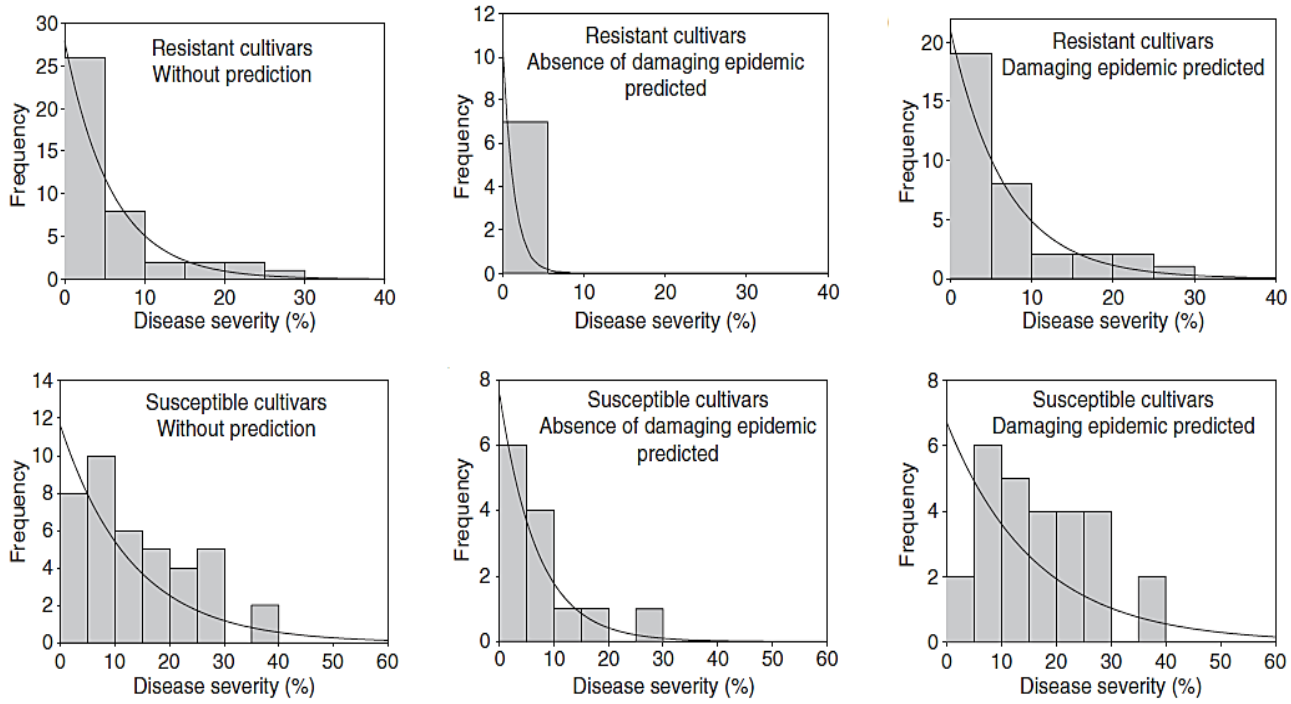

Figure 4. From te Beest et al (2009a). The probability density of a given disease severity at GS75 occurring. The grey bars are observations from the UK. The solid lines are fitted exponential distributions.

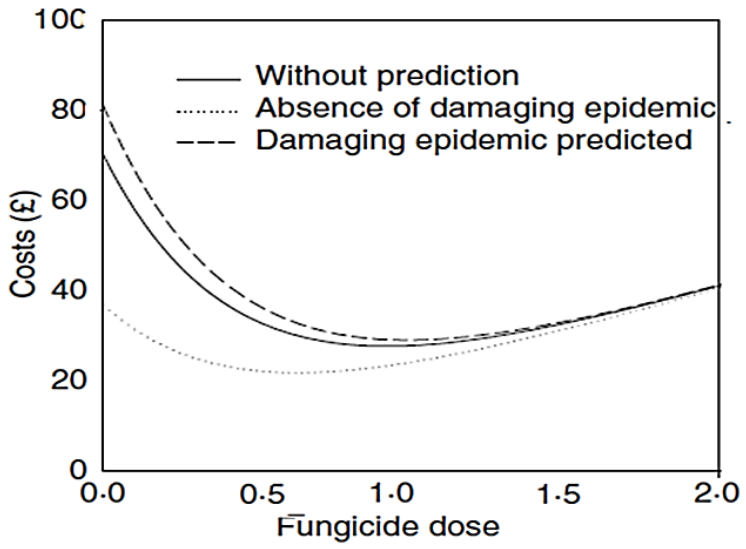

Figure 5. The cost of disease control as function of the fungicide application dose. When no disease forecast is available and when the disease forecast is available.

The fungicide resistance evolution model

The fraction of the primary inoculum of the pathogen insensitive to the fungicide is denoted by θ_n, where the subscript *n* stands for year. During the crop growing season the fraction of the population insensitive will change due to the application of fungicides. Here we describe the severity of the disease due to the sensitive and the insensitive strain at the end of the cropping season in year *n* with $S^*_{S\,n}(A)$ and $S^*_{R\,n}(A)$ respectively. We assume that the fraction insensitive in the primary inoculum is the same as the fraction insensitive at the end of the previous growing season.

The fraction of the primary inoculum of the pathogen population insensitive to the fungicide in year n+1 is then given by

$$\theta_{n+1} = \frac{S^*_{R\,n}(A)}{S^*_{S\,n}(A) + S^*_{R\,n}(A)} \qquad \text{(F15)}$$

The severity caused by the two pathogen strains at the end of the cropping season dependents on the fraction insensitive in the previous season, θ_n and on the fungicide application programme.

Consider the case where each year the same spray programme is applied. Substituting (F4) and (F5) into (F15)

$$\theta_{n+1} = \frac{\theta_n\ e^{r_R(A)\Delta}}{(1-\theta_n)\ e^{r_S(A)\,\Delta} + \theta_n\ e^{r_R(A)\Delta}} \qquad \text{(F16)}$$

This model can be explicitly solved, yielding:

$$\theta_n = \frac{\theta_0\ e^{s(A)n\Delta}}{(1-\theta_0) + \theta_0\ e^{s(A)n\Delta}} \qquad \text{(F17)}$$

where $s(A)=r_R(A) - r_S(A)$.

3.2.2. Parameter values

We parameterised the model for the *Z. tritici* on winter wheat system. See Table 2 for the parameter values and a short explanation about their estimation.

		Wheat *M. graminicola*	**Estimation or source**
C_{DA}	Cost of a dose of fungicide	Pyraclostrobin £25	Te Beest *et al* (2009)
D_A	Application dose	0 - 1	Te Beest *et al* (2009)
P	Price of grain	£100	Te Beest *et al* (2009)
Y	Yield in weight per unit area	9.15	Te Beest *et al* (2009)
μ	*Yield loss parameters*	μ=0.81	Te Beest *et al* (2009)
$1/\gamma_0$	Latent period in absence of fungicide	16.2	(1)
i	Infectious period	27.8	Eyal (1971)
βH		12.4	(2)
β_{mS}	Max fractional effect of fungicide	0.99	Te Beest *et al* (2009)
k_S	Dose response curve curvature	2.8	Te Beest *et al* (2009)
Δ	Fungicide half life	11.5	(3)

Table 2. Parameters as used in the model calculations. Many are taken from te Beest *et al* (2009a). (1) the latent period of *Z. tritici* is found in Lovell *et al* (2004) and we use the average temperature during the growing season in Cambridgeshire in the United Kingdom during the years 1984 to 2003 (Met Office, United Kingdom, published online), 15.2°C. (2) Using an average epidemic growth rate, r, of r=0.11 per day from Hobbelen et al (2011) and equation F5. (3)

3.2.3. Simulations

We present simulations for three scenarios:

(i) No disease forecast available: In this scenario the fungicide dose is used that is optimal when no forecast is available (Figure 5).

(ii) Disease forecast available. In this scenario we use a low fungicide dose (Figure 5) when the forecast is that no damaging epidemic will develop, and a higher fungicide dose (Figure 5) when a damaging disease is forecast. In this scenario the forecast may be correct or incorrect (Figure 4), and we used the actual severity that develops according to the probability that the forecast is correct or incorrect as given in Table 10.

(iii) Perfect prediction: In this scenario we assume that the prediction of the forecast is always correct. This scenario is only calculated for the mean economic gain scenario, as a perfect prediction would mean that risk averseness is not required.

The calculations are done for the resistant variety and for the susceptible variety. We calculate:

(i) The time course of the fraction of the pathogen population resistant to the fungicide (which determines how many years it takes for the fungicide to become ineffective).

(ii) The cost due to disease through time, when fungicide resistance is developing in the pathogen population.

3.3. Field experiments, variety and fungicide strategies

3.3.1. Azole-septoria field experiments year 1

Experimental approach

Field experiments were established in autumn 2013 (Table 3) using winter wheat varieties with different susceptibilities to *Z. tritici*. Plots were drilled at ADAS Boxworth, Cambridgeshire, and ADAS Rosemaund, Herefordshire, using vars. Crusoe (septoria-resistance rating RL7 in 2014 (now RL6)), Cubanita (intermediate RL 5) and Conqueror (susceptible RL4 (now RL6)), with one experiment at each site. The experiments used natural inoculum, and azole resistance to represent 'slow-shifting' type resistance. The foliar fungicide treatments within each experiment (Table 4) were designed to compare the effect on selection for fungicide insensitive strains from: a) varying levels of host resistance at the same fungicide input, b) the combined effect of host resistance and the reduction in foliar fungicide inputs enabled by host resistance, and c) reduced inputs resulting from disease forecasting approaches.

Table 3. Azole-septoria field experiments year 1 (2013-14), diaries

Task	ADAS Boxworth	ADAS Rosemaund	Timing
Drill	25-Sep-13	10-Oct-13	
First sample	30-Jan-14	23-Jan-14	first symptoms
Second sample	03-Mar-14	02-Apr-14	GS 30
GS 30 spray	11-Mar-14	04-Apr-14	GS 30
GS 31 spray	10-Apr-14	23-Apr-14	GS 31
Third sample	12-May-14	19-May-14	GS 39
GS 39 spray	14-May-14	27-May-14	GS 39
Galileo for yellow rust control	14-May-14	-	GS 39
Fourth sample	18-Jun-14	23-Jun-14	GS 51-70

Table 4. Azole-septoria field experiments year 1 (2013-14), foliar fungicide treatments

Trt.	[a]Variety	Fungicide MOA	Rate of fungicide product at each spray time (L/ha)	**Foliar treatment GS 30	**Foliar treatment GS 32	**Foliar treatment GS 39	**Total dose
1	Resistant	*Azole	0.25	-	-	0.25	0.25
2	Resistant	Azole	0.25	-	0.25	0.25	0.5
3	Resistant	Azole	0.375	-	0.375	0.375	0.75
4	Intermediate	Azole	0.375	-	0.375	0.375	0.75
5	Susceptible	Azole	0.375	-	0.375	0.375	0.75
6	Susceptible	Azole	0.5	-	0.5	0.5	1
7	Susceptible	Azole	0.5	0.5	0.5	0.5	1.5
8	Susceptible	-					UT

*Folicur (Bayer): active substance = tebuconazole, maximum individual dose = 1.0 L/ha

**foliar treatment rates given are proportions of maximum dose

[a] wheat varieties: Susceptible = Conqueror RL4, Intermediate = Cubanita RL5, Resistant = Crusoe RL7

Assessments, leaf sampling and genotyping

Assessments were made just prior to, or at each sample time. Disease and % green leaf area were assessed by individual leaf layer. Disease (including any other disease as well as *Z. tritici*) was assessed as the % area of sporulating lesions per leaf, on ten randomly selected shoots per plot, in all plots, by individual leaf layer. The assessment timings were: at first sample i.e. when first symptoms appeared; just before the GS 32 spray; just before the GS 39 spray and four weeks after the GS 39 spray or when new symptoms appeared following the application (sampling dates in Table 3).

Sampling of *Z. tritici* lesions started as soon as symptoms were seen across all plots (all untreated at that stage), in order to quantify the initial percentage of strains that were sensitive and the percentage insensitive. . All plots were sampled at each sample time, with 10 leaves with lesions collected per plot, selecting the same leaf layer across all plots. The leaves had surface water removed by pressing them gently between paper hand towels, and were then wrapped in clean paper towels (all 10 leaves together), placed in a paper envelope and posted first class to Rothamsted, for genotyping tests.

At Rothamsted, leaves were washed, surface sterilised and incubated overnight. Spores were harvested suspended in buffer and plated onto nutrient agar for colony screening and testing. DNA was isolated from 25 lesions per plot, and tested for the presence of mutations for fungicide insensitivity. Genotyping tests were done for the D134G and V136A mutations. Tebuconazole and the V136A mutation were used for this study, because presence or absence of V136A distinguishes between tebuconazole sensitive and insensitive strains, regardless of other mutations in the azole target site (unusually, absence of this mutation is associated with insensitivity and presence of the mutation with sensitivity). This enables high throughput genotyping of thousands of lesions to quantify the amount of selection for resistant strains caused by each fungicide treatment. For each plot there were two tests done, and an average result for % isolates with the mutation produced for each plot (there were 3 replicate plots per treatment). Results were expressed as the % of isolates which were insensitive (i.e. without the mutation) and analysed for treatment differences using GENSTAT analysis of variance.

3.3.2. QoI-septoria field experiments year 1

Experimental approach

Field experiments were established in autumn 2013 using winter wheat varieties with different susceptibilities to *Z. tritici*, using inoculation with *Z. tritici* spores. Plots were drilled at ADAS Boxworth and ADAS Rosemaund using vars. Crusoe, Cubanita and Conqueror, as described above for the azole year 1 experiments, with one experiment at each site. The experiments used inoculation with *Z. tritici*, and QoI resistance to represent fast 'single-step' type resistance. Prior to seedling emergence, each plot was covered with a tent with vapour permeable sides to exclude external airborne fungal spores (which are predominantly QoI resistant). The treatments within each experiment were designed to compare the effect on selection for fungicide insensitive strains from: a) varying levels of host resistance at the same fungicide input, b) the combined effect of host resistance and the reduction in foliar fungicide inputs enabled by host resistance, and c) reduced inputs resulting from disease forecasting approaches.

Small (1 m^2) field plots of the wheat varieties were inoculated with selected strains of *Z. tritici*. The plots were covered with vapour permeable fabric 'tents' which allowed air flow but excluded the influx of external ascospores. The tents remained in place during the main period for ascospore dispersal, i.e. from crop emergence to stem extension (Fraaije, 2005). Disease development was assessed at intervals, and samples of infected leaves were collected from each treatment. Samples of lesions were sent to Rothamsted Research for pathogen genotyping and determination of the proportion of samples carrying the insensitive mutation.

Spore-proof tents

'Tents' were made at ADAS Boxworth and ADAS Rosemaund, constructed from wood frames with a waterproof polyurethane top cover stapled closely in place, and breathable waterproof fabric (KBTRPMZ-F15-B16, from Fabric UK) covering each side, also stapled closely in place such that there were no gaps or openings (Figure 6). The frames were 1m^2 and 60 cm height above ground level, with at least 20 cm additional length of wooden leg at each corner to sink into the ground and secure the tent. Typical pore size in the membrane of a breathable fabric is 1.4 billion pores per cm^2 with average pore size < 10µm, typically 0.1 to 10 µm. The pores in the polymers used in breathable membranes are linked together in complex pathways (Fuller and Taylor, 2012) and as such they act as a filter and will trap or exclude *Z. tritici* spores, which on average are 2.5 x 11 µm in size. Tents were placed over plots prior to crop emergence, and apart from inoculation and irrigation procedures, stayed closed and on plots until the start of stem extension at the latest, after which the tents were removed. Openings for inoculation, sampling or irrigation purposes were created by cutting a slit in one corner of the top cover, which was then sealed immediately after use with waterproof tape.

Figure 6. Wood-framed tents with breathable fabric sides and waterproof polyurethane top, ADAS Boxworth, February 2014. Small opening in top cover is used for inoculation with *Z. tritici* spores and for irrigation purposes. Frames in 2015 had additional cross-pieces under top cover to reduce rain water collecting on top.

Z. tritici inoculum and field inoculation procedures

All strains of *Z. tritici* were supplied by Bart Fraaije at Rothamsted Research. In year 1, field experiments used QoI sensitive and insensitive strains. All isolates had been tested for sensitivity against various QoI fungicides. The three QoI-sensitive strains were CTRL1-01, LARS15 and Foli1-01. The three QoI-insensitive strains were R6-31, R3-35 and Opus 6.

Cultures on nutrient agar were sent to ADAS High Mowthorpe where they were used to initiate multiple new cultures on nutrient agar. From these new cultures, after 3 days growth at 16.5–17^{o}C, spores were scraped off the agar under sterile conditions to produce pots of spores which were then frozen ready for transport, thawing, counting and dilution at each field site. Pots were sent frozen on dry ice to each site, then kept frozen until thawing immediately prior to use on the day of field inoculation (thawed for at least 1 hour at room temperature, out of direct sunlight). For the azole experiment, on the inoculation day, one conidial suspension was prepared which contained roughly equal proportions by volume of spore concentrate of three QoI-sensitive strains, and another conidial suspension was prepared which contained roughly equal proportions of three QoI insensitive strains. The spores/ml were counted in the mixed suspensions, using a haemocytometer, and diluted to 10^6 spores/ml in distilled water (not sterile) with 1 drop of Tween 20 mixed in. These two conidial suspensions, sensitive and insensitive, were used to prepare the two inoculum suspensions required for the field plots, [1] a 95% QoI sensitive and 5% QoI insensitive mix and [2] 100% QoI sensitive.

The day for the field inoculation was selected to be when wind speed was low and average temperatures forecast to stay above 4^{o}C for at least the following week. Suitable temperatures are those between 4^{o}C and 19^{o}C. A slit was cut in the top of each tent through which the spray nozzle was inserted, and the opening was sealed immediately after inoculation with waterproof tape. Inoculum was sprayed at the rate of 50 ml/m^2 onto all appropriate plots for each treatment (dates in *Table 5*, treatment lists in *Table 6*). The sprayer was agitated every 10 seconds or so to keep the spores evenly suspended because they tended to settle quickly.

The first inoculation was planned for when the crop reached the 2–4 leaf stage and when temperatures were suitable, and a second inoculation was made a week or so later, depending on temperatures. Disease development was monitored by opening slits in the tent tops and checking the plants. Plots were irrigated by hand-held sprayers at intervals, using the openings in the top covers, to simulate rain splash which promotes spore dispersal up plants.

Sampling methods and timing were the same as described for the septoria-azole experiments.

Table 5. QoI-septoria field experiment diaries year 1 (2013-14).

TASK	BOXWORTH	ROSEMAUND	TIMING
Drill	26-Sep-13	10-Oct-13	
Spore tents on plots	07-Oct-13	18-Oct-13	
First Septoria inoculation	15-Nov-13	20-Nov-13	
Second Septoria inoculation	11-Dec-13	10-Dec-13	
First sample	14-Jan-14	22-Jan-14	first symptoms
Irrigation 100ml/plot	14-Feb-14	19-Feb-14	
TENTS REMOVED	17-Feb-14	03-Mar-14	GS 30
Second sample	18-Feb-14	04-Mar-14	GS 30
GS 30 spray	19-Feb-14	10-Mar-14	GS30
PGR spray	04-Feb-14	11-Mar-14	GS30
GS 31 spray	04-Mar-14	04-Apr-14	GS 31
Yellow rust & mildew spray	04-Mar-14	**-**	GS 31
PGR spray	01-Apr-14	12-May-14	GS 37
Third sample	16-Apr-14	15-May-14	GS 39
GS 39 spray	17-Apr-14	16-May-14	GS 39
Galileo for yellow rust	30-Apr-14	**-**	

Table 6. QoI-septoria field experiment year 1 (2013-14), foliar fungicide treatments.

Trt.	[a]Variety	Tents	[b]Septoria inoculum	Fungicide MOA	Rate of product / spray (L/ha)	Foliar trt GS 30	Foliar trt GS 32	Foliar trt GS 39	Total dose
1	Resistant	✓	✓	*QoI	**0.3125	-	-	0.25	0.25
2	Resistant	✓	✓	QoI	0.3125	-	0.25	0.25	0.5
3	Resistant	✓	✓	QoI	0.469	-	0.375	0.375	0.75
4	Intermediate	✓	✓	QoI	0.469	-	0.375	0.375	0.75
5	Susceptible	✓	✓	QoI	0.469	-	0.375	0.375	0.75
6	Susceptible	✓	✓	QoI	0.625	-	0.5	0.5	1
7	Susceptible	✓	✓	QoI	0.625	0.5	0.5	0.5	1.5
8	Susceptible	✓	✓	-		-	-	-	UT
9	Susceptible	✓	No inoculum	-		-	-	-	UT
10	Susceptible	**x**	No inoculum	-		-	-	-	UT

*Comet (BASF) max individual dose = 1.25 L/ha

**foliar treatment rates given are proportion of full dose

[a] wheat varieties: Septoria susceptible = Conqueror, RL4, Intermediate = Cubanita, RL5, Resistant = Crusoe, RL7

[b] Septoria inoculum = 95% fungicide sensitive isolates mixed with 5% insensitive isolates.

3.3.3. Azole-septoria field experiments year 2

Field experiments using natural infection and resistant, intermediate and susceptible varieties were established using winter wheat varieties and *Z. tritici* at two sites (Table 7). There were changes to the AHDB septoria resistance ratings during year 1 of the project, resulting in different varieties being selected for year 2 to give the largest differential between resistant and susceptible hosts: Cougar, RL7, Intermediate = Zulu, RL5, Susceptible = Gallant, RL4.The methods were as in year 1, i.e. comparing the effect on selection for fungicide insensitive strains from varying levels of host resistance and varying foliar fungicide inputs (Table 8), followed by sampling septoria-infected leaves post-fungicide treatments, and testing for fungicide insensitive isolates.

Table 7. Azole-septoria field experiment diaries year 2 (2014-15).

Task	BOXWORTH	TIMING	ROSEMAUND	TIMING
Drill	29-Sep		02-Oct	
Sample	10-Feb	23	-	
YR & mildew spray	12-Feb	23	-	
Assess GS30	31-Mar	30	16-Apr	30
T0 spray	02-Apr	30	15-Apr	30
Assess GS 32	22-Apr	32	11-May	32
YR overspray	23-Apr	32	-	
T1 spray	23-Apr	32	07-May	32
Assess GS 39	19-May	39	01-Jun	39
T2 spray	21-May	39	26-May	39
Assess GS39+ 4 wk	18-Jun	65	24-Jun	69

Table 8. Azole-septoria field experiment year 2 (2014-15), foliar fungicide treatments.

Trt.	[a]Variety	Fungicide MOA	Rate of product / spray (L/ha)	**Foliar treatment GS 30	Foliar treatment GS 32	Foliar treatment GS 39	Total dose
1	Resistant	*Azole	0.25	-	-	0.25	0.25
2	Resistant	Azole	0.25	-	0.25	0.25	0.5
3	Resistant	Azole	0.375	-	0.375	0.375	0.75
4	Intermediate	Azole	0.375	-	0.375	0.375	0.75
5	Susceptible	Azole	0.375	-	0.375	0.375	0.75
6	Susceptible	Azole	0.5	-	0.5	0.5	1
7	Susceptible	Azole	0.5	0.5	0.5	0.5	1.5
8	Susceptible	-					UT

*Folicur (Bayer): active substance = tebuconazole, maximum individual dose = 1.0 L/ha

**foliar treatment rates given are proportions of maximum dose

[a] wheat varieties: Septoria resistance = Cougar, RL7, Intermediate = Zulu, RL5, Susceptible = Gallant, RL4.

3.3.4. SDHI–net blotch field experiments year 2

Prior to year 2, strains of *Pyrenophora teres* (net blotch) insensitive to SDHI fungicides were reported in the UK. Experiments were therefore set up to use natural inoculum of net blotch and measure the rate of selection for SDHI resistance under different treatments. Field experiments with resistant, intermediate and susceptible varieties of winter barley were established at two sites. Fungicide treatments were planned to provide comparisons between the effect on selection for fungicide insensitive strains from: a) varying levels of host resistance at the same fungicide input, and b) the combined effect of host resistance and reduced foliar fungicide inputs enabled by host resistance. At both sites, no net blotch developed, precluding sampling and tests.

3.3.5. Economics field experiments year 1

Experimental approach

The aim of this work was to quantify the cost of switching to integrated disease management strategies. Fungicide programmes of untreated, low, moderate and high intensity were designed to have appropriately increasing numbers of applications of azoles and SDHI fungicides, with multi-site fungicides, in accordance with commercial practice. The low, moderate and high programmes had 2, 3 and 4 azole applications, and 0, 1 and 2 SDHI applications, respectively (Table 9), all using commercially realistic doses. Field trials were set up in autumn 2013 and sprayed in 2014.

Table 9. Fungicide mode of action programmes, industry partner economics field experiments

Fungicide programme intensity	**T0**	**T1**	**T2**	**T3**
Untreated	-	-	-	-
Low	-	Azole + QoI + MS	Azole + MS	-
Moderate	Mildewicide + MS	Azole + QoI + MS	SDHI + Azole + MS	Azole
High	Azole + mildewicide + MS	SDHI + Azole + QoI + MS	SDHI + Azole + MS	Azole

All of the industry partner field experiments used the same products and doses for the core programme shown in Table 9. These programmes were selected in consultation with the industry

partners to make sure products and doses were selected were commercially applicable and covered a range of products from different manufacturers. Products in Table 9 included single MOA or combined products as appropriate, as follows:
chlorothalonil (Bravo; Syngenta)
proquinazid (Talius; DuPont)
prothioconazole: (Proline, Bayer)
azoxystrobin (Amistar opti; Syngenta)
pyraclostrobin (Comet, BASF)
epoxiconazole + fluxapyroxad (Adexar, BASF)
folpet (Phoenix; Adama)
epoxiconazole (Cortez; Adama)
fluoxastrobin + prothioconazole (Firefly; Bayer)
penthiopyrad (Vertisan; Dupont)
epoxiconazole (Ignite; BASF)

The wheat varieties used across all sites in 2014 were: Crusoe, resistant (RL7), Cubanita, intermediate (RL5) and Conqueror, susceptible (RL4). There were six experiments in total: Bayer two sites, (Cambridgeshire and Herefordshire), DuPont one site (Herefordshire), BASF one site (Yorkshire), Syngenta one site (Cambridgeshire) and Limagrain one site (Suffolk). Data from the penultimate trial were variable and the last trial included non-standard treatments, so these trials were not included in the cross-site analysis. Trials were assessed for disease at the fungicide application times and plot yields were recorded. Data from the Crop Monitor disease surveys (Fera, UK) were used to compare treated disease levels on farm to disease levels in the trials. Gross margins were calculated for each plot in each trial. Gross margins are defined as *total revenue* minus *variable costs*. For this calculation the total revenue is the yield multiplied by the assumed price of wheat (£120/t). The variable costs are taken from Nix (2016) for the herbicides, insecticides, fertiliser and seed costs. For the fungicide costs, chemical distributors were asked to supply quotes, and the mean figures for each product were used in the calculations for each fungicide programme. The estimated costs reflected the intensity of the fungicide programmes applied. The results were analysed with GENSTAT analysis of variance, using average gross margins for each treatment across all sites and assuming the same estimated costs at each site for each fungicide programme.

3.3.6. Economics field experiments year 2 and 3

The same experiments were set up in autumn 2014 and in 2015 as in autumn 2013, except for a change in wheat varieties, to be the same as in the research site experiments in 2014, i.e. Cougar RL7, Zulu RL5 and Gallant RL4 (ratings are for septoria resistance). There were six experiments in total: Bayer two sites, (Cambridgeshire and Herefordshire), DuPont one site (Herefordshire) and BASF one site (Yorkshire), Syngenta one site (Cambridgeshire) and Limagrain one site (Suffolk).

An additional objective was introduced in year 2 which was to measure the effect of fungicide programmes and variety resistance on selection for septoria fungicide-resistance. *Z. tritici* samples were taken from industry partner sites in the third year of the project. Septoria isolates from sampling pre-T0 and 3 weeks post T3 fungicide applications were laboratory phenotyped for sensitivity to confirm the selection effects of low, moderate and high intensity fungicide programmes and low, moderate and high host susceptibility.

3.3.7. Phenotyping of industry partner trials

The core treatments (Table 9) were sampled at two points in the season in 2015 and 2016. Prior to T0, 100 leaf samples were collected from plots of each of the three varieties (Susceptible (Gallant), resistant (Cougar) and the moderately susceptible (Zulu)). Mid and upper leaves were selected where possible, with target samples having clear lesions on green leaves to reduce contamination from non-target pathogens. The second sample timing was approximately 2-4 weeks post T3, with this timing flexible to allow sampling to occur as late as possible before plants began senescing. At this second sampling timing 15 leaf samples per plot were collected from each of the core treatments (Table 9) of each variety. Samples were collected from the same leaf layer throughout the trial for consistency, with this leaf layer judged by making sure disease severities were suitable on the full range of treatments (i.e. disease was not too severe in the untreated susceptible, but could still be observed in the high intensity treated resistant variety). Samples from each trial at both sample timings were submitted to Rothamsted for determination of sensitivity to different fungicide concentrations. In summary (full method in Fraaije *et al* 2011), leaf segments with lesions (to give a total of at least 50 lesions) were incubated for 24 h at 20^{o}C and isolates from single spore cultures were used in in-vitro fungicide sensitivity assays. Fungicide sensitivities were determined as 50% effective concentration (EC50) for a range of fungicide concentrations. The distribution of fungicide sensitive phenotypes was compared for each combination of fungicide programme and variety-resistance.

4. Results

4.1. Modelling the effect of variety resistance on the evolution of fungicide resistance

We studied two output quantities of relevance to the effect of variety resistance on the development of fungicide resistance, the take-over time and the effective life of the fungicide.

Take-over time: The take-over time is defined as the time from the introduction of the fungicide until the moment the resistant strain has reached a frequency of X % in the pathogen population. Here we show results for X=50, T_{50}.
Effective fungicide life: The effective fungicide life is defined as the time from introduction of the fungicide until the moment the resistance has developed to such an extent that effective control of the pathogen population is no longer possible. Effective control is defined here as the HAD loss due to the pathogen being smaller than 5%.

Figure 7 plots both output variables for a range of % HAD loss in the absence of fungicides, which is a measure of variety resistance level. The figure shows that selection rate decreases, i.e. T50 increases, with increasing levels of variety resistance. Selection is lower, i.e. T50 is longer, for lower fungicide dosages. This is in line with our previous findings as well as with all published evidence (van den Bosch *et al* 214). Take over times increase from 8 years with a susceptible variety (HAD loss in absence of fungicide of 25) to 12 years with a partially resistant variety (HAD loss of 5%) in the absence of fungicide.

The effective life is also affected by the level of variety resistance. Figure 7 shows that for a very susceptible variety (HAD loss of 25-30%), a two spray program is not able to provide effective control even when fungicide resistance has not developed yet. The effective life on a susceptible variety with HAD loss of 20-25% is 6 years, whereas on a resistant variety it can be as long as 13 years. Depending on the level of variety resistance that can be achieved, the effective life of the fungicide can be doubled when resistant instead of susceptible varieties are grown.

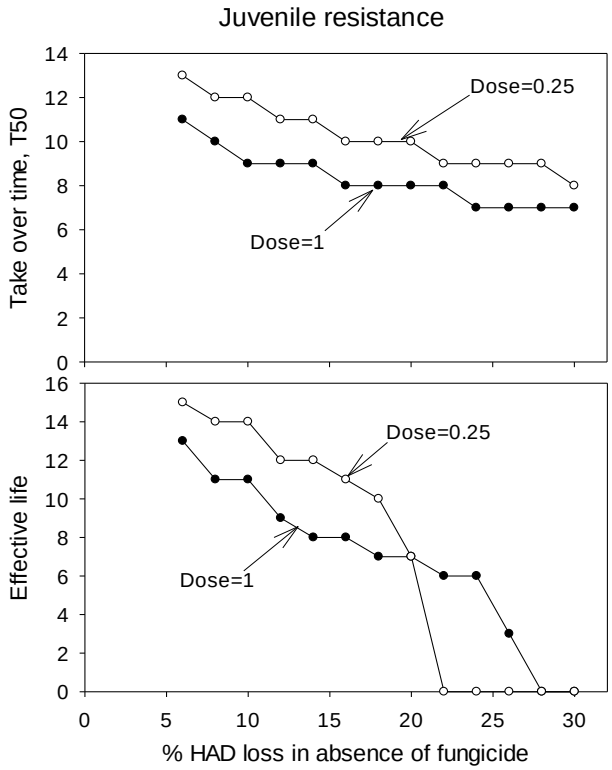

Figure 7. Juvenile resistance (where host resistance is expressed on all leaf layers) and its effect on fungicide resistance development. Take over time, T50, and effective fungicide life as a function of the % HAD loss of the variety when no fungicides are applied. The x-axis is the level of variety resistance, with susceptible variety (having a high %HAD loss) on the right hand side. Top graph shows the take over time, T50, which is defined as the time from the introduction of the fungicide until the moment the resistant strain has reached a frequency of 50% in the pathogen population. The bottom graph shows the effective life defined as the time from introduction of the fungicide until the moment the resistance has developed to such an extent that effective control of the pathogen population is no longer possible. Effective control is defined here as the HAD loss due to the pathogen being smaller than 5%.

Figure 8 shows similar trends for adult plant resistance. Here, only the top four leaves express the resistance. The figure shows that the effect of adult variety resistance on the evolution of fungicide resistance is smaller than that of the juvenile variety resistance of the same strength. The reason for this is that during the early developmental phases of the crop, up until the emergence of leaf four, the pathogen is living on a susceptible variety with the associated high selection rates for fungicide resistance.

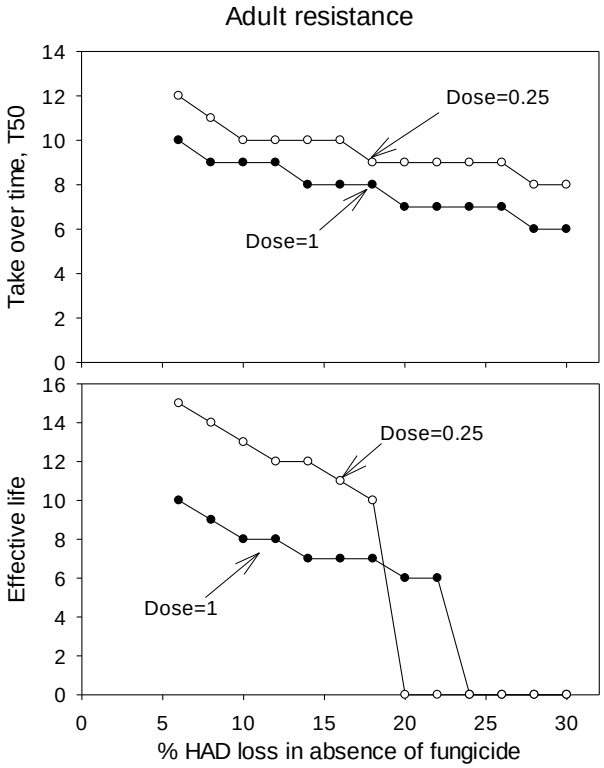

Figure 8. Adult plant resistance (where host resistance is expressed only on the top four leaves) and its effect on fungicide resistance development. Take over time, T50, and effective fungicide life as function of the % HAD loss of the variety when no fungicides are applied. The x-axis is the level of variety resistance, with susceptible variety (having a high %HAD loss) on the right hand side. Top graph shows the take over time, T50, which is defined as the time from the introduction of the fungicide until the moment the resistant strain has reached a frequency of 50 % in the pathogen population. Bottom graph shows the effective life defined as the time from introduction of the fungicide until the moment the resistance has developed to such an extent that effective control of the pathogen population is no longer possible. Effective control is defined here as the HAD loss due to the pathogen being smaller than 5%.

In summary, growing partially resistant varieties slows down the development of fungicide resistance in the pathogen population, as compared to a situation where susceptible varieties are grown. Juvenile resistance is more effective in slowing down the development of fungicide resistance than adult plant resistant varieties, if the strength of expression of host resistance is similar.

4.2. Modelling the effect of disease forecasting on the evolution of fungicide resistance

Resistance evolution

With resistant varieties, making use of the disease forecasting system (i.e. applying fungicides only when needed) greatly delays the development of fungicide resistance in the pathogen population (Figure 9A, 'current'). When no forecasting system is available (resulting in more frequent applications of fungicides than necessary) the pathogen population consists of 50% resistant strains after 8 years (Figure 9A, 'no'). If using forecasting, it takes 14 years before this level of resistance is reached. If the accuracy of the forecast can be improved there is an additional two years before the 50% resistance level is reached (Figure 9A, 'perfect').

With susceptible varieties, the situation is less positive because the forecasting system provides very little delay in the build-up of resistance (Figure 9B). However, if it would be possible to develop a forecasting system that is always accurate, there would be a lot to gain from using forecasting.

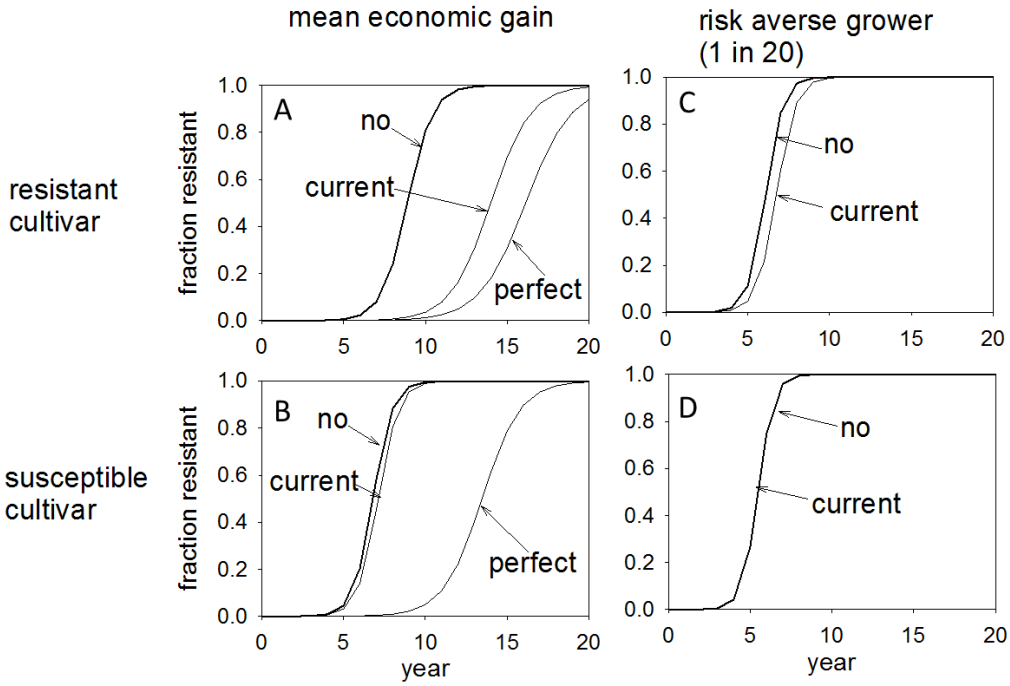

Figure 9. Fraction of the pathogen population resistant to the fungicide as a function of time. Panel **A** is for the resistant varieties, when fungicide treatments to obtain mean economic gain are simulated. **B**: susceptible varieties when treatments to obtain the mean economic gain are calculated. **C**: resistant varieties for risk averse growers. **D**: susceptible varieties for risk averse growers. The line 'no' is for the case where no forecast is available; 'current' is when the currently available forecast is applied; 'perfect' is when a perfect forecasting system would be in place.

The considerable difference between the resistant and the susceptible varieties can be understood by considering Table 10. For the resistant variety there is only 13% of the cases where 'no' damaging epidemic is predicted but in reality a damaging epidemic does develop. For the susceptible varieties this is the case in 48% of the cases for the 'no' damaging epidemic prediction. For the susceptible varieties the predictive scheme is not accurate enough to provide any confidence in the prediction of the absence of a damaging epidemic.

When growers are risk averse, in this case they are assumed here to protect their crop against the 1 in 20 years high severity epidemics, the forecasting system does not delay the build-up of fungicide resistance in the population. This is not surprising because the dosages used in the case of risk averseness are considerably larger than in the case of no risk averseness.

Resistant cultivar (6,7)

prediction \ field	Epidemic	No-epidemic
Epidemic	0.60	0.40
No-epidemic	0.13	0.87

Susceptible cultivar (3-5)

prediction \ field	Epidemic	No-epidemic
Epidemic	0.74	0.26
No-epidemic	0.48	0.52

Table 10. The accuracy of the forecasting model. On the right hand side for the varieties with resistance rating 6,7 (resistant varieties). On the left hand side for the varieties with resistance rating 3-5 (susceptible varieties). Data from te Beest *et al* 2013.

Costs due to disease

Figure 10B (resistant variety) and Figure 10C (susceptible variety) show the costs to disease and the dotted lines mark the situation where no fungicide treatment program is in place. All cases are calculated relative to the no fungicide treatment case, which is marked as 1.0.

Figure 10B and Figure 10C show that the use of fungicides provides an economic return with or without a forecasting system being used. The cost to disease are about halved for the resistant variety when fungicide treatments are applied and no resistance has developed yet. In the following years, resistance is building up (Figure 10A), and the gain from using the fungicide slowly erodes. Around year 8 the 'cost-to-disease' line crosses the line marking where no fungicide is used. This implies that from 8 years onward the use of fungicides does not bring an economic gain due to pathogen insensitivity and that its use should be abandoned.

When the disease forecast system is used the cost-to-disease is lower than when no forecast is used. Even at the point where no resistance has developed yet there is an additional economic gain of about 20% from using the forecast instead of an application program that is optimal when no forecast is available. As the resistance in the pathogen population builds up more slowly when the forecast is used (Figure 10A), the gain from using the fungicide also erodes more slowly. A measure of the total gain from the fungicide is the area between the line representing the cost-to-disease when no fungicide is used and the line tracing the cost through time when the fungicide programme is in place. Figure 10B clearly shows that the total gain of applying fungicide is more than twice as large when the disease forecasting system is used than in the case where such a system is not in place.

There is a potential additional gain if it was possible (hypothetically) to develop a forecasting system that always predicts the presence or absence of a damaging epidemic completely accurately (Figure 10B). For the susceptible varieties the situation is less positive, with little economic gain from using the current forecast. However, if a more accurate forecasting system were available there could be a considerable economic gain. This again reflects the fact that the present forecasting system is not very good at predicting a damaging epidemic on susceptible varieties.

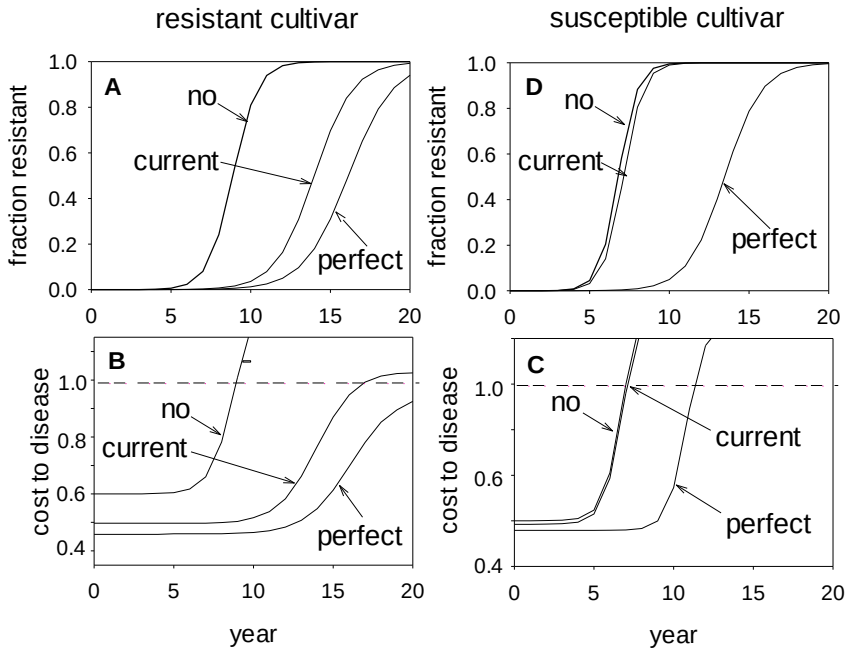

Figure 10. Fraction of the pathogen population resistant to the fungicide (top row) and the relative cost to disease (bottom row) as a function of time. Panel A and B is for the resistant varieties, panel C and D for the susceptible varieties. The line ‘no’ is for the case where no forecast is available, ‘current’ is when the currently available forecast is applied, and ‘perfect’ represents the maximum possible gain if a perfect forecasting system would be in place.

4.3. Field experiments

4.3.1. Azole-septoria field experiments year 1

Consistent results from these experiments relying on natural infection were obtained across the two sites. Disease severity was clearly related to wheat variety susceptibility rating at both the Boxworth (Figure 11) and Rosemaund sites (Figure 13). But severity tended to increase with more fungicide applications and a higher total dose applied (Figure 11). This decrease in efficacy was explained by the genotyping results which showed a clear increase in selection for less sensitive isolates with increased total dose, increased number of applications, and with a more susceptible variety, at the Boxworth (Figure 12) and Rosemaund sites (Figure 14).

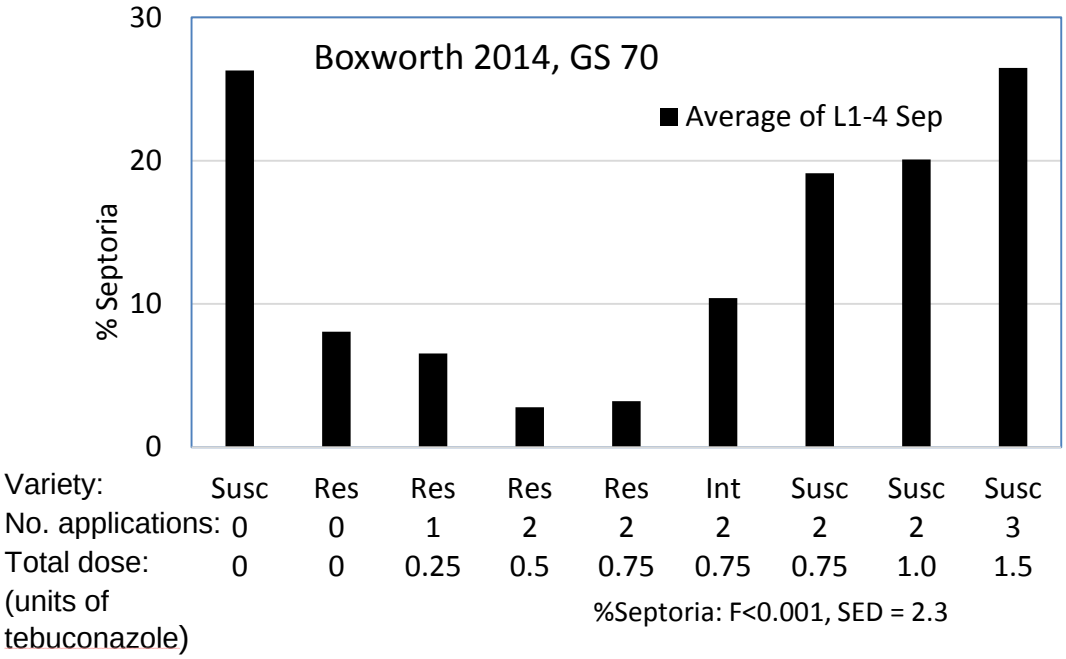

Figure 11. Azole effects on septoria leaf blotch severity ADAS Boxworth year 1 (2014). Wheat variety = Crusoe, resistant; Cubanita, intermediate; Conqueror, susceptible. Total dose = total foliar fungicide dose received as a proportion of full dose (e.g. total dose of 1.5 = 3 applications each of 0.5 dose).

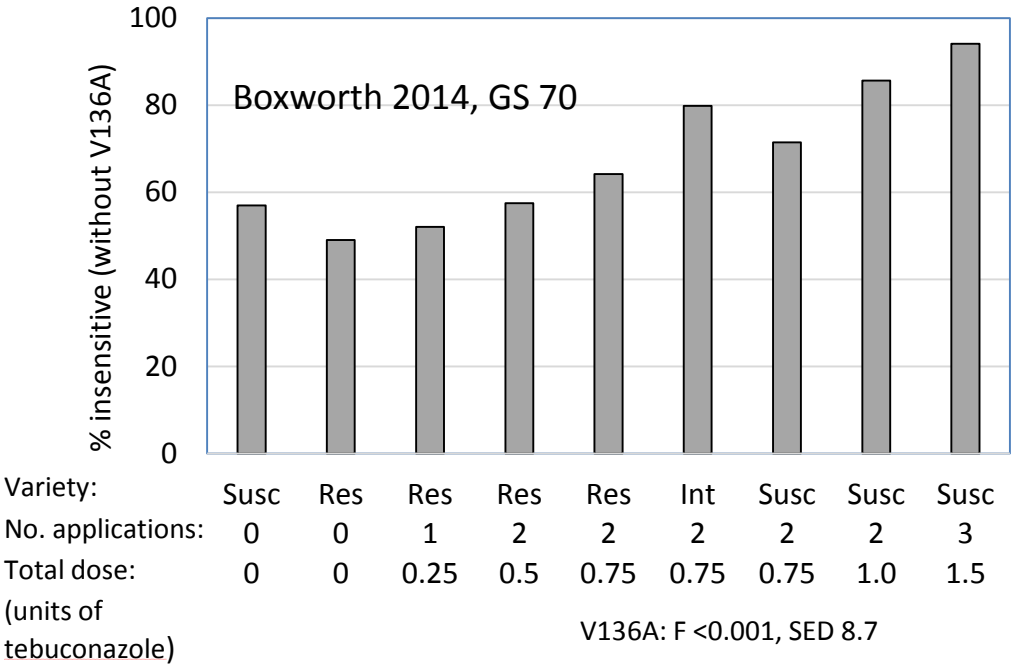

Figure 12. Selection for azole insensitive septoria, ADAS Boxworth year 1 (2014). Wheat variety = Crusoe, resistant; Cubanita, intermediate; Conqueror, susceptible. Total dose = total foliar fungicide dose received as a proportion of full dose.

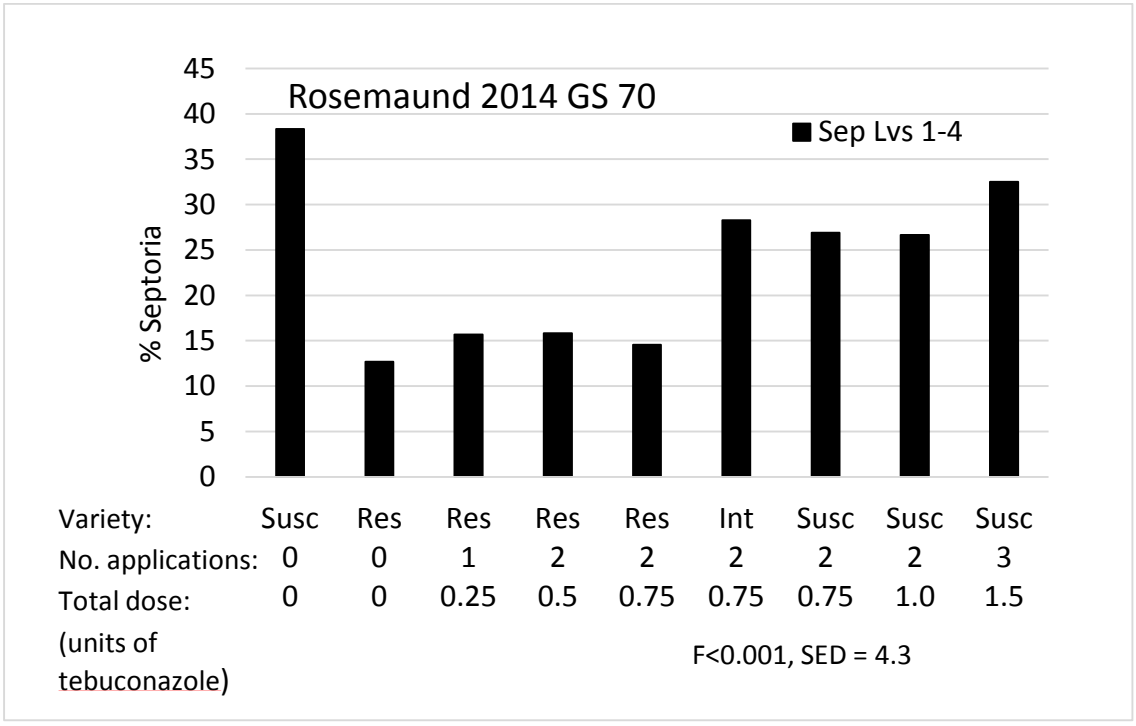

Figure 13. Azole effects on septoria leaf blotch severity ADAS Rosemaund year 1 (2014). Wheat variety = Crusoe, resistant; Cubanita, intermediate; Conqueror, susceptible. Total dose = total foliar fungicide dose received as a proportion of full dose.

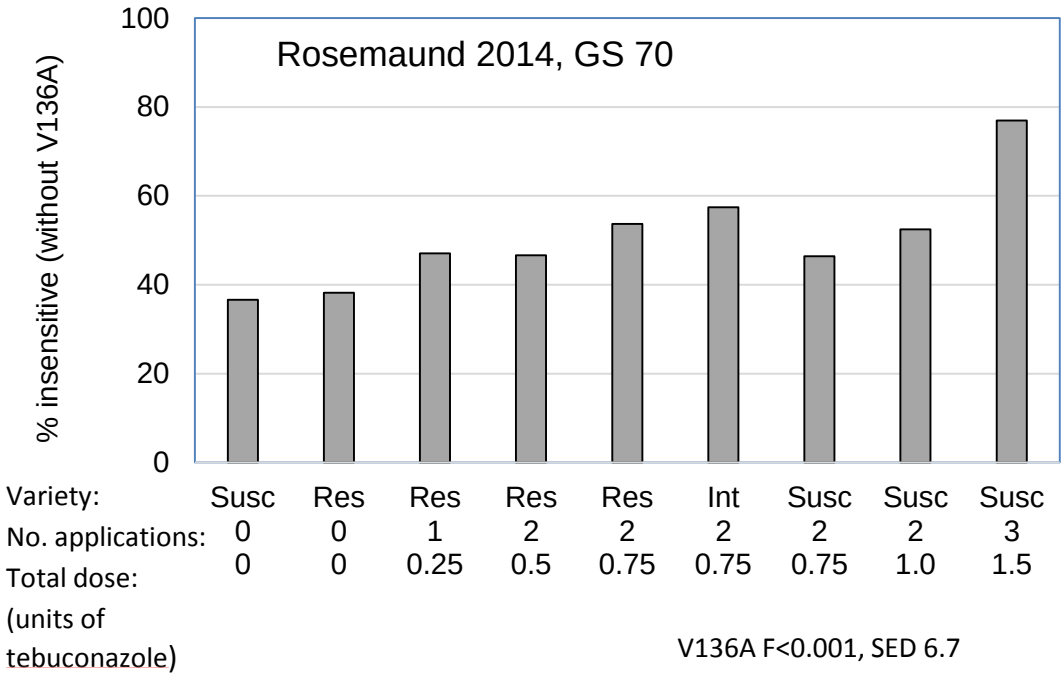

Figure 14. Selection for azole insensitive septoria, ADAS Rosemaund year 1 (2014). Wheat variety = Crusoe, resistant; Cubanita, intermediate; Conqueror, susceptible. Total dose = total foliar fungicide dose received as a proportion of full dose.

4.3.2. QoI-septoria field experiments year 1

The results of these inoculated experiments were prejudiced by influx of the natural pathogen population after tent removal in the spring, under the exceptional septoria pressure experienced in 2014. For example, at the Rosemaund site at GS 65, septoria leaf blotch severity was variable at 8-19% across all fungicide treatments for the inoculated tented plots but was 16% on the non-inoculated tented plots (Figure 15) which were expected to have zero or very low septoria severities. As the influx was of QoI resistant strains, the fungicide treatments had little effect on severity.

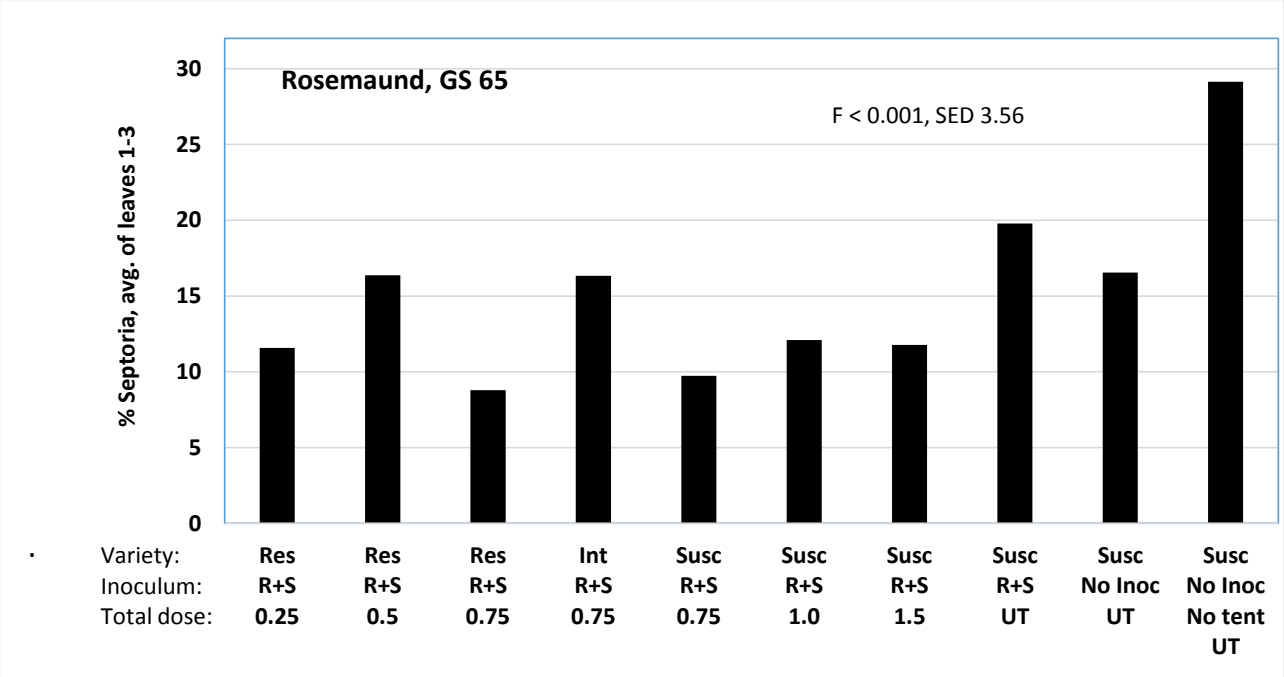

Figure 15. Septoria leaf blotch severity ADAS Rosemaund year 1 (2014) on treatments differing in variety, presence/absence of inoculation and QoI treatment. Wheat variety = Crusoe, resistant; Cubanita, intermediate; Conqueror, susceptible. Inoculum R = resistant (isolates R6-31, R3-35, Opus 6); S = sensitive (isolates CTRL1-01, LARS15, Foli1-01); mix = 95% sensitive and 5% sensitive. Total dose = total foliar fungicide dose received as a proportion of full dose.

4.3.3. Azole-septoria field experiments year 2

The azole-septoria results in 2015 were consistent with the results in 2014, although the genotyping results were more variable than in 2014. This variability was likely to have resulted from lower disease severity in 2015 than in 2014, with average septoria severity on leaves 1-3 on untreated susceptible var Gallant of 8% at Boxworth (Figure 16) and 12% at Rosemaund (**Error! Reference source not found.**). This made it more difficult to obtain representative samples. At Boxworth, there was some indication of increased selection for fungicide insensitive isolates with increased total dose and increased number of applications, but the frequencies of insensitive isolates were not consistent across treatments (Figure 17). Selection results at Rosemaund were also variable, but demonstrated the least selection for resistance with no fungicide treatment, and the most selection with the highest azole dose and number of treatments (Figure 19).

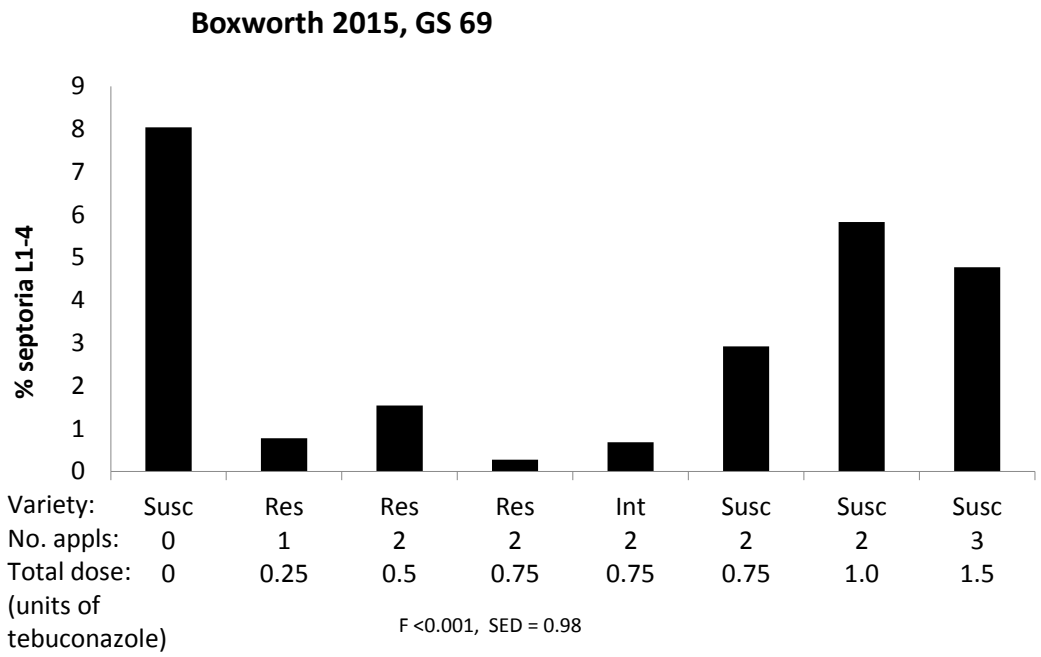

Figure 16. Azole effects on septoria leaf blotch severity ADAS Boxworth year 2 (2015). Wheat variety = Cougar, resistant; Zulu, intermediate; Gallant, susceptible. Total dose = total foliar fungicide dose received as a proportion of full dose.

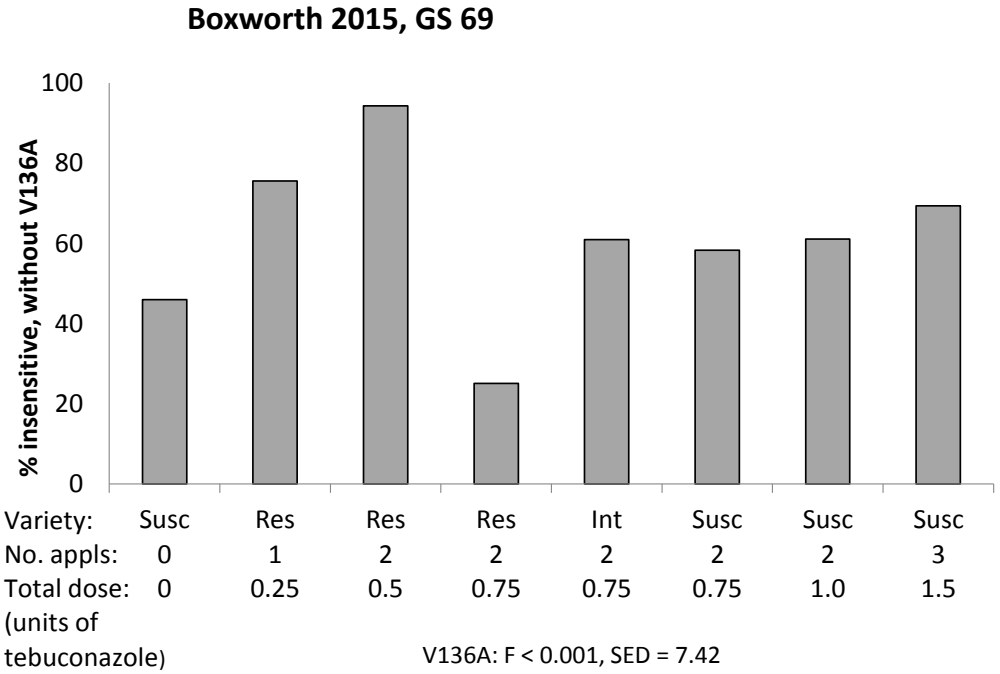

Figure 17. Selection for azole insensitive septoria, ADAS Boxworth year 2 (2015). Wheat variety = Cougar, resistant; Zulu, intermediate; Gallant, susceptible. Total dose = total foliar fungicide dose received as a proportion of full dose.

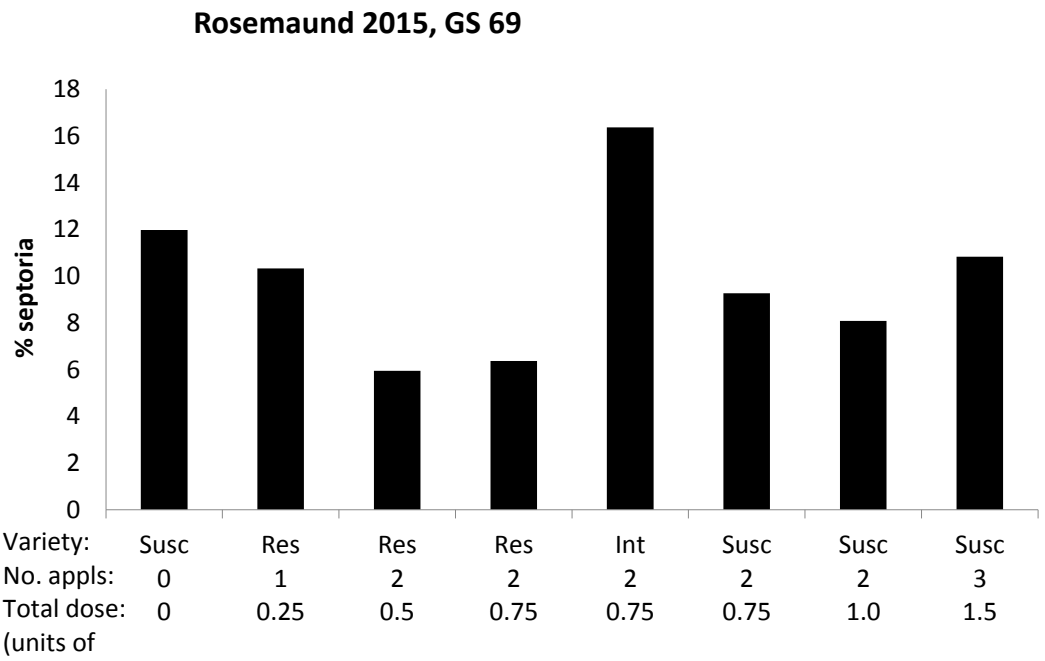

Figure 18. Azole effects on septoria leaf blotch severity ADAS Rosemaund year 2 (2015). Wheat variety = Cougar, resistant; Zulu, intermediate; Gallant, susceptible. Total dose = total foliar fungicide dose received as a proportion of full dose. Avg Leaves 1-3, F <0.001, SED = 0.96.

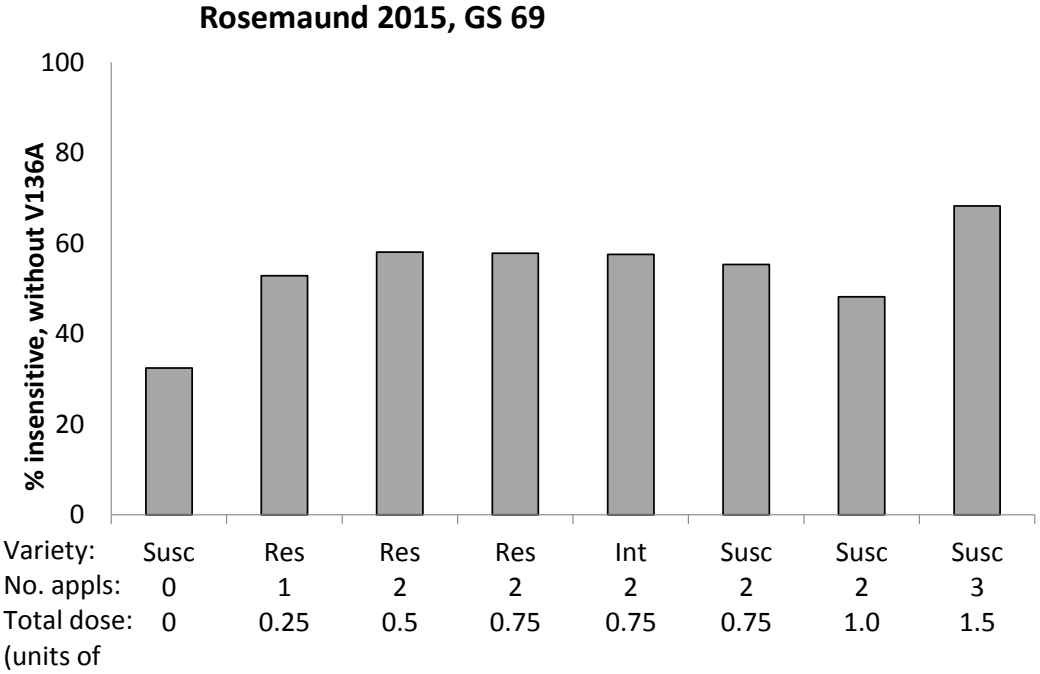

Figure 19. Selection for azole insensitive septoria, ADAS Rosemaund year 2 (2015). Wheat variety = Cougar, resistant; Zulu, intermediate; Gallant, susceptible. Total dose = total foliar fungicide dose received as a proportion of full dose. Avg Leaves 1-3, F <0.001, SED = 0.60.

4.3.4. SDHI–net blotch field experiments year 2

No net blotch developed at the barley sites, so it was not possible to take infected leaf samples and no genotyping tests were done.

4.3.5. Economics field experiments year 1

2014 was a high septoria disease year, with nearly 35% leaf area affected on average on leaf 2 of the untreated susceptible variety by mid-grain filling, with almost double this observed in the most affected individual trial. However, good control was still achieved within the high intensity fungicide programme (Figure 20). For a wheat price scenario of £120/t the most *Z. tritici* resistant wheat variety (Crusoe) returned the highest gross margin when comparing untreated varieties. When the varieties were fungicide treated, the moderate and resistant varieties Crusoe and Cubanita achieved gross margin optimum from the moderate intensity fungicide treatment, with a gross margin reduction seen from applying a high intensity fungicide programme to the more resistant varieties. For the susceptible variety conqueror there were gross margin benefits from applying the most intense fungicide programme (Figure 21).

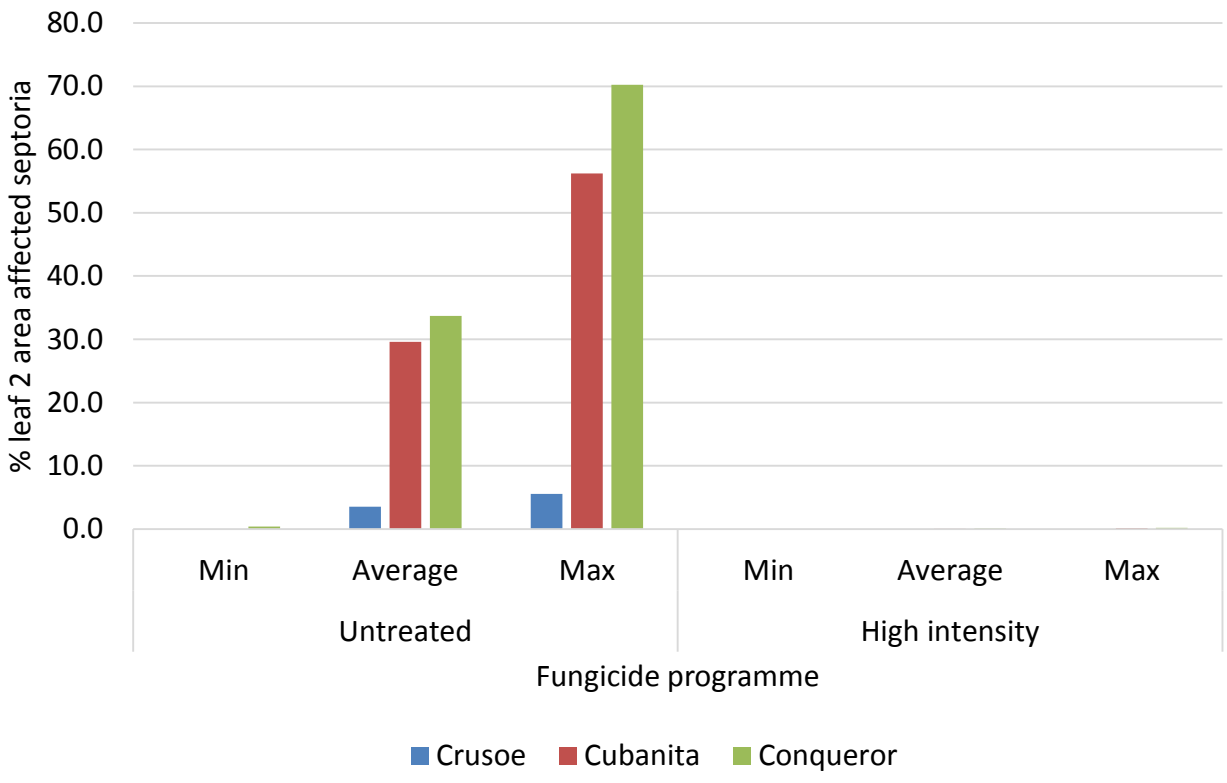

Figure 20. Mean, min and max leaf 2 septoria severity on untreated and high intensity treated plots from three trials in 2014 (Bayer Cambridgeshire, Bayer Herefordshire, DuPont Herefordshire).

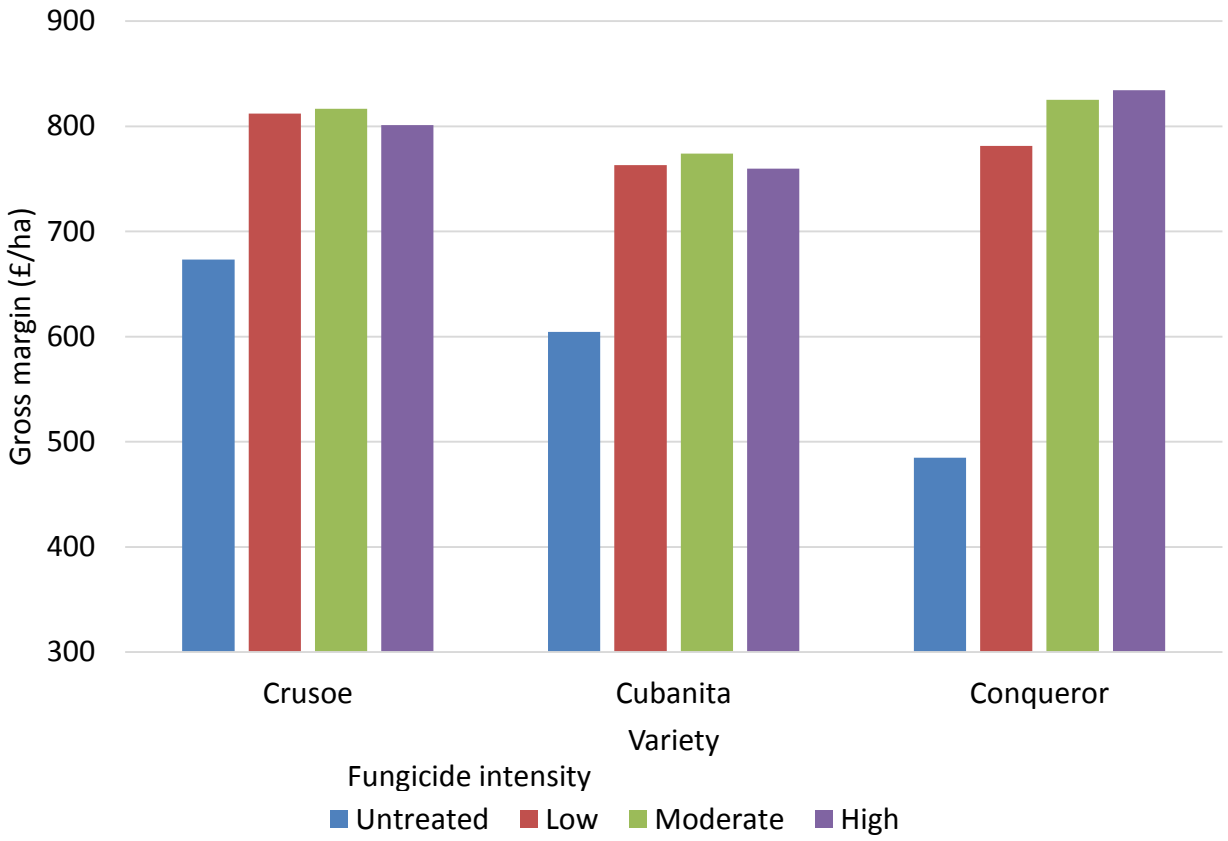

Figure 21. Gross margin analysis for wheat at £120/t, 2014, industry partner sites. Wheat variety = Crusoe, resistant; Cubanita, intermediate; Conqueror, susceptible. Fungicide: Fpr <0.001, LSD 46.75. Variety: Fpr 0.006, LSD 30.17. Fungicide.Variety: Fpr <0.001, LSD 66.29.

4.3.6. Economics field experiments year 2

The wheat varieties used in 2015 were changed because of adjustments to the RL ratings for septoria resistance after the 2014 AHDB variety trial data was included. Different varieties were selected to provide as much of a spread as possible between the high and low ratings. The varieties used were: resistant, Cougar RL7; intermediate, Zulu RL5; susceptible, Gallant RL4.

In contrast to the previous year, 2015 had low septoria disease pressure, with less than 10% leaf area affected on average in the untreated susceptible standard, and 32% leaf area on the most susceptible untreated variety in the individual trial with the highest disease severity (**Figure 22**). At £120/t, there was little or no economic benefit from applying fungicides on the most resistant variety Cougar and the moderately resistant variety Zulu. For the susceptible variety Gallant there was a gross margin benefit from applying the low intensity fungicide programme, although the economic return was negative when programme strength was increased above the low intensity programme. The untreated resistant variety had the highest gross margin in the 2015 trials (Figure 23).

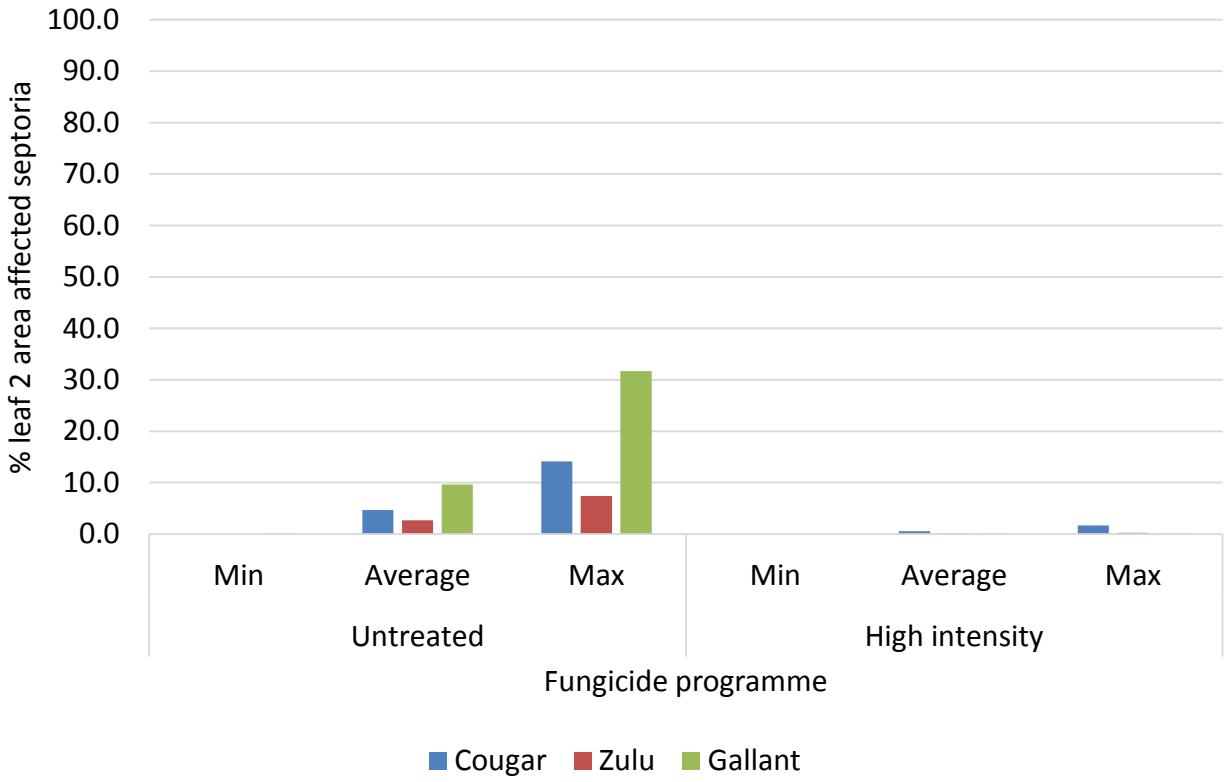

Figure 22. Mean, min and max leaf 2 septoria severity on untreated and high intensity treated plots from Four trials in 2015 (Bayer Cambridgeshire, Bayer Herefordshire, DuPont Herefordshire, Syngenta Herefordshire).

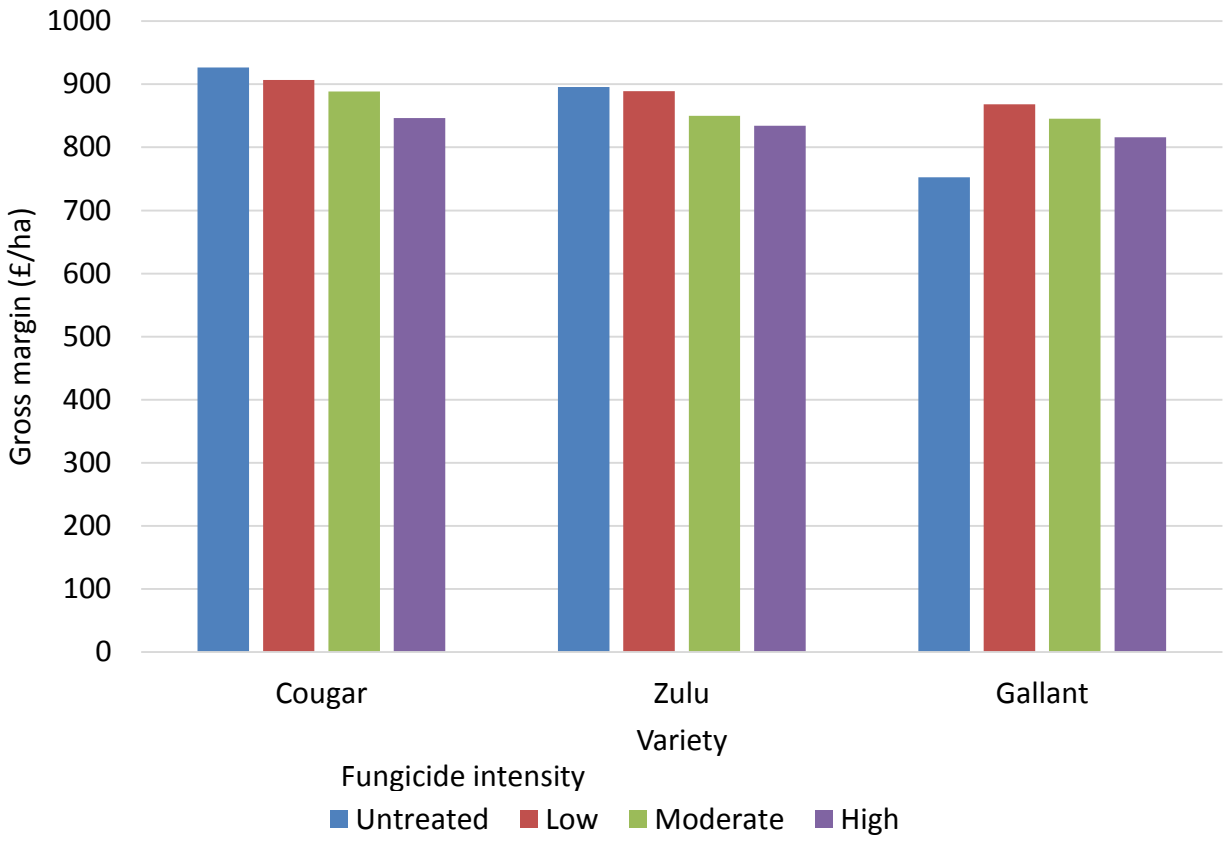

Figure 23. Gross margin analysis for wheat at £120/t, 2015, industry partner sites. Wheat varieties = Cougar, resistant; Zulu, intermediate; Gallant, susceptible. Fungicide: Fpr 0.024, LSD 35.3. Variety: For <0.001, LSD 30.57. Fungicide.Variety: Fpr 0.007, LSD 61.14.

4.3.7. Economics field experiments year 3

In contrast to the previous year, 2016 had relatively high septoria disease pressure at many of the trial sites, similar to levels seen in 2014 (Figure 24). At £120/t, there was an economic benefit from applying fungicides on all varieties, although the optimal intensity of fungicide application varied across the varieties. On the resistant variety Cougar there were positive economic benefits for increasing fungicide intensity to the moderate intensity programme. For the moderately resistant variety, Zulu, there was a trend for the low intensity programme to have the greatest economic return. For the susceptible variety Gallant the economic return from fungicide treatments was greater with the increased intensity of the fungicide programme, with the high intensity programme having the greatest economic benefit (Figure 25).

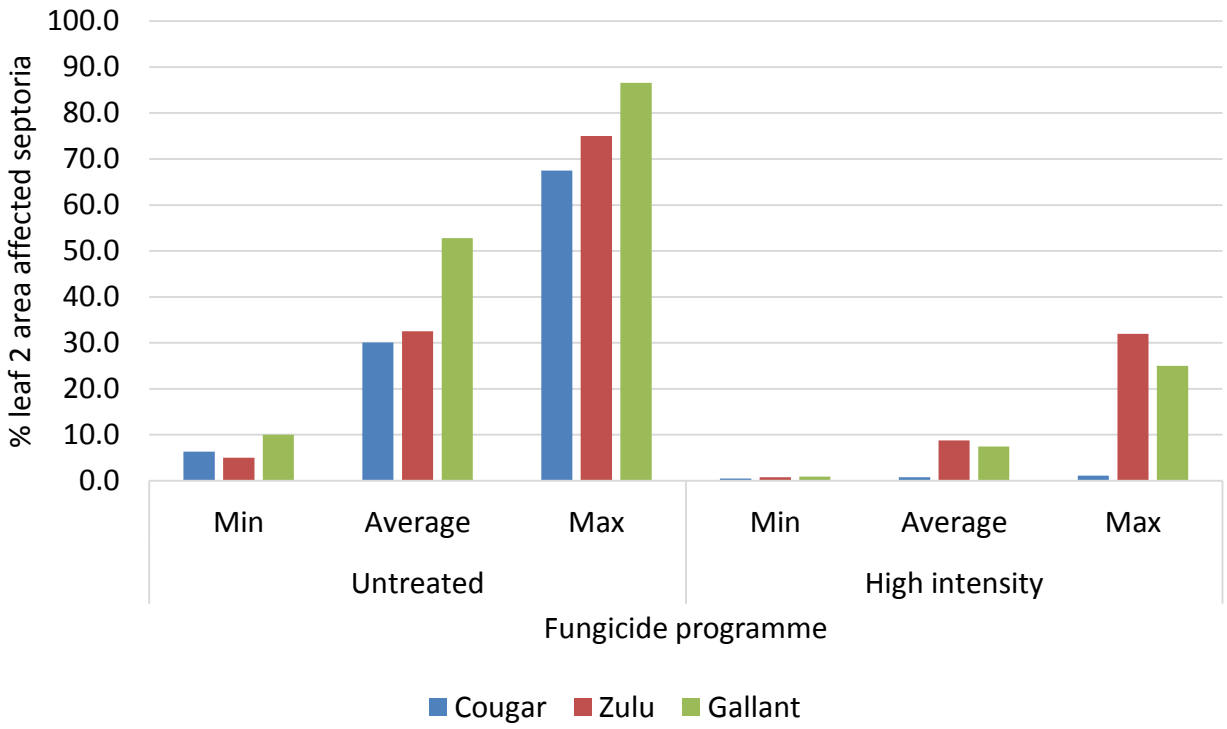

Figure 24. Mean, min and max leaf 2 septoria severity on untreated and high intensity treated plots from three trials in 2016 (Bayer Cambridgeshire, Bayer Herefordshire, DuPont Herefordshire).

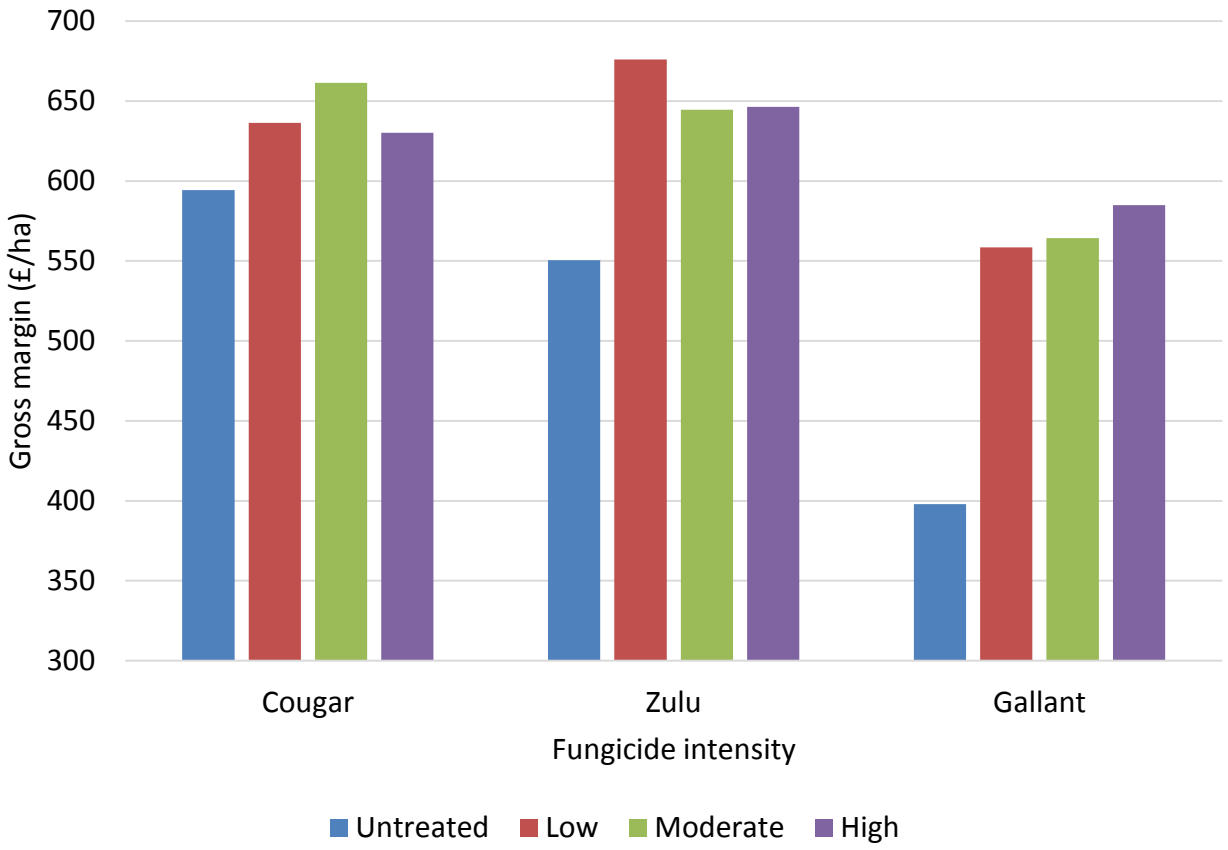

Figure 25. Gross margin analysis for wheat at £120/t, 2016, industry partner sites. Wheat varieties = Cougar, resistant; Zulu, intermediate; Gallant, susceptible. Fungicide: Fpr <0.001, LSD 21.92. Variety: Fpr <0.001, LSD 18.98. Fungicide.Variety: Fpr <0.001, LSD 37.97.

4.3.8. Effect of wheat price on industry partner gross margin trials

Gross margin data from across all years of the project was averaged across varieties at two grain prices, £100/t and £200/t to demonstrate how grain pricing effects the justification for intensity of fungicide programmes. Data presented in Figure 26 are the gross margin averages from across varieties from all industry trials in this project (n14). When grain prices are assumed to be £100/t, as fungicide intensity was increased above the low intensity fungicide programme there was a trend for the gross margin to decline significantly between both the low and moderate intensity programmes, and also the moderate and the high intensity programmes. When the price of grain was assumed to be £200/t there was a trend for the moderate and high intensity programmes to have the most economic return, with both these programmes having a significantly higher gross margin than the low intensity programme, although no significant differences were observed between the moderate and the high intensity programmes. It is important to note that the averages in **Figure 26** cover three different seasons, and six different varieties so results from individual varieties in each trial in each seasons and location will vary. Gross margins for each variety category (resistant, intermediate or susceptible), for £100/t and £200/t, are shown in Appendix 1.

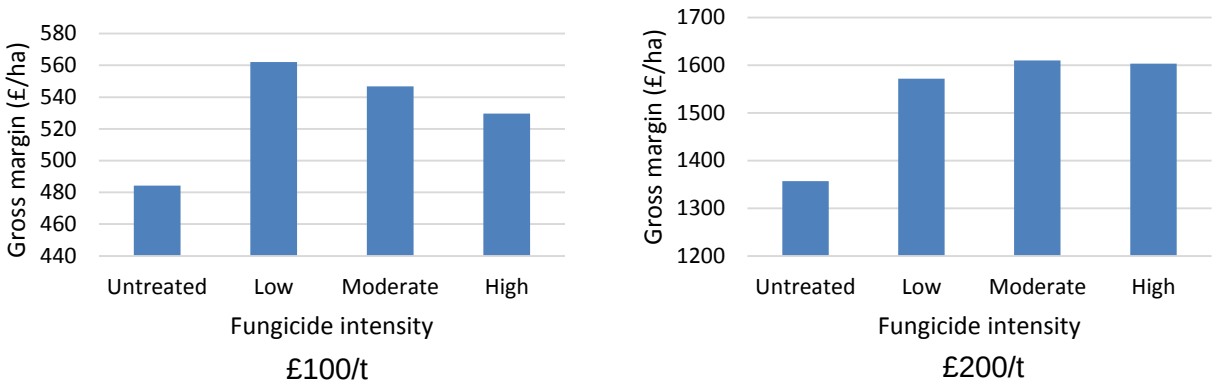

Figure 26. Gross margin analysis comparing programme economics at £100/t (left) and £200/t (right), industry partner sites. Average across all varieties in all 14 trials from 2014-2016. £100 Fpr <0.001, LSD 15.08. £200 Fpr <0.001, LSD 30.16.

4.3.9. *In-vitro* sensitivity phenotyping results from industry partner field experiments

The effects of the low, moderate and high intensity fungicide programmes (using mixtures of MOA) at the industry partner sites were smaller than at the research experiment sites where the focus was on single MOA fungicide treatments. Over one season there was a small shift towards insensitivity of septoria isolates at some sites and for some fungicide MOA, but not all. For example, at ADAS Rosemaund, on variety Zulu (intermediate resistance RL5) there was a small shift towards epoxiconazole, prothioconazole and bixafen insensitivity with treatment, mainly with the highest intensity treatment 4, but no shift in folpet sensitivity (**Error! Reference source not found.Error! Reference source not found.**). The effects were small and therefore need to be interpreted with caution. Samples for testing were not available from all sites. See Appendix 1 for phenotyping results from other industry partner sites and other wheat varieties.

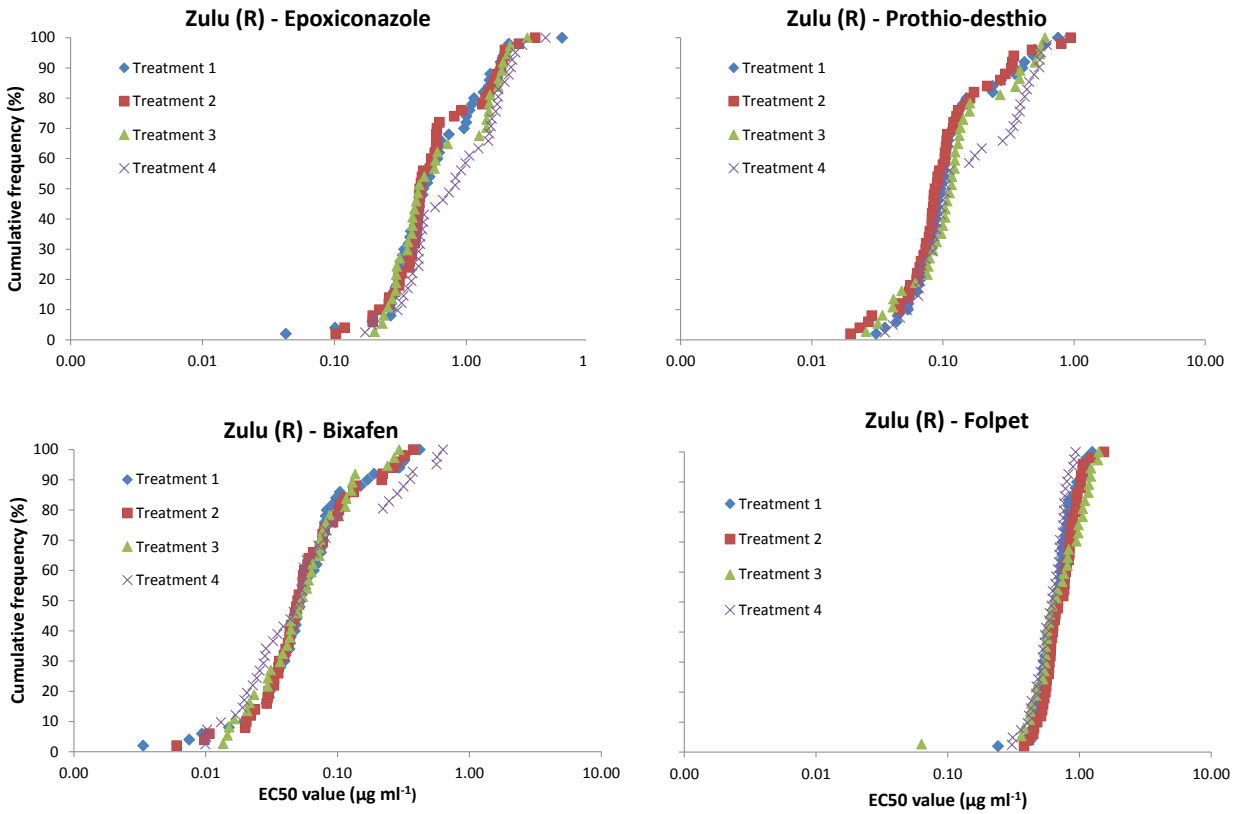

Figure 27. Cumulative frequency distributions of fungicide sensitivity of isolates of *Z. tritici* to four different active substances, on variety Zulu (intermediate resistance to septoria: RL5). Samples were taken late season at ADAS Rosemaund 2015 following fungicide programmes of low to high intensity on variety and tested in vitro. Treatments 1, 2, 3 and 4 = zero, low, intermediate and high intensity fungicide programmes, respectively.

For the isolates tested from trials in 2016, the frequency of mutations associated with varying degrees of SDHI insensitivity was low for all treatments at each of the industry partner trial sites. The C-H152R mutation, which is associated with a high degree of insensitivity to SDHIs, was not detected in any of the samples tested. Signals from the pyrosequencing for mutations associated with low or moderate insensitivity (C-T79N, C-W80S, C-N86S) were found, but these were in most cases below the detection threshold of 4%, so it is uncertain whether they were false positive signals. No significant variety or fungicide treatment effects were observed when mutation frequency was analysed for each mutation separately. Where results from each mutation were combined to give a total mutation frequency a trend was observed for the most resistant variety (Cougar) to have a lower mutation frequency than the moderate (Zulu) and the most susceptible variety (Gallant) but the results should be interpreted with caution. No treatment trends in mutation frequency were observed from fungicide treatments.

5. Discussion

5.1. Simulation modelling

The modelling work provided quantification of the extent to which integrated disease control (fungicide strategies, variety resistance and disease forecasting) could prolong the effective lives of new fungicide modes of action. For the first time, the work has linked models for fungicide action with models of variety disease resistance and disease forecasting systems. Models have been developed and applied previously for fungicide insensitivity (van den Bosch & Gilligan 2008; Hobbelen *et al*. 2011; van den Bosch *et al*. 2011b), for crop resistance (van den Bosch & Gilligan 2003; Lo Iacono *et al*. 2012) and disease forecasting (Pietravalle *et al*. 2003; te Beest *et al*. 2009b). In previous work, disease control options were considered in isolation, but in practice they should be integrated.

It should be emphasised that the model cannot predict how many years resistance will take to develop, but it should predict to a useful degree, the relative effectiveness of different strategies. The model was parameterised to represent evolution of QoI resistance, which is an example of resistance occurring in a large single step (where the new resistant strain is insensitive to any field dose). The rate at which resistance evolves is determined by the difference in fitness (quantified as the intrinsic rate of growth of the population) of the sensitive and insensitive strains in the presence of the mode of action. The model is not, therefore, specific to a particular MOA or resistance with a specific genetic basis. Hence, the modelling results are relevant to different types of fungicide MOA; the qualitative relationships should stay the same. Further exploration of the model with different sets of parameters suggests that the rank order of effectiveness of different strategies should be similar for different pathogen/crop/fungicide MOA combinations).

The modelling showed that development of insensitive pathogens following fungicide treatment is slowed down by using resistant varieties. If fungicide treatments are applied at the same intensity on disease resistant and susceptible varieties, then a high degree of variety disease resistance may be required to have a substantial effect on slowing fungicide resistance. However, if variety disease resistance allows the intensity of fungicide treatments to be reduced (number of treatments and/or dose per treatment) then a greater reduction is obtained in the rate of selection for insensitive strains. Hence, the effective life of a fungicide mode of action can be substantially prolonged. This conclusion is supported by the results from the field experiments testing the effect of variety and intensity of fungicide programmes on selection for azole insensitive isolates. In those experiments, the effect of variety resistance on selection was unclear when the same fungicide programme was applied to three varieties contrasting in septoria resistance. Whereas, a more intense fungicide programme on a susceptible variety resulted in substantially more selection than a less intense programme on a

septoria resistant variety. In terms of efficacy, in a location where there are already fungicide-insensitive pathogens present, increasing the fungicide dose on a resistant crop variety will reduce infection or have no impact. However, on a susceptible variety, increasing the fungicide dose will either have no impact or will increase infection.

Combining resistant varieties with other management strategies, particularly the use of disease forecasting to guide fungicide treatment, would further slow the rate of fungicide resistance development. This project has demonstrated for the first time the synergistic benefits of combining variety disease resistance and forecasting for managing fungicide resistance. The forecasting scheme used in the modelling work was a previously published scheme, which could be implemented for practical use. Many forecasting schemes have been advocated for different diseases, but have often fallen into disuse when users experience economic losses from a false negative prediction (when the forecast predicts that a damaging epidemic will not occur, but a damaging epidemic does occur). The work here shows that such outcomes are quite likely, and can be very economically deleterious, leading to no net economic benefit from use of the system, when growing disease susceptible varieties. Whereas, the predictions are more reliable and can provide net economic benefits when growing disease resistant varieties.

Hence, there are three mechanisms by which variety resistance can slow fungicide resistance:

- A sufficient degree of host resistance can reduce the rate of selection for fungicide resistant strains.
- Reducing the intensity of fungicide treatments on disease resistant varieties, reduces selection further.
- There are good economic benefits from using disease forecasting schemes with disease resistant varieties, and adjusting fungicide inputs according to disease risk reduces unnecessary treatments and, thus, further reduces selection for resistant strains.

5.2. Field experiments and evidence for selection effects

The main aim of the field experiments was to provide evidence on the consequences for fungicide resistance from using resistant wheat varieties, in combination with different fungicide dose rates and application frequency, to underpin modelling work. Field experiments each spanning one season were repeated in different years and sites, with the aim of corroborating modelling work which showed the effective life of fungicide MOA in years.

Field experiments using natural infection of *Z. tritici*, an increased total dose of fungicide MOA and an increased number of applications resulted in a clear increase in selection for less sensitive isolates, associated by a decrease in efficacy. Previously published field research on a range of

pathogens, crops and fungicide modes of action, also showed that increasing the number of foliar applications of the same mode of action results in increased selection for insensitive strains of pathogens (van den Bosch, Oliver *et al*., 2014, Hunter *et al.,* 1987, Sanders *et al.,* 1985).

When the same treatments of increased total dose of fungicide MOA and an increased number of applications were applied to varieties differing in resistance to *Z. tritici*, the more susceptible variety generally resulted in the highest selection for less sensitive isolates, compared with the same treatments on moderate or high resistance varieties. This contributes to the evidence that use of resistant varieties can substantially slow the development of pathogen fungicide resistance. The underlying rationale is that using a resistant variety will slow down the speed at which insensitive strains become dominant in the pathogen population (because the difference in relative epidemic growth rates between fungicide sensitive and fungicide insensitive strains is decreased). In addition, resistant varieties allow effective control to be obtained with a lower total fungicide dose (fewer treatments per crop or lower dose per treatment) (van den Bosch *et al*, 2011).

The use of mixtures can only ameliorate the selection for pathogen resistance to fungicides to some degree. The small shifts seen in sensitivity phenotype from the industry partner trials were found for the single-site acting fungicides, but not the multi-site. This may indicate selection for strains which have efflux mechanisms (which usually only affect sensitivity to single site acting fungicides). The high fungicide programme included two SDHI treatments and four azole treatments, all of which add to the selection pressure for such strains.

There has been some progress towards more use of resistant varieties and the results from this project will encourage this by highlighting the benefits of using these varieties. The results provide further evidence for plant breeders to emphasise the identification and deployment of resistance genes, but the case for resistant varieties will be strengthened by identifying genes with a low physiological cost to the plant, i.e. developing resistant varieties with higher yield potential. Fungicide input costs show no signs of stabilising, despite variations in disease pressure from year to year. The percentage of cereal crops treated has been increasing since 2001 and was highest in 2015 (Defra Agricultural statistics 2015), yet 2015 was a low septoria year. Increasing use of fungicides will encourage the development of fungicide resistant pathogens and consequent loss of control, so the use of resistant varieties may become increasingly important.

5.3. Economic analysis of industry partner field experiments

The gross margin results show how the fungicide inputs could be adjusted to take account of variety septoria resistance, thus reducing the intensity of fungicide programmes in certain situations, without decreasing the economic return from the crop. By choice of varieties, high risk of yellow and brown rust was avoided, despite many of the trial sites being in regions where yellow rust occurred. In 2014

the highest level of septoria recorded over the last 25 years was observed in treated farm crops (Crop monitor, 2016). Despite this exceptional disease pressure, the gross margin trial results in 2014 demonstrated that on a septoria resistant or moderately resistant variety, optimum gross margins could be achieved from a moderately intense fungicide programme, with no further economic benefit from using a high intensity fungicide programme. Where a susceptible variety was grown in a high disease situation, a high intensity fungicide programme was economically justified. By contrast, in a low septoria year such as 2015 there were no economic benefits from applying a fungicide programme above that of a low intensity programme, regardless of the variety resistance rating.

In summary, the results of the research partner trials provide strong evidence that high intensity fungicide programmes speed up the development of fungicide resistance by pathogens. The results of the industry partner field trials show that in many cases, particularly on more disease resistant varieties, high intensity fungicide treatments on crops are not justified economically.

5.4. Key messages and guidance

- Fungicide resistance is driven by the number of treatments and dose.
- Selection for pathogen insensitivity is ameliorated by effective mixtures of modes of action.
- Current fungicide programmes in the UK are economical for high disease pressure seasons on susceptible varieties.
- In some countries, pesticide regulators are restricting fungicide programmes in order to manage fungicide resistance. The preferred approach is to retain flexibility of number of treatments, but ‘use responsibly’.
- Uncertainty about future disease progress drives risk-averse intensive fungicide programmes: this uncertainty can be reduced by implementing disease forecasting systems.
- Variety disease resistance:
- makes intensity of spray programme less critical
- makes the use of disease forecasting economically viable
- reduces losses if fungicide timing is not optimal (due to lack of spray windows)
- Integrated disease management strategies, which combine disease resistant varieties and fungicide use guided by forecasting, could substantially slow the selection for fungicide resistance.

5.5. Future research

- Field work and modelling evidence is needed to determine the effect of resistance management strategies (e.g. mixtures of fungicides with different MOA) when resistance is evolving concurrently against two or more MOA. This situation is occurring, for example, with the continued evolution of azole insensitivity in *Z. tritici* and the selection for new strains which have some degree of insensitivity to SDHIs. This is being addressed in an AHDB and industry-funded project, 'Managing resistance evolving concurrently against two or more modes of action, to extend the effective life of new fungicides', AHDB project 21120052 (01/01/17 to 31/03/21).
- Field experiment evidence to validate model findings is still a priority. There is a window of opportunity to use naturally occurring resistance evolution to quantify resistance selection from different treatments in field experiments. This window of opportunity lasts from the point at which a new insensitive strain becomes reasonably widespread, to the time at which the proportion of the resistant strain becomes too high, thus limiting further selection. Such a window of opportunity is occurring now for SDHI insensitive *Z. tritici*. This window of opportunity is being exploited in the AHDB and industry-funded project (see above).
- Uptake of disease resistant varieties, the economics of crop production, and the effective life of new fungicide modes of action could all be improved by the development of varieties with resistance genes which have a low physiological cost to the plant, i.e. disease resistant varieties with high yield potential.
- Accurate forecasting of septoria infection is essential. An analysis to determine which of the current septoria forecast models work best in the UK would help increase the uptake of forecasting, by demonstrating the benefits of combining variety disease resistance with disease forecasting for managing fungicide inputs.

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7. Appendix 1. Gross margin results from industry partner trials

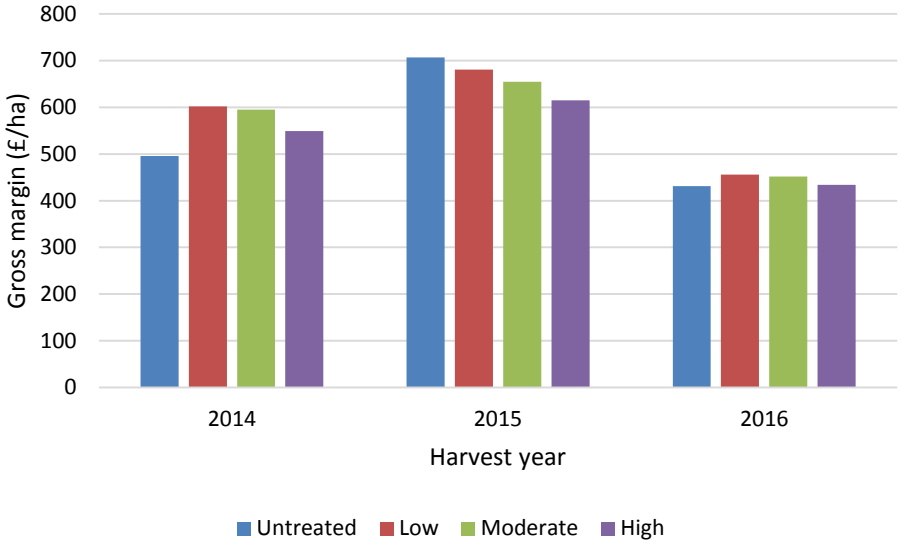

Figure 28. Gross margin analysis for least susceptible wheat varieties at £100/t in years 2014, 2015 and 2016 in industry partner sites. Year: Fpr 0.003, LSD 99.2. Fungicide: Fpr 0.045, LSD 33.1. Year.Fungicide: Fpr 0.017, LSD 104.5.

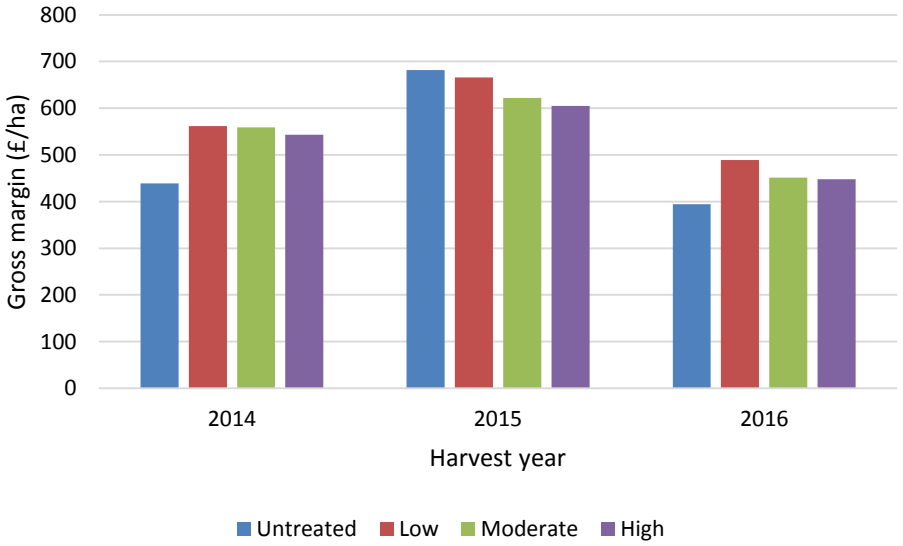

Figure 29. Gross margin analysis for moderately susceptible wheat varieties at £100/t in years 2014, 2015 and 2016 in industry partner sites. Year: Fpr <0.001, LSD 58.2. Fungicide: Fpr <0.001, LSD 22.8. Year.Fungicide: Fpr <0.001, LSD 63.1.

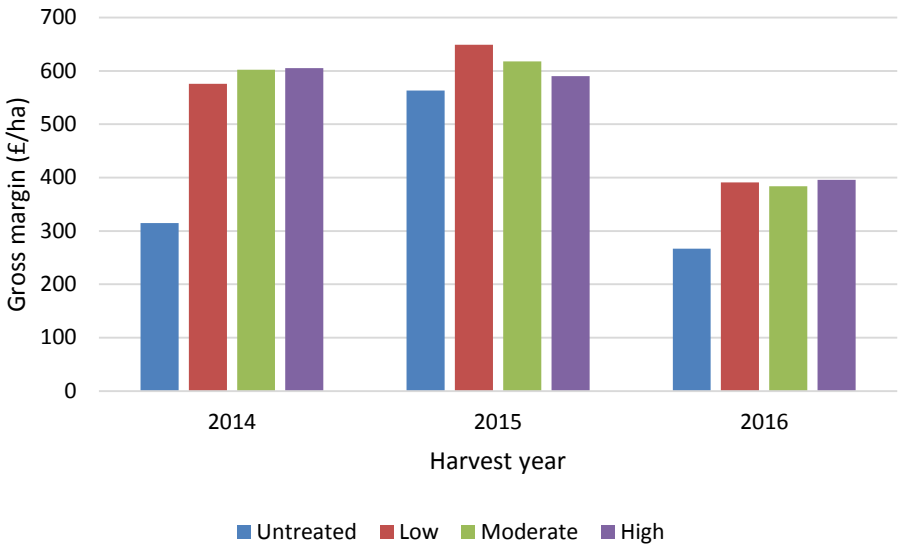

Figure 30. Gross margin analysis for susceptible wheat varieties at £100/t in years 2014, 2015 and 2016 in industry partner sites. Year: Fpr <0.001, LSD 68.5. Fungicide: Fpr <0.001, LSD 39.9. Year.Fungicide: Fpr 0.001, LSD 84.9.

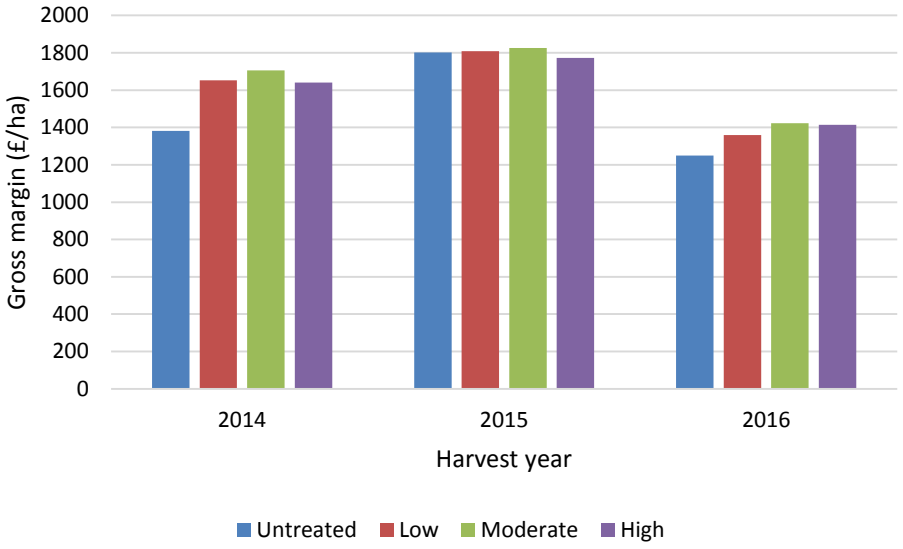

Figure 31. Gross margin analysis for least susceptible wheat varieties at £200/t in years 2014, 2015 and 2016 in industry partner sites. Year: Fpr 0.003, LSD 198.5. Fungicide: Fpr <0.001, LSD 66.1. Year.Fungicide: Fpr 0.017, LSD 209.0.

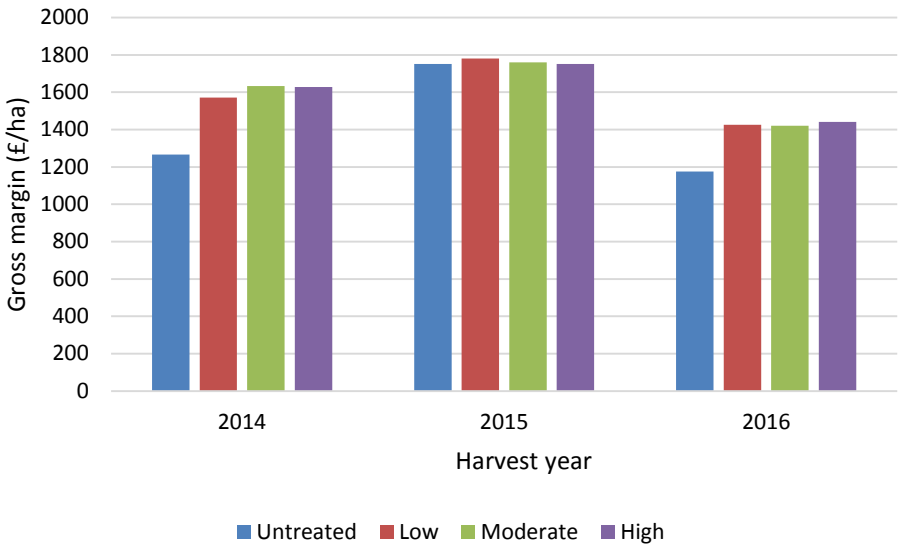

Figure 32. Gross margin analysis for moderately susceptible wheat varieties at £200/t in years 2014, 2015 and 2016 in industry partner sites. Year: Fpr <0.001, LSD 116.3. Fungicide: Fpr <0.001, LSD 45.6. Year.Fungicide: Fpr <0.001, LSD 126.2.

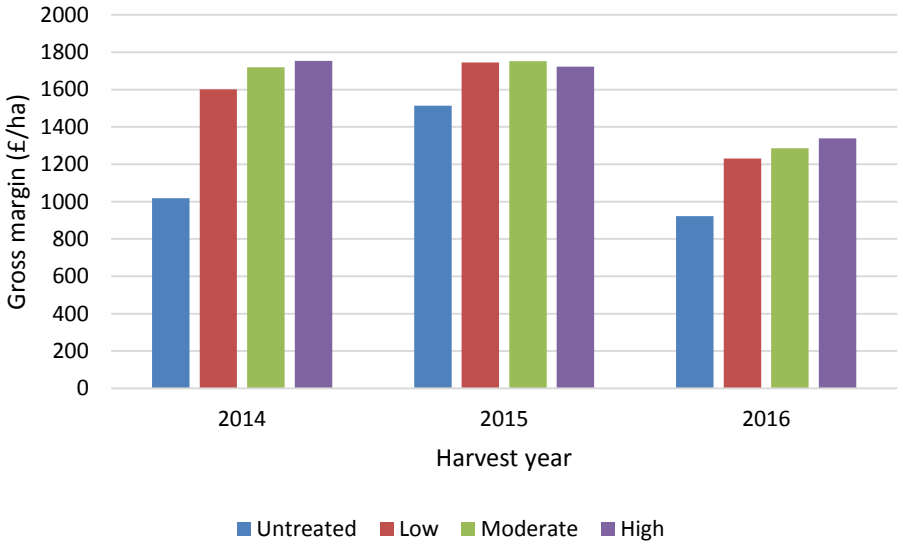

Figure 33. Gross margin analysis for susceptible wheat varieties at £200/t in years 2014, 2015 and 2016 in industry partner sites. Year: Fpr <0.001, LSD 136.9. Fungicide: Fpr <0.001, LSD 79.8. Year.Fungicide: Fpr 0.001, LSD 169.8.

8. Appendix 2. Additional phenotyping results from 2015 industry partner field trials

The ECC50 data for septoria isolates from the industry partner trials provided some evidence for a shift to more resistant septoria isolates (higher EC50 values) following treatment with fungicides, mainly with epoxiconazole and prothioconazole-desthio. In addition to the late-season (= post-treatment) data shown for variety Zulu in Figure 27 (in report section 4.3.9), isolates from some, but not all, of the other sites and varieties also had a shift to higher EC50 values following treatment with some fungicide modes of action (MOA) with the most intensive fungicide programme compared with untreated, for example, for epoxiconazole, prothioconazole-desthio and bixafen on variety Gallant (relatively susceptible to Septoria) in Herefordshire (Figure 34); similarly for variety Cougar in Herefordshire (relatively resistant) (Figure 35) and variety Zulu (intermediate resistance) in S Yorks. (Figure 37).

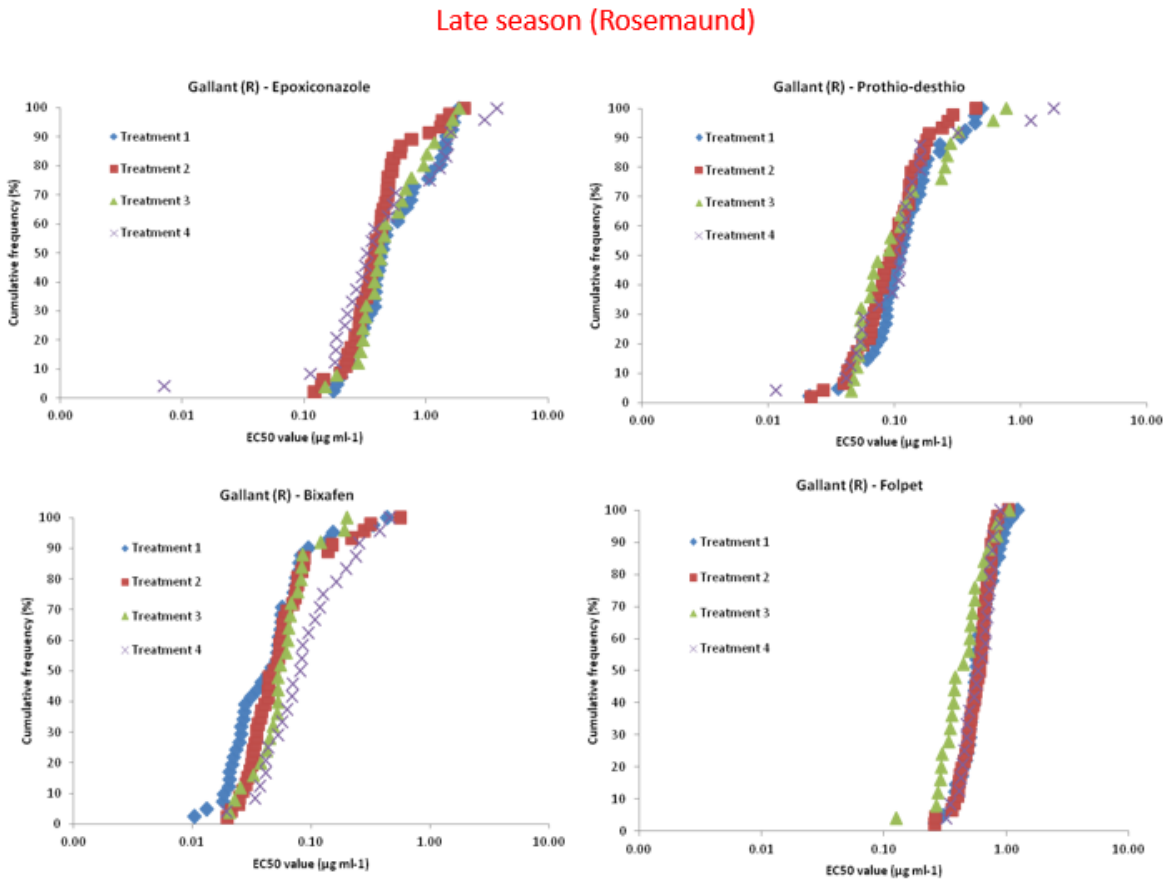

Figure 34. Cumulative frequency distributions of fungicide sensitivity of isolates of *Z. tritici* to four different active substances, on variety Gallant (susceptible to septoria: RL4). Samples were taken late season at ADAS Rosemaund 2015 following fungicide programmes of low to high intensity on variety and tested in vitro. Treatments 1, 2, 3 and 4 = zero, low, intermediate and high intensity fungicide programmes, respectively.

On a resistant variety, Cougar, there was a difference in the distribution of EC50 values for isolates of Septoria for epoxiconazole from untreated, low, moderate or high intensity fungicide treatments (Figure 35.).

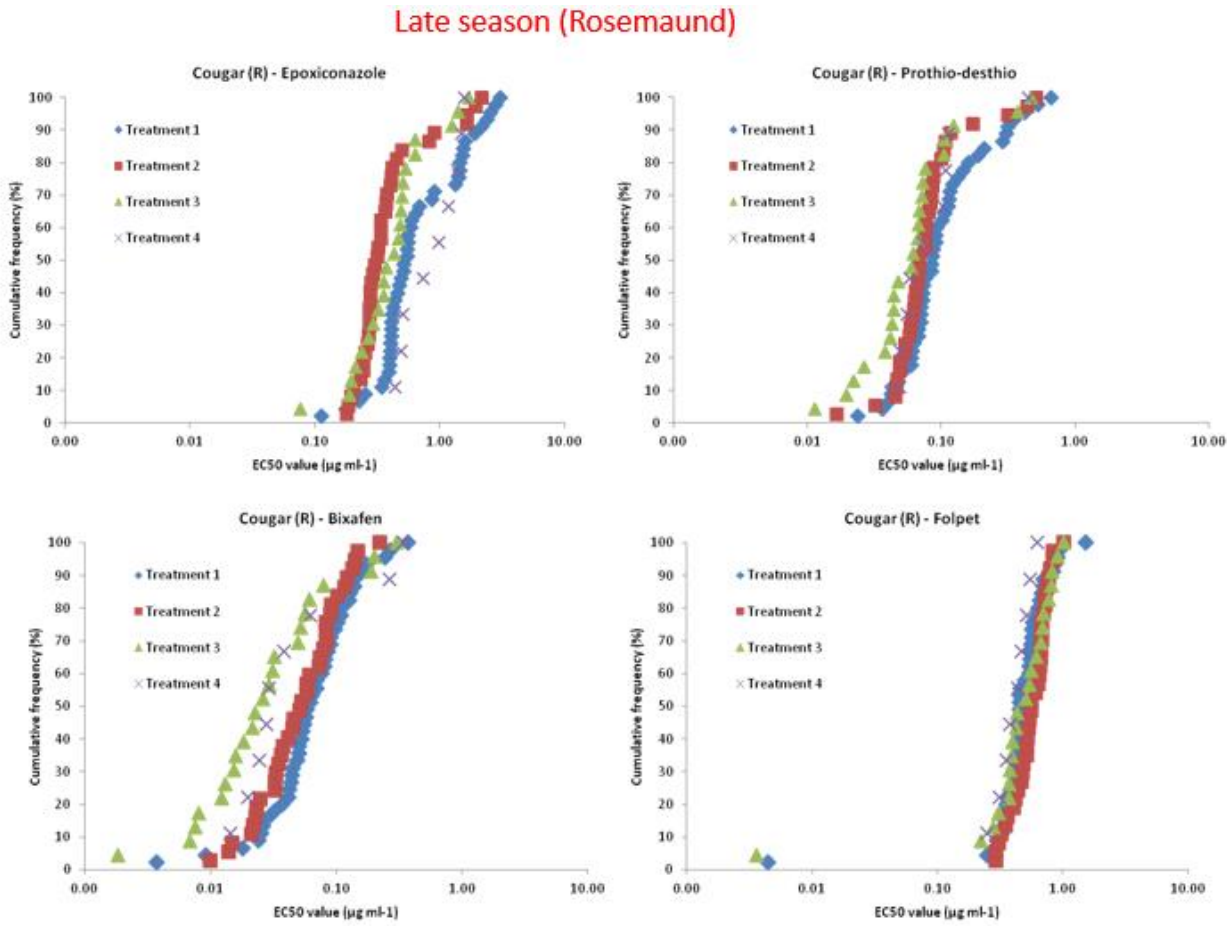

Figure 35. Cumulative frequency distributions of fungicide sensitivity of isolates of *Z. tritici* to four different active substances, on variety Cougar (resistant to septoria: RL7). Samples were taken late season at ADAS Rosemaund 2015 following fungicide programmes of low to high intensity on variety and tested in vitro. Treatments 1, 2, 3 and 4 = zero, low, intermediate and high intensity fungicide programmes, respectively.

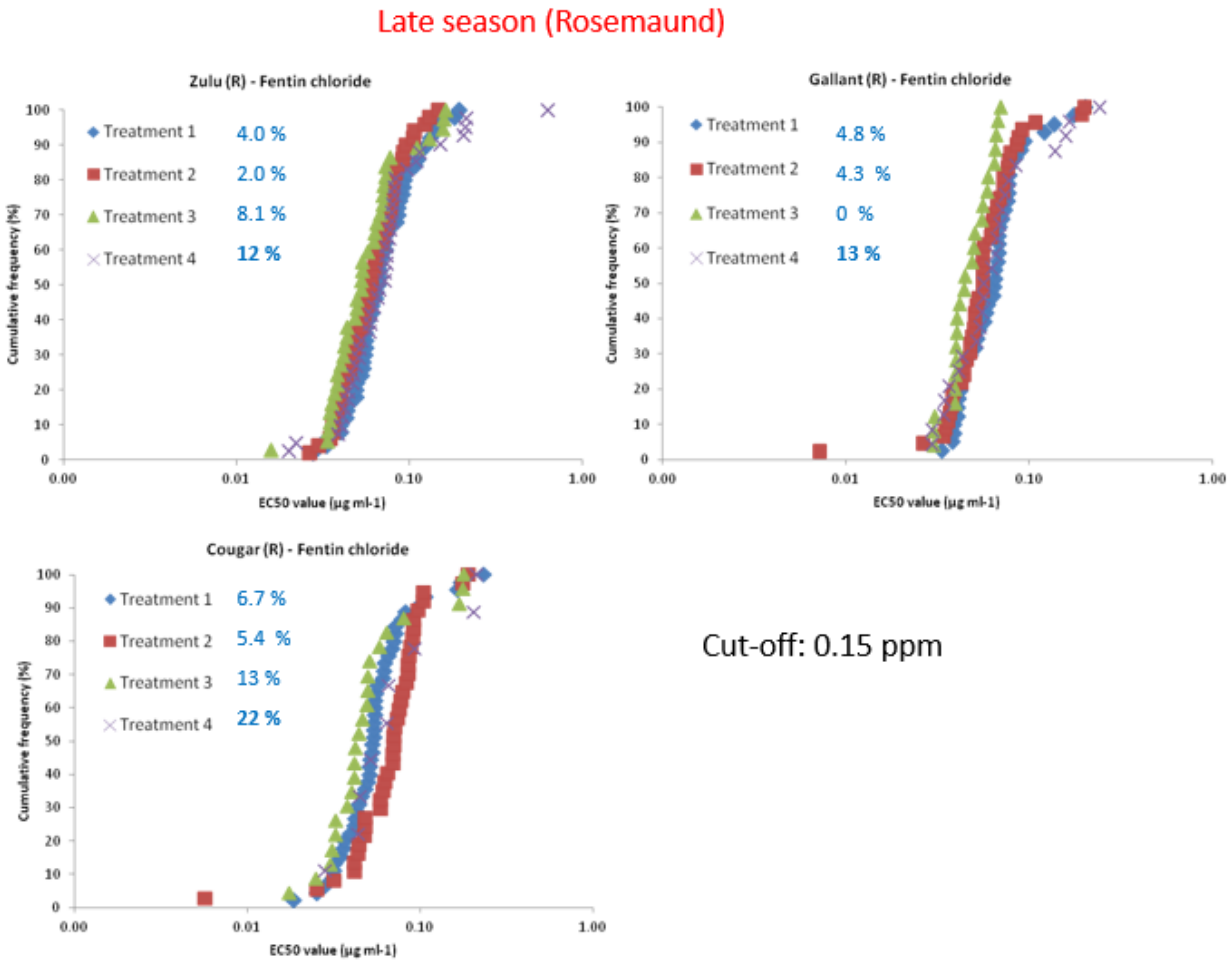

Figure 36. Cumulative frequency distributions of fungicide sensitivity of isolates of *Z. tritici* to fentin chloride, on varieties Zulu, Gallant and Cougar (resistance ratings to septoria: RL 5, 4 and 7, respectively). Samples were taken late season at ADAS Rosemaund 2015 following fungicide programmes of low to high intensity on variety and tested in vitro. Treatments 1, 2, 3 and 4 = zero, low, intermediate and high intensity fungicide programmes, respectively.

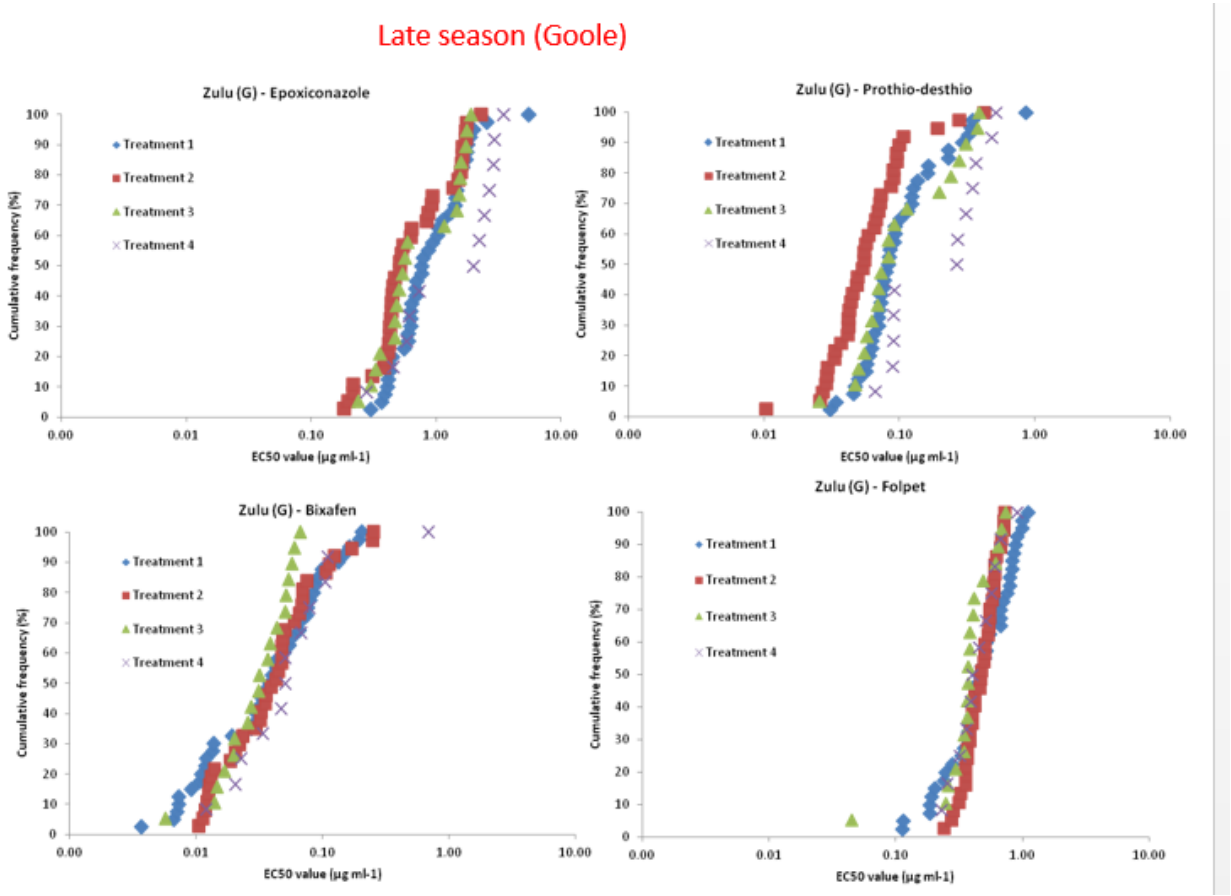

Figure 37. Cumulative frequency distributions of fungicide sensitivity of isolates of *Z. tritici* to four different active substances, on variety Zulu (intermediate resistance to septoria: RL5). Samples were taken late season at Goole, S Yorks, 2015, following fungicide programmes of low to high intensity on variety and tested in vitro. Treatments 1, 2, 3 and 4 = zero, low, intermediate and high intensity fungicide programmes, respectively.

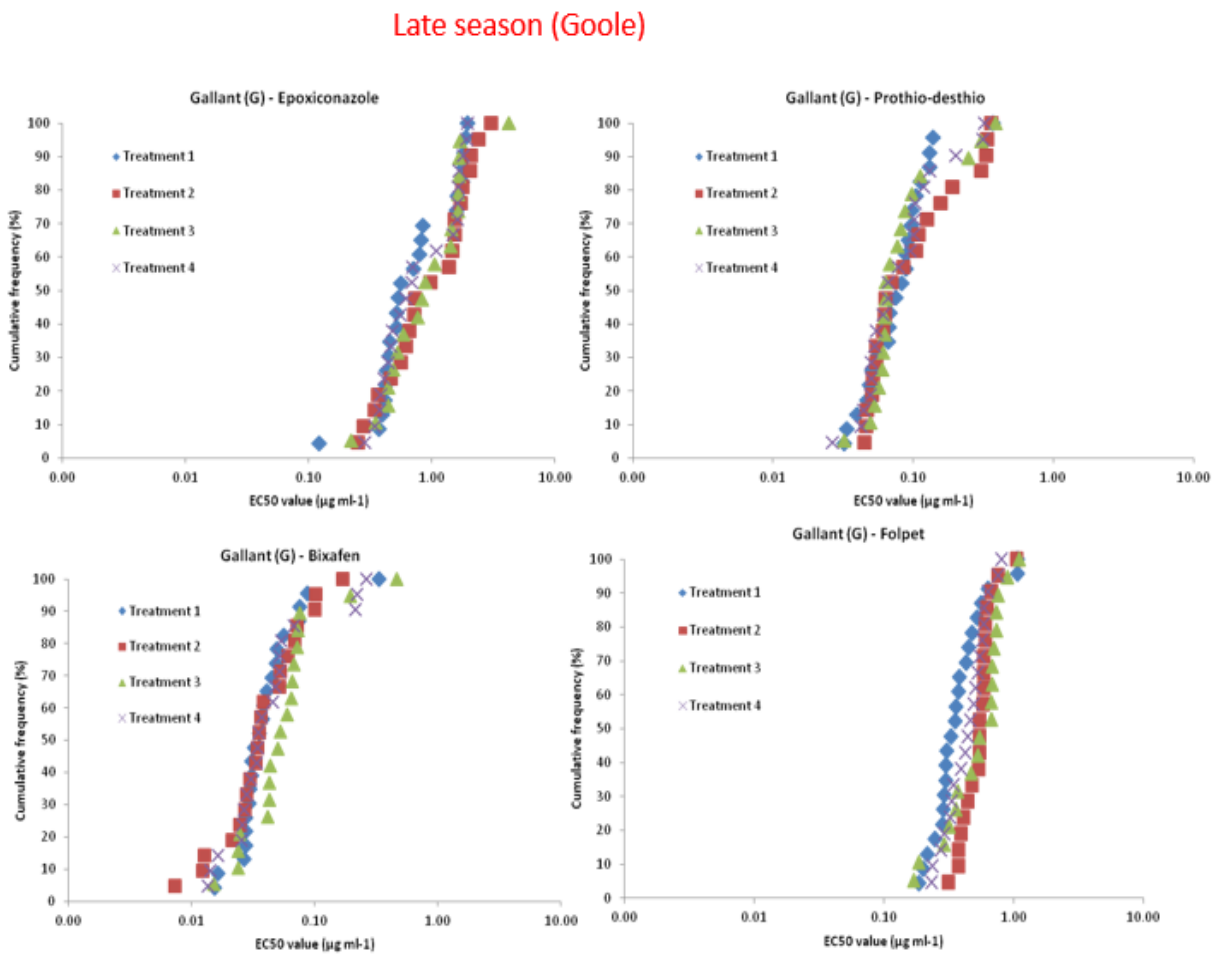

Figure 38. Cumulative frequency distributions of fungicide sensitivity of isolates of *Z. tritici* to four different active substances, on variety Gallant (susceptible to septoria: RL4). Samples were taken late season at Goole, Yorks, 2015, following fungicide programmes of low to high intensity on variety and tested in vitro. Treatments 1, 2, 3 and 4 = zero, low, intermediate and high intensity fungicide programmes, respectively.

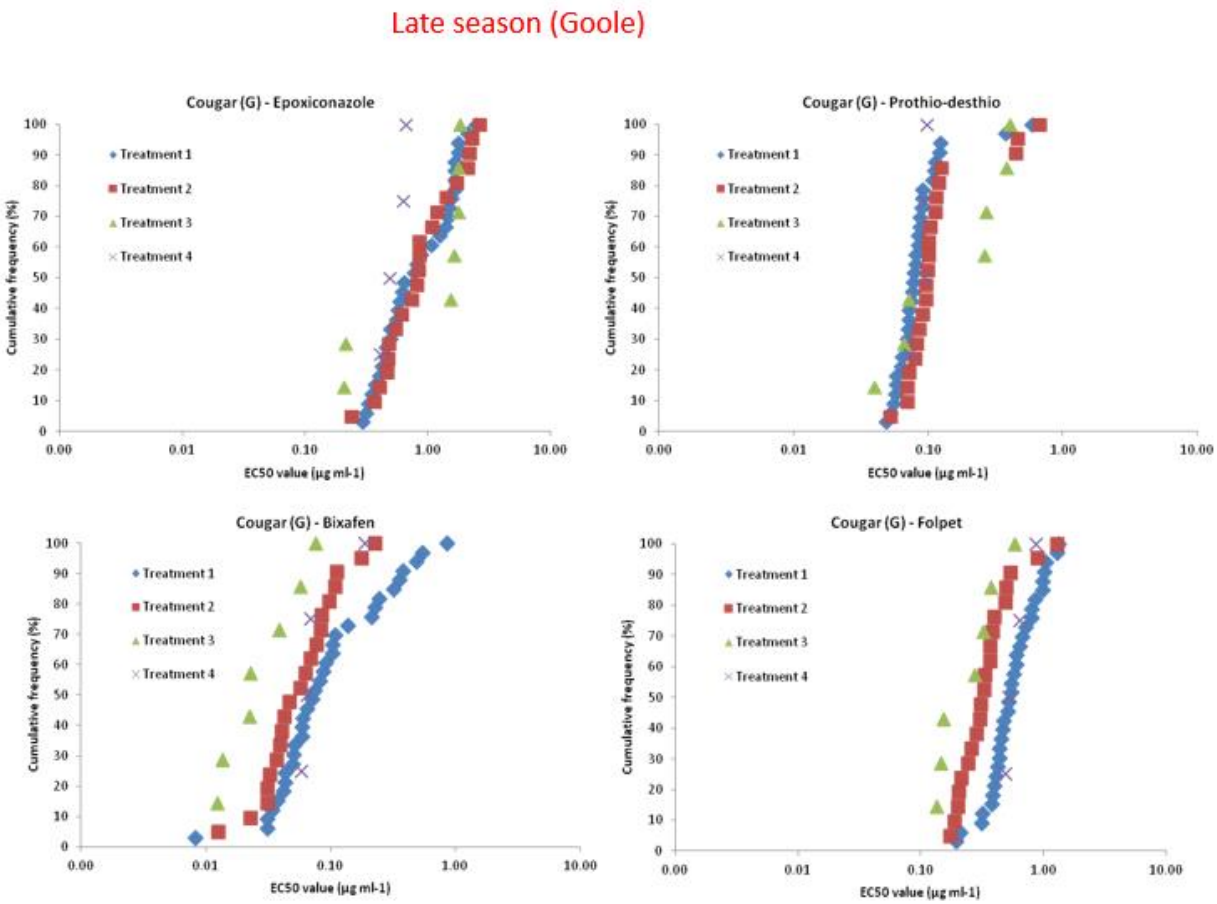

Figure 39. Cumulative frequency distributions of fungicide sensitivity of isolates of *Z. tritici* to four different active substances, on variety Cougar (resistant to septoria: RL7). Samples were taken late season at Goole, Yorks, 2015 following fungicide programmes of low to high intensity on variety and tested in vitro. Treatments 1, 2, 3 and 4 = zero, low, intermediate and high intensity fungicide programmes, respectively.

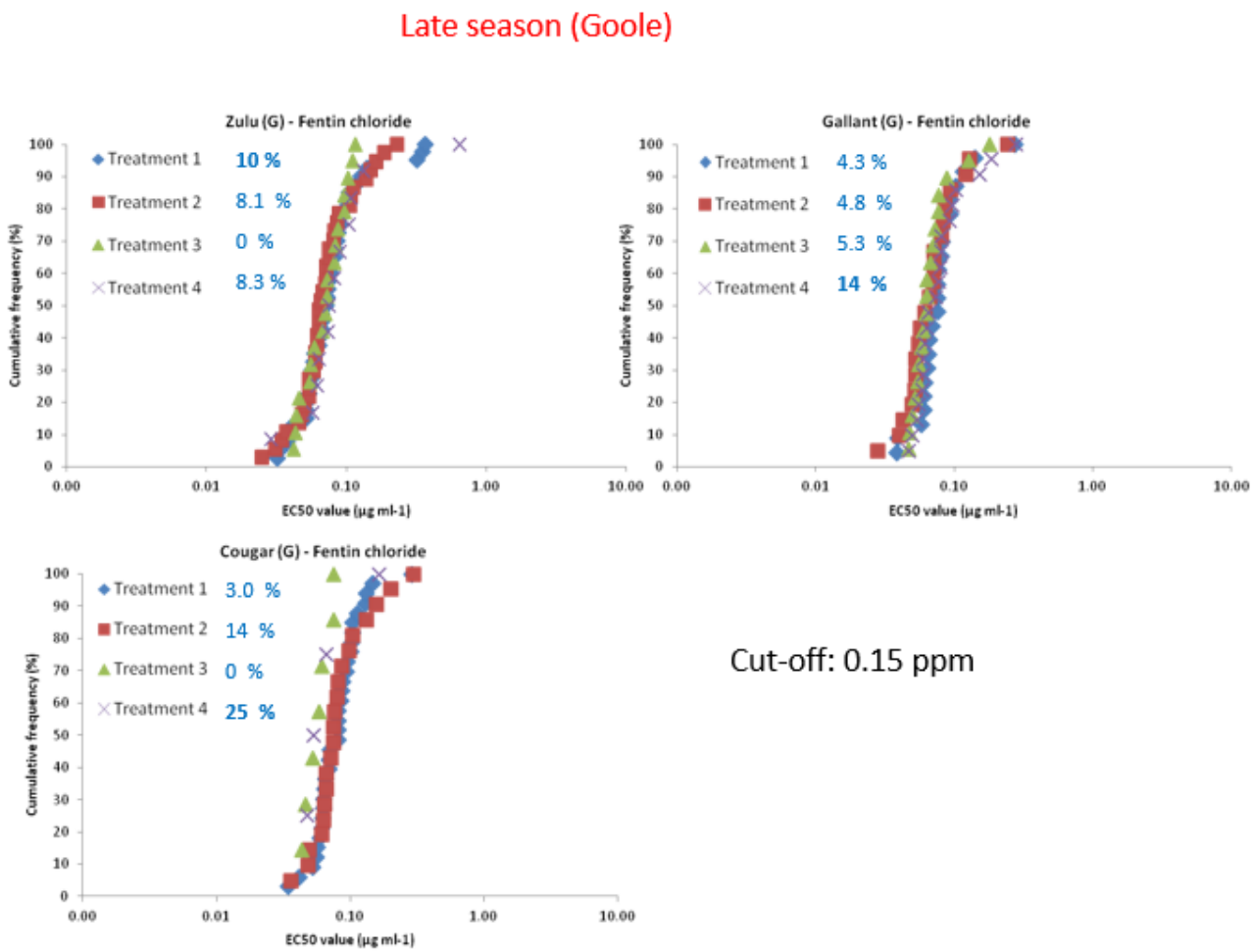

Figure 40. Cumulative frequency distributions of fungicide sensitivity of isolates of *Z. tritici* to fentin chloride, on varieties Zulu, Gallant and Cougar (resistance ratings to septoria: RL 5, 4 and 7, respectively). Samples were taken late season at Goole, Yorks, 2015 following fungicide programmes of low to high intensity on variety and tested in vitro. Treatments 1, 2, 3 and 4 = zero, low, intermediate and high intensity fungicide programmes, respectively.