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Fungicide performance on winter wheat

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1. ABSTRACT

Between 2008 and 2010 the efficacy of eight fungicides was tested in 21 replicated trials in wheat in the UK and Ireland. Four sites each year tested products on Septoria tritici. Three sites (one site per year per disease) tested product efficacy on powdery mildew, brown rust and yellow rust. The fungicides chosen were either new active ingredients, from the succinate de-hydrogenase inhibitor (SDHI) group, or new mixtures of existing chemistry, tested alongside core treatments representing current standards. The new fungicides have since been approved and are registered for use. Fungicides were evaluated as single applications at a range of doses, so that dose response curves could be fitted for each disease. In contrast to previous fungicide performance work, the trials tested products at T1 and T2 spray timings, and also included treatments applied at half dose at two application timings, to better reflect how products would perform in a more commercial situation. The main conclusions were as follows:

On Septoria tritici, the rank order of performance of fungicides was similar whether applied at T1 or T2 timings, and in both eradicant and protectant activity. Bravo (chlorothalonil) continued to show useful protectant activity. Opus (epoxiconazole) and Proline (prothioconazole) still provided good eradicant and protectant activity, and were equally matched. Brutus (epoxiconazole and metconazole) and Ennobe (epoxiconazole and prochloraz) provided better eradicant and protectant activity than could be explained simply by the higher triazole loading of these products. This may be due to a change of formulation or a synergy between the two triazoles. The new SDHI products Aviator Xpro (bixafen and prothioconaozole), Seguris (isopyrazam and epoxiconazole) and Adexar (fluxapyroxad and epoxicaonazole) gave better eradicant and protectant activity than the current standards, and there were higher than expected yield increases from these products when compared to Brutus and Ennobe.

On yellow rust all products were effective where a half dose or more of the product was applied. Products containing epoxiconazole (Brutus, Opus and Adexar) appeared to provide the highest levels of control. The addition of fluoxastrobin to prothioconazole (in Firefly) improved the level of control compared to prothioconazole (Proline) applied alone.

On brown rust, the strobilurin (e.g. pyraclostrobin) and SDHI (e.g. isopyrazam) fungicides tested were effective. When tested in mixture with prothioconazole (as Firefly and Aviator Xpro) or epoxicaonazole (as Adexar), they added significantly to activity compared to the triazole alone.

On powdery mildew, the specific mildewicides Talius (proquinazid), Cyflamid (cyflufenamid), Tern (fenpropidin) and Flexity (metrafenone) were effective in 2008, the only season with significant disease levels. SDHIs also appeared to control powdery mildew.

A fungicide calculator was developed to predict how sequences and mixtures of fungicides might perform. Data from the trials were used to validate the calculator. The calculator generally predicted performance within the variation between replicates of the experimental data.

Significant differences in Septoria tritici sensitivity to azoles were recorded between sites. Additionally the use of specific azoles appeared to result in higher proportions of certain mutations in the remaining population. This could lead to the build up of insensitive isolates where the same azoles, (or azoles that select in the same way) are used repeatedly.

2. FUNGICIDE PERFORMANCE IN WINTER WHEAT

2.1. Introduction

An effective disease control strategy plays an important role in winter wheat management, and current high wheat prices make it important to invest in fungicides that minimise yield loss to disease. Triazoles have a broad spectrum of activity and form the foundation for most fungicide programmes, although strobilurins still give useful control of rusts and chlorothalonil adds useful protectant activity against Septoria tritici. New fungicide products continue to be registered for use, in particular the Succinate Dehydrogenase Inhibitors (SDHIs) launched for wheat in 2011. These have a different mode of action, which broadens the armoury to control foliar diseases. New fungicides typically demand a premium price, so it is important that growers and agronomists have access to independent data on the performance of different fungicide products to enable a cost-benefit analysis.

Applying appropriate fungicide products at the right time and correct dose is critical for effective disease control. Use of sub- or super-optimal doses can result in reduced margins either through reduced yields or unnecessary expenditure.

Effective control of disease in wheat usually relies on using mixtures of fungicides that combine both protectant and eradicant ability. All diseases are most effectively controlled by protecting against infection, however, some products give effective eradicant activity that can prevent disease developing, even if the treatment is applied after infection. It is therefore important that differences between products in protectant and eradicant activity are determined so that the correct fungicides for each situation are selected at the right dose to ensure effective disease control.

This report provides a summary of the main findings from three years of fungicide performance experiments in harvest years 2008, 2009 and 2010. Dose response curves and graphs of sequential treatments show the activity of a range of fungicides against the major economic diseases in the UK. A full set of data can be found on the HGCA website (www.hgca.com).

2.2. Materials and methods

2.2.1. Experimental sites, cultivars and target diseases

Experiments were conducted over three harvest years (2008-2010) at 7 sites in the UK and Ireland. Sites and cultivars were selected to create high disease pressure for one of the following main foliar diseases of winter wheat – Septoria tritici, yellow rust, brown rust and mildew (table 1).

Table 1. Site numbers, locations, harvest years, cultivars and target diseases

Site number	Location	Harvest year	Cultivar	Target disease
1	Rosemaund, Herefordshire	2008	Ambrosia	Septoria tritici
2	Andover, Hampshire	2008	Ambrosia	Septoria tritici
3	Fife, Perthshire	2008	Consort	Septoria tritici
4	Terrington, Norfolk	2008	Robigus	Yellow rust
5	Biggleswade, Bedfordshire	2008	Alchemy	Brown rust
6	Fife, Perthshire	2008	Claire	Mildew
7	Carlow, Ireland	2008	Humber	Septoria tritici
8	Rosemaund, Herefordshire	2009	Ambrosia	Septoria tritici
9	Andover, Hampshire	2009	Ambrosia	Septoria tritici
10	Fife, Perthshire	2009	Consort	Septoria tritici
11	Terrington, Norfolk	2009	Robigus	Yellow rust
12	Biggleswade, Bedfordshire	2009	Hereford	Brown rust
13	Fife, Perthshire	2009	Claire	Mildew
14	Carlow, Ireland	2009	Einstein	Septoria tritici
15	Rosemaund, Herefordshire	2010	Ambrosia	Septoria tritici
16	Andover, Hampshire	2010	Ambrosia	Septoria tritici
17	Fife, Perthshire	2010	Consort	Septoria tritici
18	Terrington, Norfolk	2010	Robigus	Yellow rust
19	Biggleswade, Bedfordshire	2010	Alchemy	Brown rust
20	Fife, Perthshire	2010	Claire	Mildew
21	Kilkenney, Ireland	2010	JB Diego	Septoria tritici

2.2.2. Site selection and establishment

To minimise the risk of site variation due to take-all disease, trials were located in fields entering wheat following at least a one year break from wheat and barley. Where this was not possible, in Ireland, seed was treated with Latitude (containing the active substance silthiofam) to reduce the impact of the disease. Soil was sampled for pH and analysed for major nutrients, and plots were drilled using a suitable plot drill (eg Øyjord) at a seed rate appropriate for the locality and soil type. Plot sizes were in the range 20-60m^2. All inputs other than fungicides were applied to ensure that the crop remained as free as possible from nutritional deficiencies, or pest or weed infestations. In spring 2010, pots of yellow rust infected plants were planted out in a regular grid pattern in the yellow rust trial to maximise the chance of yellow rust development. All other trials relied on natural infection.

2.2.3. Experiment design

Due to the size of the field trials, and to prevent significant field variation effects within replicates, a randomised block design, with blocking within replicates of spray timings, was developed by BIOSS and HGCA. Each trial incorporated between 45 and 79 treatments with three replicates.

2.2.4. Fungicide treatments

Eight fungicides were tested against the four main foliar pathogens of wheat, Septoria tritici*,* yellow rust, brown rust and mildew. Sprays were applied in 200-300 litres water/ha using hand-held pressurised plot spraying equipment fitted with flat fan nozzles, selected to produce a medium spray quality at 200-300kPa pressure. Each fungicide product was applied at quarter, half, full and double the label recommended dose. Double dose treatments were applied for experimental purposes (to ensure accurate dose-response curve fitting). The double dose treatments are shown in this report so that the fit of the curves to the data is transparent. However, treatments above a full label recommended dose are NOT permitted for farm crops. Grain from plot areas that received double dose treatments or new products tested prior to approval was disposed of safely at harvest. Experimental products were applied under an Automatic Experimental Permit. Four sites tested fungicides aimed at Septoria tritici control*,* reflecting its relative importance in UK crops. In 2008 and 2009, to fit with commercial application timings, all sites tested products at T1 (leaf 3 emerged) or T2 (flag leaf emerged) timings, and two Septoria tritici sites (Hampshire and Herefordshire) tested dose responses at both timings. In 2010, six sites tested products at T1 or T2 with two Septoria tritici sites (Hampshire and Perthshire) testing dose responses at both timings. The site in Herefordshire used a new trial design in 2010 where dose responses were tested at leaf 2 emerged (growth stage 37), with half doses of each product applied at 10 and 20 days before, and 10 and 20 days after leaf 2 emerged (Tottman & Broad, 1987). This was designed to provide more accurate information on the flexibility of spray timing for the products tested.

In each season, the number of products that could be tested alongside core treatments was limited. Therefore, product selection for inclusion in the trials was prioritised in the following order:

1. Products containing a new broad-spectrum active substance
2. Products containing a new pathogen-specific active substance
3. Filling data 'gaps' for recently approved active substances, or recently approved new mixtures of existing active substances where data for particular diseases are inadequate
4. Products included for comparing older active substances against their baseline performance

Where a new active substance was only available commercially as a formulated mixture, the relevant mixture partner/s were also included in the trials. Spectrum of activity was used to determine the target diseases (and hence trial sites/varieties) against which each new product should be tested.

Fungicides tested included a range of existing products that were commercially available and those in the process of registration. Since the start of the project, the products Aviator Xpro (bixafen + prothioconazole) from Bayer CropScience, Seguris (isopyrazam + epoxiconazole) from Syngenta, Ignite (a new formulation of epoxiconazole) and Adexar (fluxapyroxad + epoxiconazole) from BASF have become commercially available. Ignite was only tested in 2010. A tank mix of Opus and isopyrazam was tested in 2008 and 2009, whilst in 2010 a co-formulation product of epoxiconazole and isopyrazam (Seguris) in the same proportions was used. Aviator Xpro tested in all years contained prothioconazole 150g/l and bixafen 75g/l, the same proportions as the product registered for use in Ireland. In the UK however, the registered product is Aviator 235 Xpro which contains a slightly higher loading of prothioconazole (prothioconazole 160g/l + bixafen 75g/l).

Fungicide doses stated in this report are all expressed as a proportion of full label rates. Full label rates for each product tested are given in Table 2.

Table 2. Full label rates l/ha, and active substance g/ha, for products tested for the control of one or more diseases between 2008 and 2010.

Product tested	Full label rate l/ha	Active substance (a.s.) g/ha at full label rate
Adexar	2.00	epoxiconazole 62.5g/l + fluxapyroxad 62.5g
Aviator Xpro	1.25	prothioconazole 187.5g + bixafen 94g
Bravo	2.00	chlorothalonil 1000g
Brutus	3.00	epoxiconazole 113g + metconazole 83g
Comet 200	1.25	pyraclostrobin 250g
Cyflamid	0.50	cyflufenamid 25g
Ennobe	1.80	epoxiconazole 113g + prochloraz 405g
Firefly	1.50	prothioconazole 150g + fluoxastrobin 75g
Flexity	0.50	metrafenone 150g
Ignite	1.50	epoxiconazole 125g
Isopyrazam	1.00	isopyrazam 125g
Opus	1.00	epoxiconazole 125g
Proline / Proline275*	0.80 / 0.72	prothioconazole 200g /198g
Seguris	1.00	epoxiconazole 90g + isopyrazam 125g
Talius	0.25	proquinazid 50g
Tern	1.00	fenpropidin 750g
Torch	1.50	spiroxamine 750g
Tracker	1.50	epoxiconazole 100g + boscalid 350g

*Proline and Proline275 were considered equal as they both delivered more or less the same loading of a.s. at full label rate. This was a concentration rather than a formulation change.

2.2.5. Assessments and records

Assessments of leaf disease and green leaf area

Levels of foliar disease, % green leaf area and stem-base diseases were assessed on 25 shoots randomly selected from across the untreated experiment area immediately prior to fungicide application. At the time of the T2 application, all untreated plots and those treated at T1 were assessed for foliar diseases. All plots were assessed for foliar diseases approximately three and six weeks after T2 applications. Precise assessment timings were at the discretion of the site manager, so that the first of these assessments recorded disease expression on eventual leaf 3, and the second assessment recorded optimal disease expression on leaves 1 and 2. Optimal disease expression was determined as the point at which good disease expression had occurred but it is still possible to assess untreated plots prior to the leaves senescing due to disease. For the new design trial in Herefordshire in 2010, all plots sprayed prior to the growth stage 37 application were assessed at the time of this application, and all plots were assessed at approximately three and six weeks later. Disease assessments were carried out by randomly sampling 10 shoots per

plot and estimating the average percentage leaf area affected by disease symptoms (including any necrosis or chlorosis attributable to disease) on each leaf layer.

Assessment of ear diseases

Diseases were assessed on a random sample of 10 ears per plot at GS 85 if more than 10% ear area was affected by a particular disease.

Stem-base disease

Stem-base diseases (eyespot, sharp eyespot and *Fusarium spp.*) were assessed on 25 stems per plot in untreated plots at GS75. If over 25% of stems had moderate or severe lesions, or if over 10% of stems had severe lesions, then all plots were assessed.

Lodging

If plots were affected by lodging, the percentage plot area affected was recorded just prior to harvest.

Yield

All plots were harvested using a plot combine harvester. Grain samples were taken for moisture determination and grain quality assessments. Yields were calculated at 85% dry matter.

Grain quality

Specific weight of grain was measured for each plot and adjusted to 85% dry matter.

Agronomic records

Details of site, soil type and all agrochemical inputs were recorded.

2.2.6. Data handling

Disease, green leaf area, yield and grain quality data were collected manually or directly onto portable computers. All data were transferred to Microsoft Excel worksheets after collection.

2.2.7. Statistical analysis

Individual season and site assessments

For all sites and seasons, each assessment of disease by leaf layer, and yield, was summarised by analysis of variance and the validity of the analysis was checked by the examination of residuals.

For each disease assessment, dose response curves were plotted for each fungicide. Exponential curves of the form $y = a + be^{kx}$ were fitted, where y = % disease and x = proportion of the recommended dose. Exponential curves were also fitted to the green leaf area, yield and specific weight data. All curves were constrained to pass through the mean of the untreated (dose = 0) plots.

Variables that did not contribute useful or reliable information were excluded from further analysis. This was considered on a site by site basis, as a guide data were excluded where there was no significant effect of treatment, and where there was an average of less than 3% or more than 70% disease on untreated plots. In addition, assessments where more than one disease was recorded on a particular date were examined to determine if results for either disease were compromised by an interaction. Any assessments thought to be compromised were excluded from the analysis.

For each site, the mean disease and green leaf area were calculated, based on the categorisation of leaf layers as indicating eradicant, protectant or mixed activity from the fungicide. This was based on leaf emergence relative to spray timing and, in the case of Septoria tritici, chlorothalonil (Bravo) a fungicide known to have only protectant activity, was used as a check. For Septoria tritici, means were calculated separately for protectant fungicide activity (leaves just emerged, or still to emerge at time of treatment), and eradicant fungicide activity (the first two non-protectant leaves down the stem). For other diseases, the eradicant and protectant categories were combined.

Each season, results from all sites were combined to provide an across site mean for disease and yield. Analysis from previous fungicide performance projects has shown that, whilst no transformation is needed for yield or specific weight, a logit transformation of % disease and % green leaf area provides a more valid analysis. Therefore, disease and green leaf area were analysed on a logit scale and back-transformed for ease of evaluation. This process provided a more equal weighting between sites.

Combining results from different seasons

Residual maximum likelihood (REML) has been developed for the analysis of across year data analysis. The REML method has the advantage of including information on product differences that may be available in site means and of calculating the appropriate weight to give this information in the combined means. REML means are always between the individual site means and the combined means. If the variability between sites was small relative to the variation within sites, REML means would be close to the unadjusted means.

REML analysis is sensitive to the proportion of the data matrix that is missing. Although it is theoretically possible to include all the data from individual assessment dates and leaf layers at each site, the resulting matrix is sparse and investigation has shown that the method does not converge to give a solution. The average percentage disease was calculated from the leaves categorised as showing eradicant, protectant or mixed activity at each site. This provided a suitable measure of disease for combining over experiments using the REML method. Exponential curves were fitted to the REML adjusted means to provide over-site means and season summaries.

2.3. Results

2.3.1. Septoria tritici experiments

Disease control

In 2008, favourable conditions for Septoria tritici resulted in high disease pressure in all trials. The two triazole standards tested, Opus (epoxiconazole) and Proline (prothioconazole), were equal in their protectant and eradicant activity. Bravo (chlorothalonil) provided useful protection (Figure 1). Brutus (epoxiconazole + metconazole) and Ennobe (epoxiconazole + prochloraz) gave higher levels of protectant and eradicant activity than Opus or Proline (Figures 1 and 2). The size of this effect was higher than could be explained by the higher azole loading of these products alone, as metconazole and prochloraz had previously been found to be less effective than epoxiconazole in controlling Septoria tritici. The performance of the SDHI-based products Aviator Xpro (prothioconazole + bixafen), isopyrazam and Seguris (epoxiconazole + isopyrazam) indicated that the SDHI components were adding significantly to the protectant and eradicant activity of Proline and Opus. All products were relatively less effective on lower leaves where eradicant activity was required.

In 2009 disease pressure was lower, Opus and Proline were again equal in their protectant and eradicant activity, and Bravo continued to provide useful protectant activity (Figure 3). Brutus gave higher levels of protectant and eradicant activity than Opus or Proline (Figures 3 and 4). Aviator Xpro and Adexar, when compared to Brutus showed similar protectant activity, but appeared to give a higher level of eradicant activity. Aviator and Adexar showed similar protectant activity, although Adexar appeared to have slightly more effective eradicant activity when compared at the same proportion of label rates. Isopyrazam gave similar protectant and eradicant activity to Opus or Proline, although the isopyrazam + Opus tank mix gave a higher level of protectant and eradicant activity, which was similar to Brutus.

In 2010, a dry spring resulted in low Septoria tritici levels and protectant activity was tested more than eradicant activity. As a result, data on eradicant activity was only obtained from two sites. Opus and Proline again gave very similar levels of protectant and eradicant activity (Figures 5 and 6). Aviator Xpro, Adexar, Brutus, isopyrazam, Seguris and Ignite (reformulation of epoxiconazole) all appeared to have improved protectant activity over Opus and Proline. Eradicant activity followed a similar pattern, although Brutus, Aviator Xpro and Adexar appeared more effective. Adexar again appeared to show more effective eradicant activity than Aviator Xpro when compared at equivalent proportions of label rates. There were no improvements in protectant or eradicant activity with Seguris (also containing epoxiconazole) compared to isopyrazam alone.

Cross-site analysis over three years showed that Opus and Proline gave similar levels of protectant and eradicant control (Figures 7 and 8). Aviator Xpro, isopyrazam, Seguris (or Opus + isopyrazam as a tank mix) and Brutus gave higher levels of protectant activity compared to Opus or Proline. Aviator Xpro, Seguris (or Opus + isopyrazam as a tank mix) and Brutus gave a higher level of eradicant activity than Opus or Proline. Isopyrazam performed best as a protectant but showed some eradicant activity, almost equivalent to that seen for Opus.

Comparison at half doses of the fungicides at T1 and T2 showed a similar rank order of products in the control of disease. In 2008 and across all 3 years, all products tested reduced Septoria tritici to significantly lower levels than the untreated, but there were no significant differences between treatments (Figures 9 and 10).

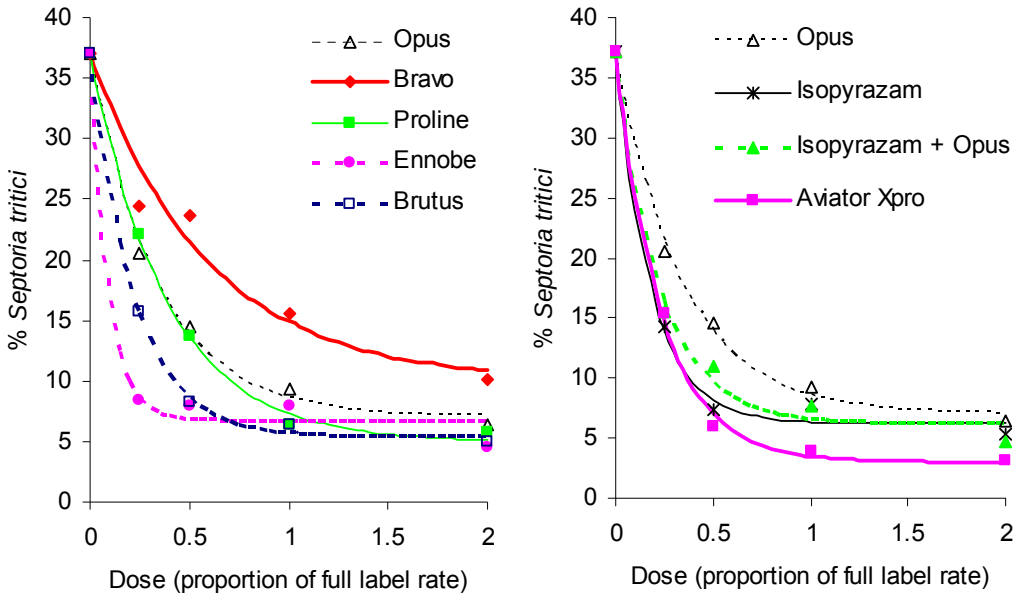

Figure 1. Fungicide dose-response curves for protectant activity against Septoria tritici in 2008 (overall means across sites 1, 2, 3 and 7)

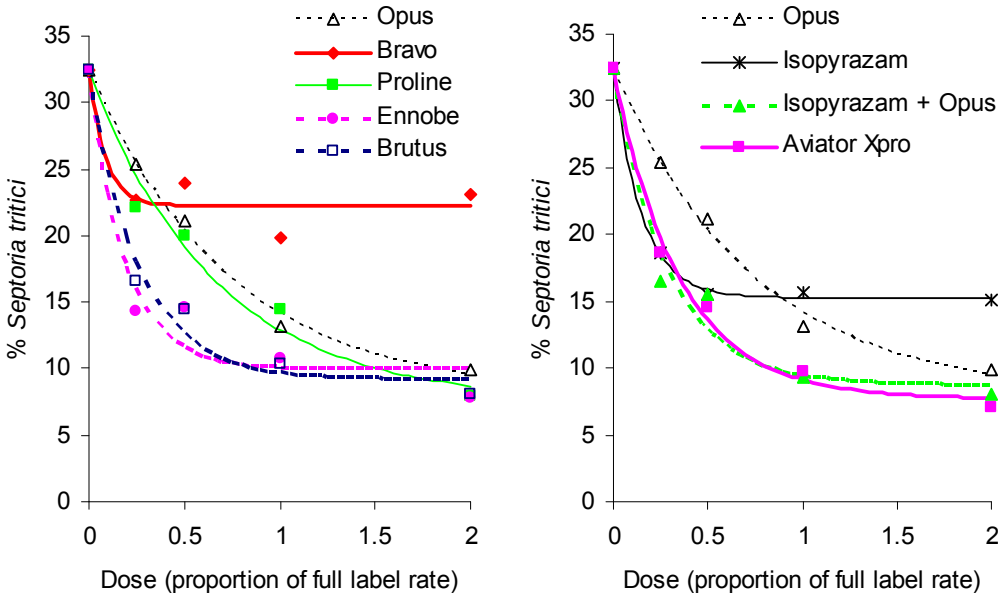

Figure 2. Fungicide dose response curves for eradicant activity against Septoria tritici in 2008 (overall means across sites 1, 2, 3 and 7)

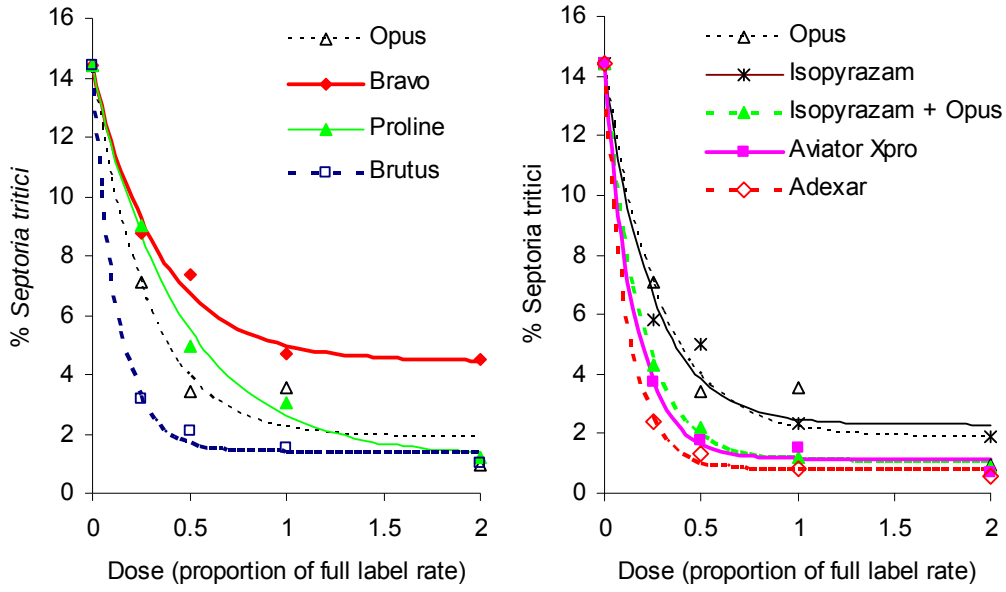

Figure 3. Fungicide dose-response curves for protectant activity against Septoria tritici in 2009 (overall means across sites 8, 9, 10 and 14)

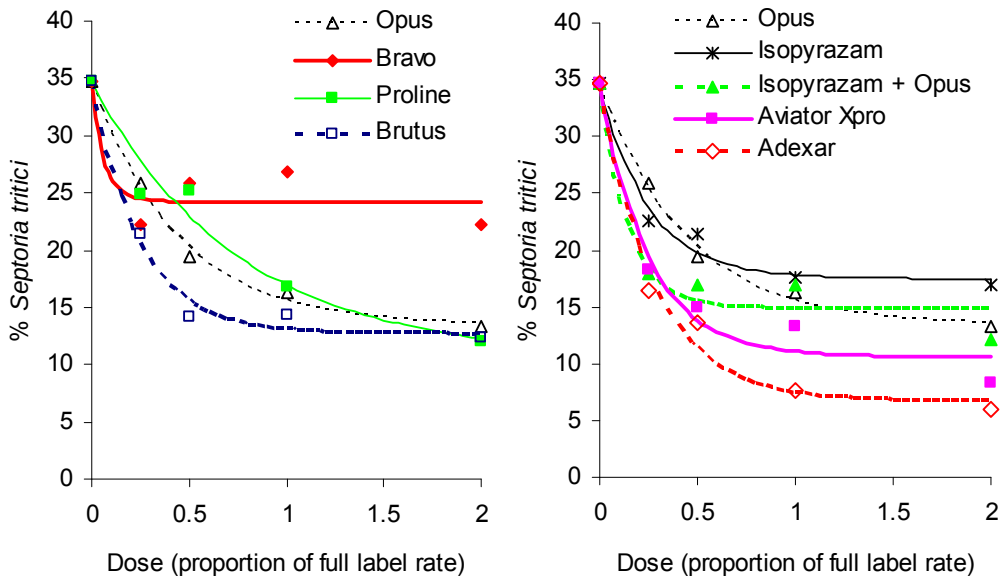

Figure 4. Fungicide dose-response curves for eradicant activity against Septoria tritici in 2009 (overall means across sites 8, 9, 10 and 14)

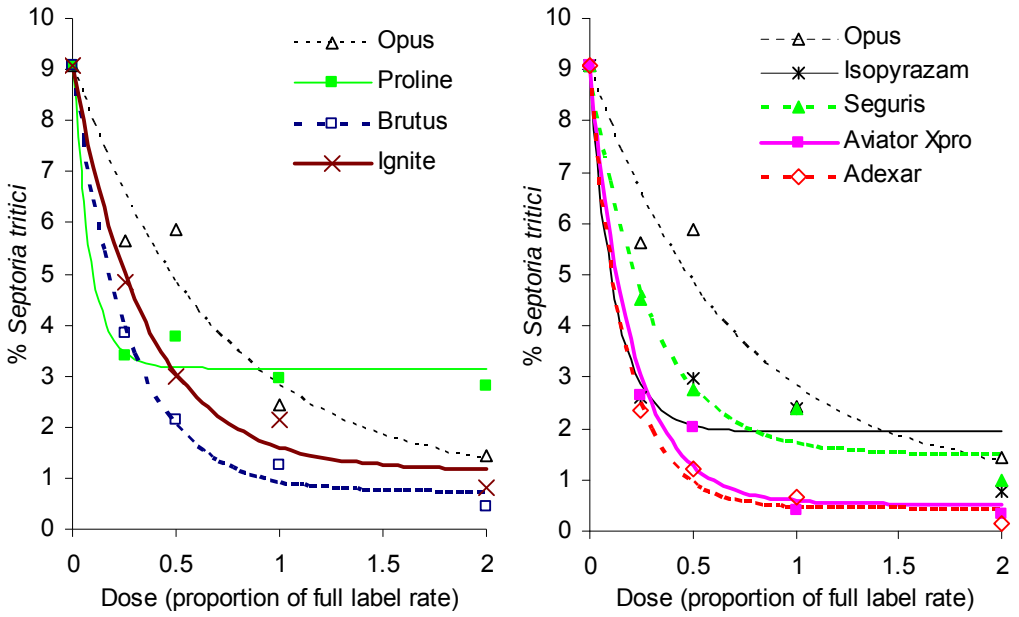

Figure 5. Fungicide dose response curves for protectant activity against Septoria tritici in 2010 (overall means across sites 15, 16, 17 and 21)

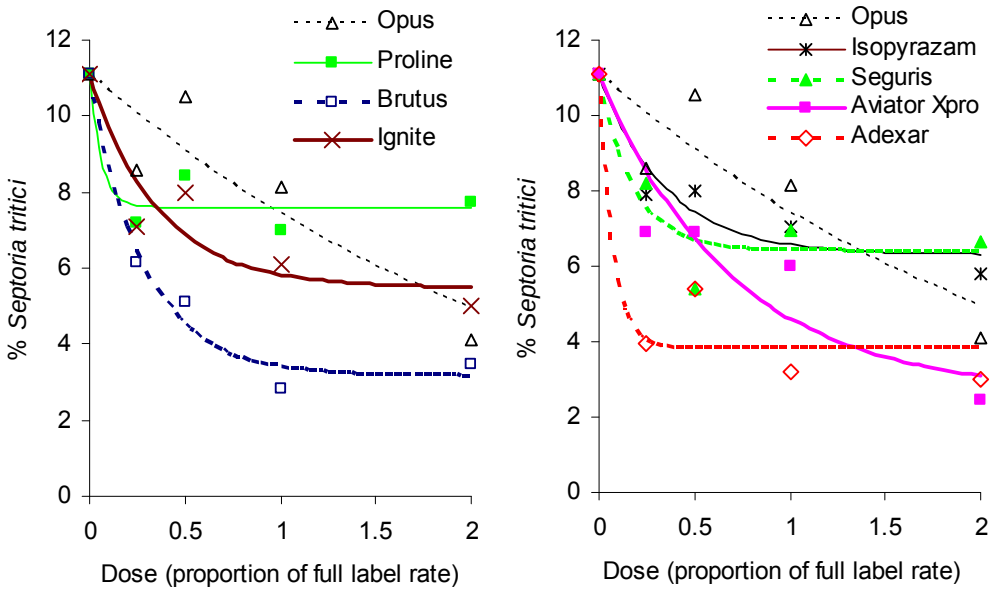

Figure 6. Fungicide dose response curves for eradicant activity against Septoria tritici in 2010 (overall means across sites 16 and 17)

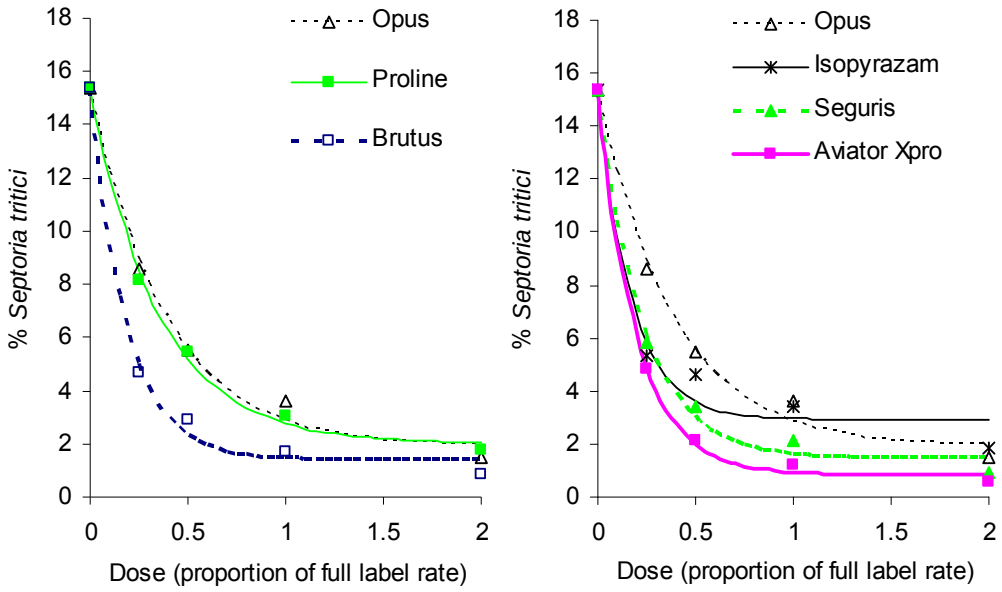

Figure 7. Fungicide dose-response curves for protectant activity against Septoria tritici; 2008-2010 (overall means across sites 1, 2, 3, 7, 8, 9, 10, 14, 15, 16, 17 and 21)

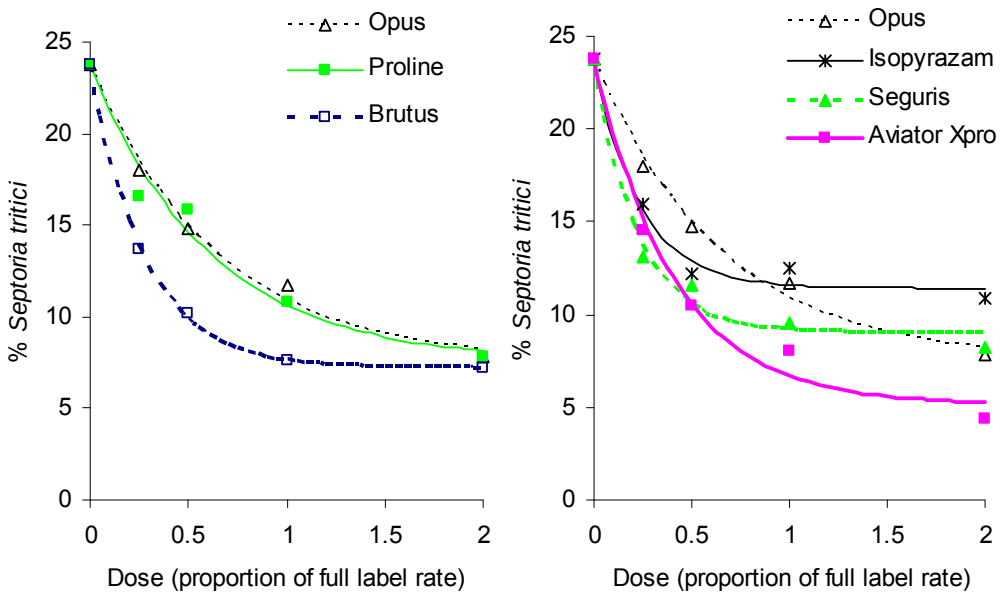

Figure 8. Fungicide dose-response curves for eradicant activity against Septoria tritici; 2008-2010 (overall means across sites 1, 2, 3, 7, 8, 9, 10, 14, 16 and 17)

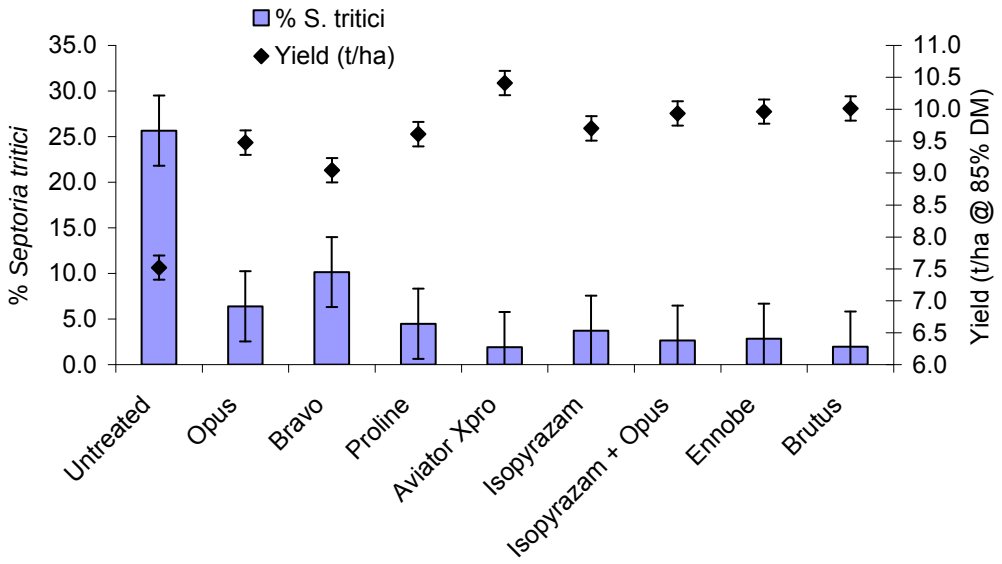

Figure 9. Septoria tritici levels as percent leaf area and corresponding grain yields for plots treated with half doses of fungicides at T1 and T2 in 2008 (overall means across sites 1, 2, 3 and 7, Error bars indicate the LSD @ 5%)

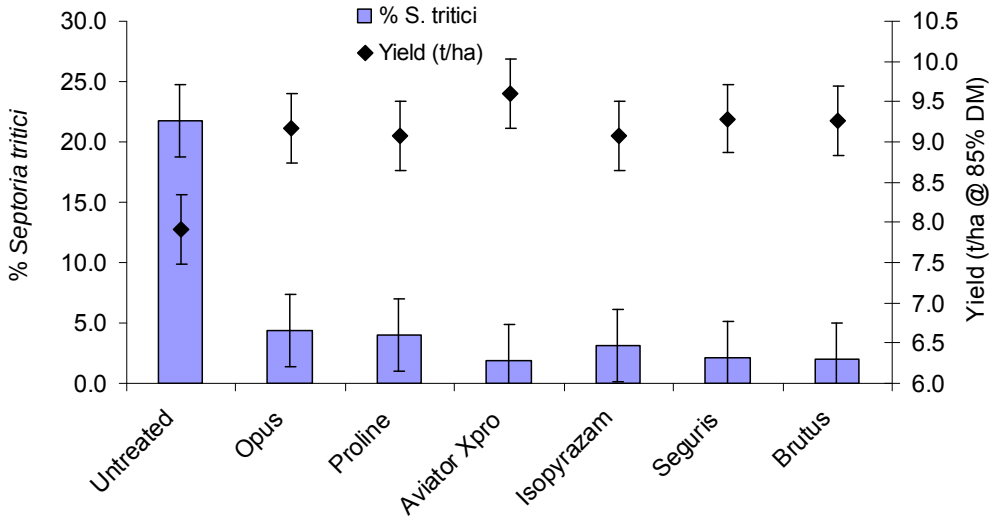

Figure 10. Septoria tritici levels as percent leaf area and corresponding grain yields for plots treated with half doses of fungicides at T1 and T2; 2008-2010 (overall means across sites 1, 2, 3, 7, 8, 9, 10, 14, 15, 16, 17 and 21)

Yield

In practice, most wheat disease control strategies involve between two and four fungicide application timings, with products often being applied in mixtures. Yield responses to single spray applications, which do not fully control disease, will not reflect yield responses that will be seen in practice where a more comprehensive strategy is employed. However, yield response data can support disease control information on products, and can identify effects on yield that may not be attributable to disease control alone.

In 2008, yield increases from applying single full dose applications ranged from 1.0 tonne/ha to over 2.0 tonnes/ha for the most effective products. Isopyrazam, Opus and Proline all gave a similar yield response at full dose (1.45, 1.58 and 1.70 tonnes/ha respectively) (Figure 11). Ennobe, Brutus and isopyrazam + Opus gave slightly higher yield responses (1.8, 1.84 and 1.84 tonnes/ha respectively), and Aviator Xpro gave the highest yield response (2.24 tonnes/ha).

Yield responses were lower in 2009, with yield increases from full dose applications ranging from 0.6 tonnes/ha to 1.6 tonnes/ha. Opus, Proline and Isopyrazam all gave similar yield responses (1.07, 1.13, and 1.05 tonnes/ha) (Figure 12). Isopyrazam + Opus and Brutus gave a slightly higher yield increase from full dose applications (1.39 and 1.32 tonnes/ha), whilst Aviator Xpro and Adexar gave the highest yield responses with 1.55 tonnes/ha and 1.60 tonnes/ha increases respectively when applied at full dose.

Very low Septoria tritici levels in 2010 resulted in low yield responses to fungicide treatment. Yield increases from full dose applications ranged from 0.26 to 0.53 tonnes/ha, with Aviator Xpro and Adexar again giving the highest yield response (Figure 13).

Mean yield data for all Septoria tritici sites over all three years showed that yield increases from full dose applications ranged from 1.02 (Opus alone) to 1.51 tonnes/ha (Aviator Xpro) (Figure 14). Opus, Proline and isopyrazam gave similar yield responses with a full dose increasing yield by 1.02, 1.09 and 0.95 tonnes/ha respectively. Brutus and Seguris gave a slightly higher yield response with a full dose increasing yield by 1.18 and 1.22 tonnes/ha respectively.

Where half dose applications were made at T1 and T2, yields reflected differences in the disease control observed. In 2008, all treatments were significantly higher yielding than the untreated. Bravo increased yield by 1.52 tonnes/ha, whilst Opus, Proline and isopyrazam were similarly matched, increasing yield by 1.92, 2.09 and 2.18 tonnes/ha respectively (Figures 9 and 10). Isopyrazam + Opus, Ennobe and Brutus significantly increased yield compared to Bravo and Opus (2.41, 2.44 and 2.49 tonnes/ha), and Aviator Xpro treated plots had a yield response significantly higher than all other treatments, with 2.89 tonnes/ha. When yield responses were averaged over

the three years, all treated plot yields were significantly higher than the untreated. However despite clear differences between products were single applications were applied (figure 14), there were no significant differences between treatments in the three year T1 + T2 averages. However yields followed a similar rank order to the significant differences observed in 2008.

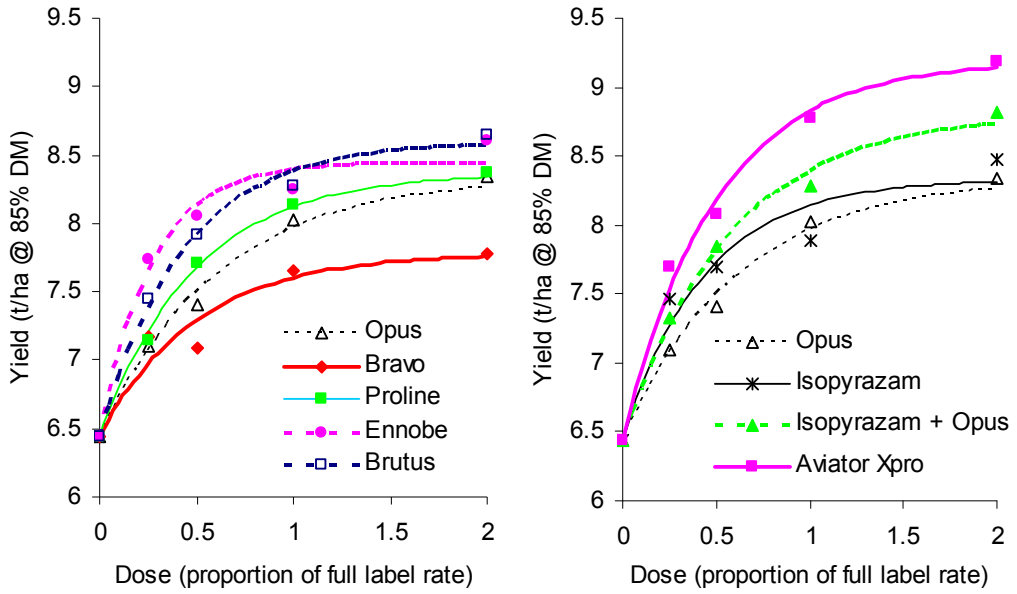

Figure 11. Fungicide dose response curves for yield in 2008 (overall means across sites 1, 2, 3 and 7)

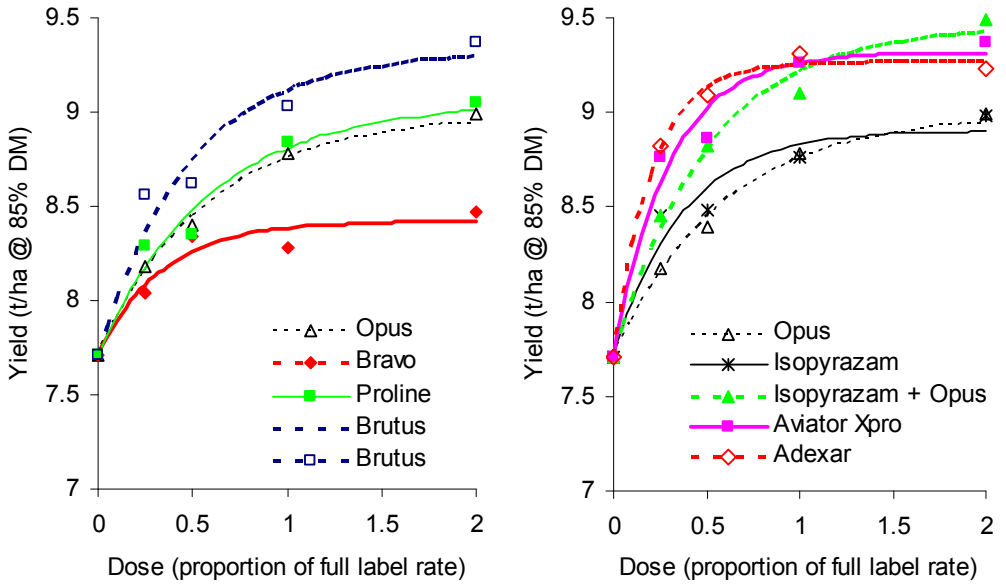

Figure 12. Mean fungicide dose response curves for yield in 2009 (overall means across sites 8, 9, 10 and 14)

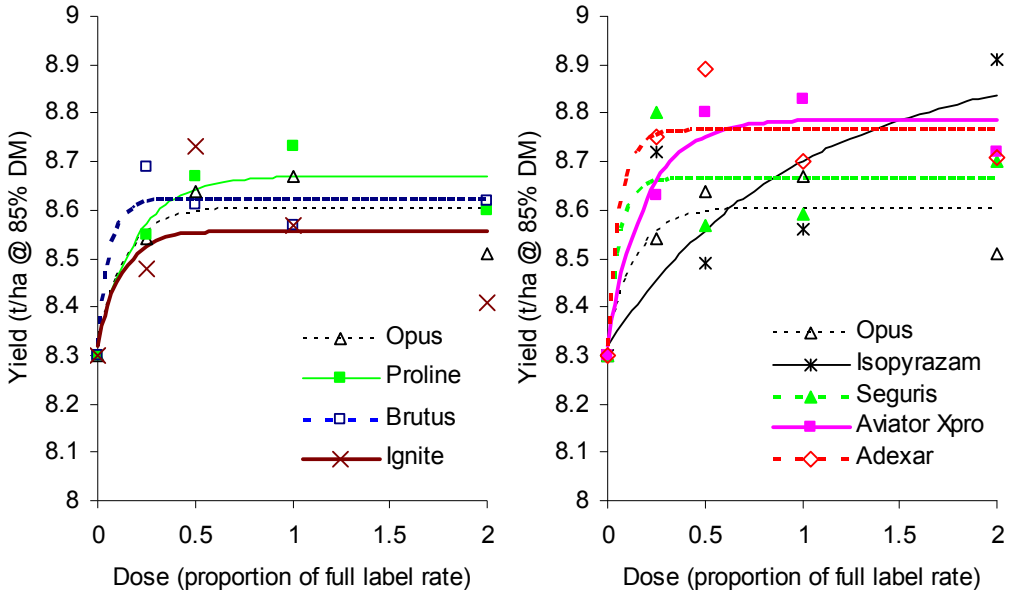

Figure 13. Fungicide dose response curves for yield in 2010 (overall means across sites 15, 16, 17 and 21)

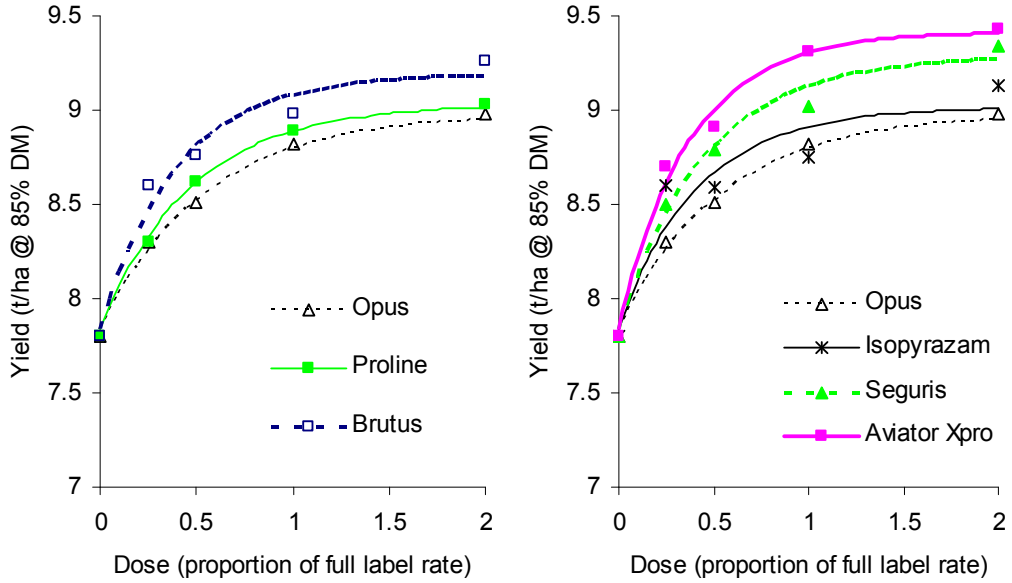

Figure 14. Fungicide dose response curves for yield; 2008-2010 (overall means across sites 1, 2, 3, 7, 8, 9, 10, 14, 15, 16, 17 and 21)

2.3.2. Yellow rust experiments

Disease Control

In 2010 yellow rust levels were too low for response curves to be fitted. Yellow rust protectant and eradicant data were combined for 2008 and 2009, as disease control showed a similar pattern in both years. Opus (epoxiconazole) provided very effective control of yellow rust, with Brutus (epoxiconazole + metconazole) showing a similar level of activity (Figure 15). Both of these products controlled yellow rust effectively at a quarter of the full label dose. The strobilurin Comet 200 (pyraclostrobin), also gave good control of yellow rust with a half dose keeping levels below 5% leaf area. Proline (prothioconazole) did not control yellow rust as effectively as Comet 200 or products containing epoxiconazole. The addition of fluoxastrobin to Proline (as in FireFly) improved the control of yellow rust. Of the SDHIs, isopyrazam alone was shown to have a useful level of yellow rust activity, with yellow rust control that was similar to Proline. In contrast there was little or no benefit from the addition of bixafen to Proline (as Aviator Xpro) at below quarter dose, although it appeared to give a small improvement in yellow rust control above half dose. Adexar showed good activity on yellow rust giving a level of control similar to Opus and the strobilurins. Tracker (epoxiconazole + boscalid) was only tested in 2008 and gave very effective protectant and eradicant activity against yellow rust. The level of control given by Tracker was similar to that given by Firefly and Comet 200, with a quarter dose keeping levels below 2% leaf area (data not shown). In 2008 and 2009, where T1 and T2 treatments were applied at a half dose, all products tested showed good activity and controlled yellow rust to levels significantly lower than the untreated (Figures 16 and 17). This suggests that product timing may be as important as product selection in the control of yellow rust. In 2008, yellow rust levels reached 26.1% in untreated plots, with just 0 to 4.6% in treated plots and no significant differences between treatments. In 2009, 59% of leaf area was infected with yellow rust in untreated plots, with most treatments controlling levels to between 5.8% and 8.8%. The low level of yellow rust control where half dose T1 + T2 applications of isopyrazam were applied was unexpected, given the level of control seen in the dose response results.

Yield

For half dose applications applied at T1 and T2, all treatments significantly increased yield over the untreated in 2008 and 2009. In both years, yields broadly reflected differences in disease control. Yield responses from treatment ranged from 2.5 to 3.3 tonnes/ha in 2008 and 1.84 to 3.51 tonnes/ha in 2009. In 2008, there were no statistically significant differences between treatments, although Opus, Tracker, Brutus and Aviator Xpro treated plots appeared to be higher yielding (Figure 16). In 2009, there were statistically significant differences in the yields between treatments, with isopyrazam increasing yield by around 1.5 t/ha over the untreated, but this was significantly less than all other treatments (Figure 17).

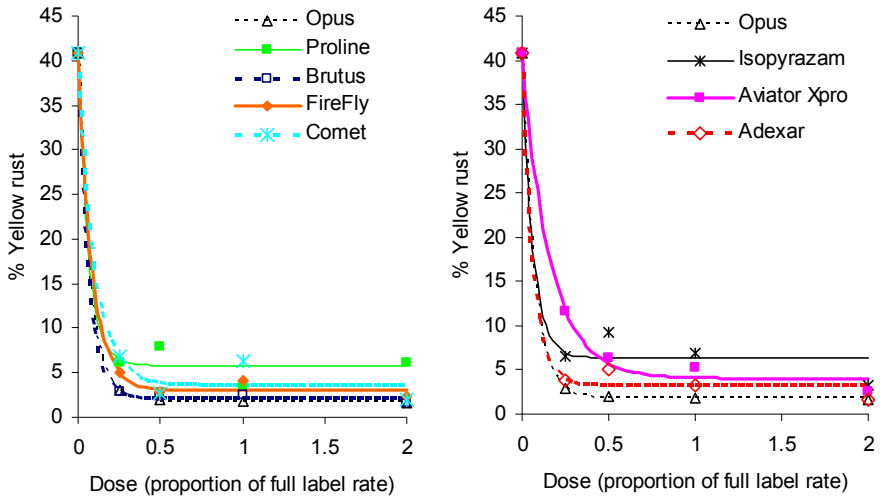

Figure 15. Fungicide dose response curves for yellow rust control at sites 4 in 2008 and 11 in 2009

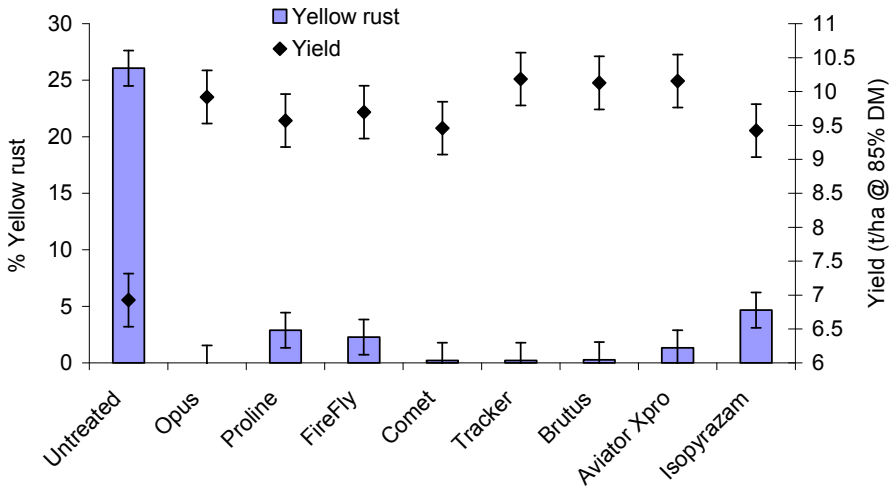

Figure 16. Yellow rust levels as percent leaf area and corresponding grain yields for plots treated with half doses of fungicides at T1 and T2 at site 4 in 2008

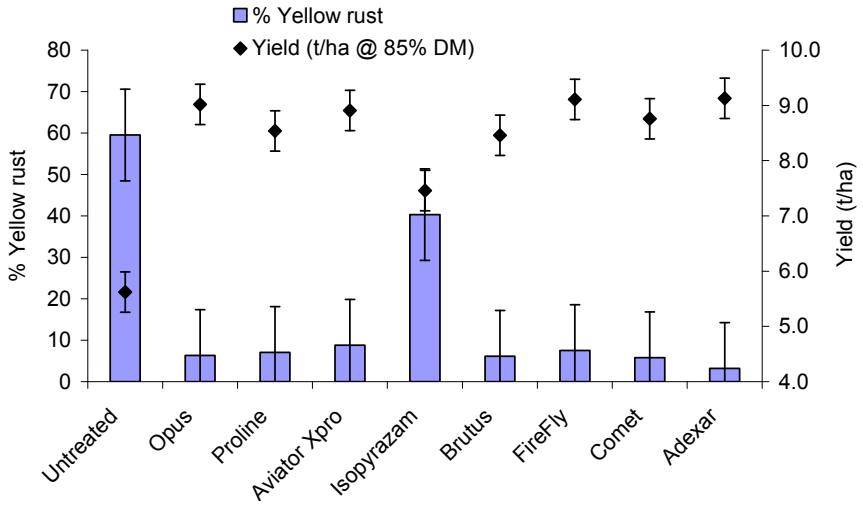

Figure 17. Yellow rust levels as percent leaf area and corresponding grain yields for plots treated with half doses of fungicides at T1 and T2 at site 11 in 2009

2.3.3. Brown rust experiments

Disease control

Brown rust was present in 2008 and late in 2009, with data from both years showing a similar rank order of products. Across both years, Opus (epoxiconazole) controlled brown rust more effectively than Proline (prothioconazole), with a full dose of Opus reducing brown rust levels to 1.8% of leaf area affected, compared with 4.7% for Proline. Aviator Xpro (prothioconazole + bixafen), Adexar (epoxiconazole + fluxapyroxad), isopyrazam, Firefly (prothioconazole + fluoxastrobin) and Comet 200 (pyraclostrobin) all controlled brown rust more effectively than Opus and Proline, especially when applied at between half and full dose (Figure 18). A half dose of these products reduced disease levels below 2%, compared to 4% where Opus was applied. Unlike all other products, increasing the dose of Comet 200 beyond half gave no further improvements in disease control. Brutus (epoxiconazole + metconazole) and Adexar controlled brown rust more effectively than all other products, with even a quarter dose reducing levels to 1%. When half doses were applied at T1 and T2, all products reduced brown rust levels to significantly less than the untreated in 2008 and 2009. The triazoles (Opus and Proline) and strobilurins (Comet 200 and Firefly) were particularly effective on brown rust in 2008 (Figure 19). Although there were no statistically significant differences between treatments, two applications of isopyrazam or Brutus appeared to provide excellent control of brown rust. There was no clear benefit from the addition of boscalid to epoxiconazole (as in Tracker). Similar trends were seen in 2009 and there were statistically significant differences between treatments, with Proline controllling brown rust less effectively than all other treatments (Figure 20). In 2010, low levels of brown rust resulted in no brown rust data for that season.

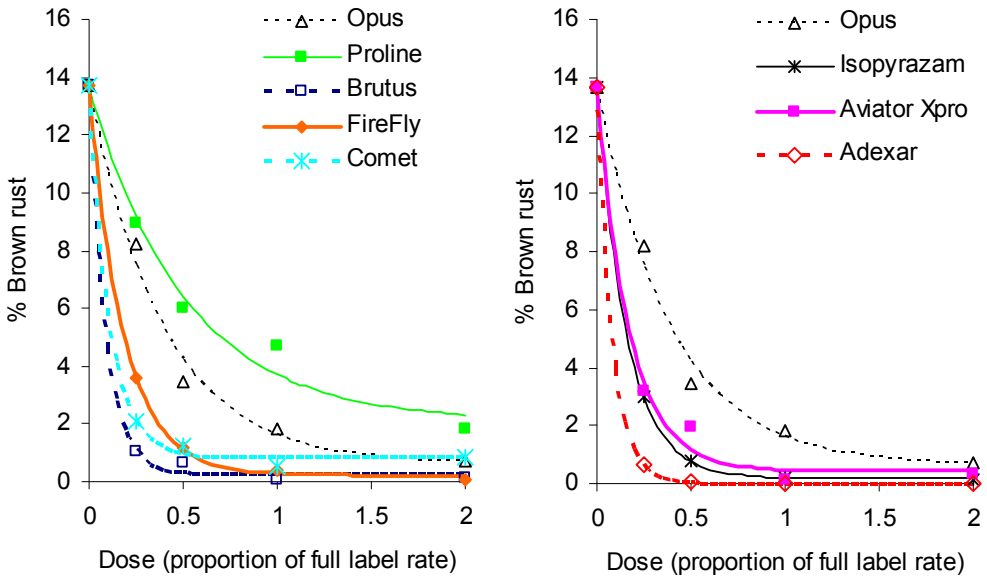

Figure 18. Fungicide dose response curves for brown rust control at site 5 in 2008 and site 12 in 2009

Yield

Yield responses from half rate dose applications at T1 and T2 reflect the control of both brown rust and Septoria tritici. All products gave significant increases in yield with responses ranging from 0.8 to 2.0 tonnes/ha (Figures 19 and 20).

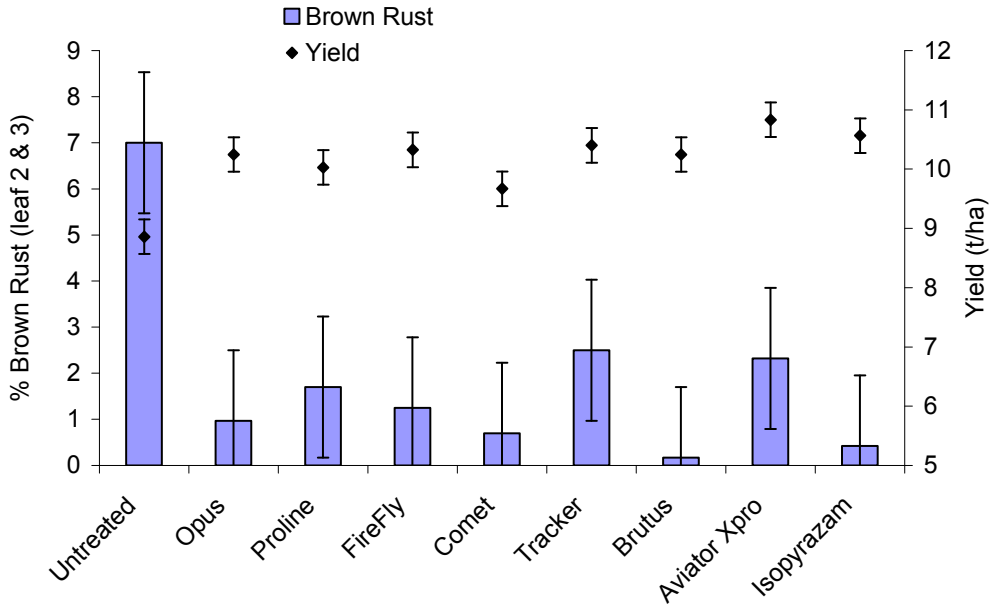

Figure 19. Brown rust levels as percent leaf area and corresponding grain yields for plots treated with half doses of fungicides at T1 and T2 at site 5 in 2008

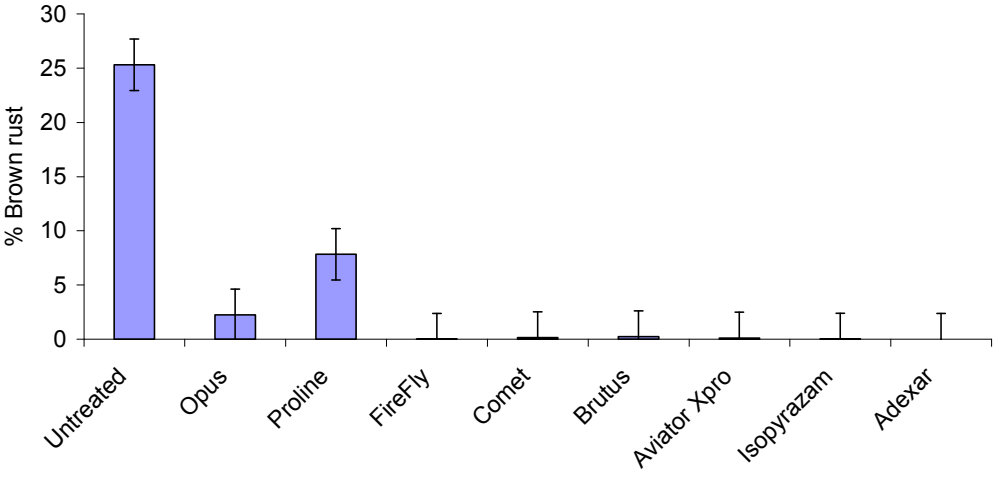

Figure 20. Brown rust levels as percent leaf area for plots treated with half doses of fungicides at T1 and T2 at site 12 in 2009

2.3.4. Mildew

Disease control

Mildew levels were generally low in all years. As a result, few clear product differences were observed. In 2008, there was a moderate level of mildew present in the plots, and all products controlled the disease effectively (Figure 21). Where T1 + T2 treatments were applied at half dose, all products showed good activity and reduced mildew to significantly lower levels than the untreated. There were no statistically significant differences between treatments, although the specific mildew products Talius (proquinazid) and Cyflamid (cyflufenamid) appeared to control the disease more effectively. The SDHIs isopyrazam and bixafen both showed some useful mildew activity.

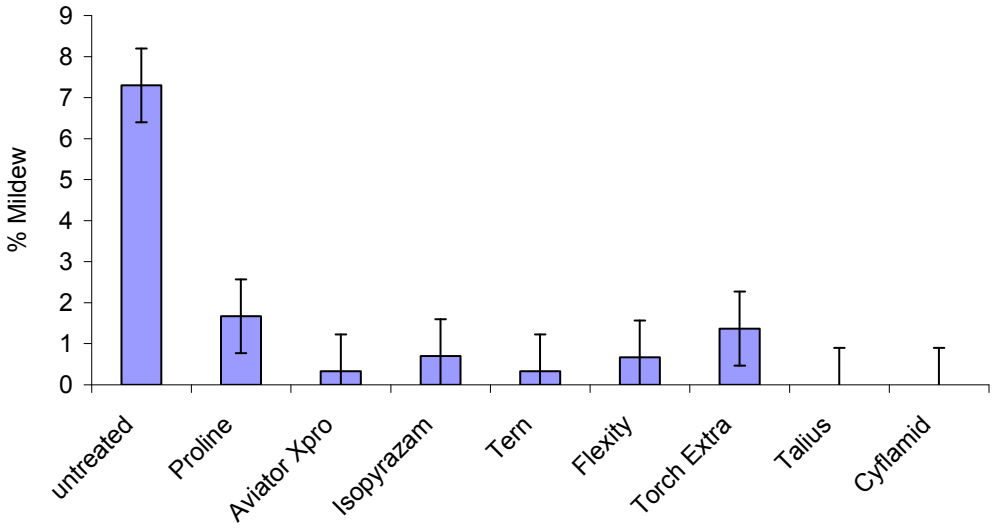

Figure 21. Mildew levels as percent leaf area for plots treated with half doses of fungicides at T1 and T2 at site 16 in 2008

2.4. Conclusions

All experiments used varieties that were susceptible to the target disease and were located in areas of the country where natural pressure from that disease was high. As a result, response to fungicides in terms of disease control and yield are higher than those observed on less susceptible varieties, and in lower pressure sites in these seasons.

2.4.1. Septoria tritici

- Chlorothalonil (Bravo) continued to provide effective protectant activity against Septoria tritici. The low risk of resistance developing against chlorothalonil due to its multi site mode of action means that it still has an important role in providing an alternative mode of action in sequences and mixtures with higher resistance risk groups such as the SDHIs and triazoles.
- Products based on epoxiconazole and prothioconazole performed equally in trials, both providing effective protectant and eradicant activity against Septoria tritici.
- Brutus (epoxiconazole + metconazole) and Ennobe (epoxiconazole + prochloraz) gave better protectant and eradicant activity than can be explained by higher triazole loading alone. Increased disease control may be due to the change of formulation or a synergy between the two azoles.
- The new SDHI products Aviator Xpro (prothioconazole + bixafen), Seguris (epoxiconazole + isopyrazam) and Adexar (epoxiconazole + fluxapyroxad) provided a higher level of protectant and eradicant activity than existing products. Aviator Xpro, Seguris and Adexar gave similar protectant activity, although Adexar appeared stronger in eradicant situations and Aviator Xpro appeared marginally more effective than Seguris in eradicant situations
- In high disease pressure situations, the protectant and eradicant activity of isopyrazam was greatly improved by the addition of Opus (as in Seguris).
- Yield responses were generally in line with disease control, although in some cases yield increases from Aviator Xpro and Adexar were higher than expected.

2.4.2. Yellow rust

- Products containing epoxiconazole (Opus and Brutus and Adexar) provided excellent control of yellow rust, even at low doses
- The strobilurin pyraclostrobin (Comet 200) also controlled yellow rust effectively.
- Control with prothioconazole (Proline) was improved by the addition of fluoxastrobin as in FireFly.
- The SDHI, isopyrazam gave useful additional control of yellow rust indicating that this may add to yellow rust control when used in mixture with triazoles.

2.4.3. Brown rust

- The most effective control of brown rust was provided by the strobilurins, strobilurin + triazole mixtures (Comet and FireFly) and the SDHIs isopyrazam, bixafen (in combination with prothioconazole in Aviator Xpro) and fluxapyroxad (in combination with epoxiconazole in Adexar).
- Prothioconazole controlled brown rust less effectively than epoxiconazole (Opus) and all other products tested.
- Isopyrazam effectively controlled brown rust in both T1 + T2 sprays and in dose response trials, indicating that it will add excellent activity against brown rust when applied in mixtures with triazoles, such as in Seguris.

2.4.4. Mildew

- Specific mildewicides proquinazid (Talius) metrafenone (Flexity), fenpropidin (Tern) and cyflufenamid (Cyflamid) provided very effective control of mildew in 2008.
- SDHIs showed some mildew activity in addition to partner products. Note that Seguris does not have a label claim for mildew control.

2.5. Acknowledgements

The UK work was funded by HGCA, and the Irish site by Teagasc. The considerable effort given by ADAS, SAC, NIAB TAG and Teagasc staff in spraying and assessing the experiments is gratefully acknowledged. Thanks are also due to Jim McVittie for data management and statistical analysis of the data and BIOSS for statistical support.

3. FUNGICIDE MIXTURES AND SEQUENCES CALCULATOR

3.1. Introduction

Project RD-2004-3026 (Knight *et al*., 2008) included development of a fungicide mixture calculator which predicts the combined dose-response of fungicide tank mixes with reasonable accuracy from the dose-responses of the constituent active ingredients. Previous research by Paveley *et al*. (2003) described a method to predict the effective dose for two fungicides applied in sequence. For this project, the fungicide mixture calculator was extended to calculate the effectiveness of sequences of fungicide applications using a simple model of disease and fungicide interaction. It used the data for individual trial sites and seasons to predict results that were then compared with data from the two-spray treatments in the trials.

3.2. Methods

3.2.1. Dose-response curves

A dose-response curve was fitted to the raw data for the three replicates of each fungicide (9) × timing (T1, T2) × leaf (1–4) × site (2) × season (2008, 2009) combination (see Chapter 1) using the later observation for each leaf where there were two. In addition, curves were fitted to the sum of the disease on leaves 1 and 2 as an indicator of potential yield loss. In 2009 there were no useful data for leaves 1–3 from the Andover site from the T1-only treatments, so there were three complete data sets and one partial one.

The dose-responses are exponential curves of the form

$$u = a + be^{-kx} \quad (1)$$

where x is dose, u is the disease remaining (% of leaf), a, b and k are positive fitted parameters. The interpretation of the parameters is that (a+b) is the disease with no treatment, a is the disease that cannot be controlled even at very high doses and k controls the steepness of the curve. If k is large, the curve falls very steeply, meaning that the fungicide achieves most of its potential efficacy at low doses. Note that (a+b) must be the same for all treatments of the same disease, since it is the untreated disease level, so there are only two independent parameters.

In order to combine dose-response curves, it is useful to convert the disease remaining to the proportion z of the original disease that survives (the survival rate), which is u/(a+b), so

$$z = \frac{a + be^{kx}}{a + b} \quad (2)$$

There are two ways of combining dose-response curves, known as the additive dose method (ADM) and the multiplicative survival method (MSM) (Milne *et al*., 2007), both of which were used in the calculator for mixtures. The MSM assumes that one fungicide controls the disease that survives the other with the same proportional effectiveness as it would control the total disease. So if the survival rates for the two fungicides at particular doses are z_1 and z_2, the survival rate for the combined application is z_1z_2. This method is most appropriate when the two fungicides have different modes of action, because it assumes that the fungi respond independently to the two fungicides.

The MSM model is inappropriate for mixtures of active substances of the same mode of action. This can be illustrated by considering the notional case of applying two simultaneous doses of the same fungicide. Applying the MSM gives a lower survival rate than considering it as one dose of double the amount, unless $a = 0$. The ADM addresses this issue and is more suitable for chemicals of the same mode of action. First consider two identical fungicides applied simultaneously at doses x_1 and x_2. The combined effect is clearly that of a single application of dose x_1+x_2. This is generalised to two different chemicals by converting the less effective one into the equivalent (lower) dose of the more effective one, that is, the dose giving the same survival rate, then adding the dose of the more effective chemical and calculating resulting survival rate. If applied to fungicides with different modes of action, this usually gives a higher survival rate than the MSM.

When considering a sequence of two applications at different times, it is appropriate to treat these as acting independently and use the MSM, even for two applications of the same fungicide (Paveley *et al*., 2003). This is the method used in the calculator.

3.2.2. The calculator program

The calculator developed for RD-2004-3026 was revised to meet the requirements of this project. The program was originally written in Microsoft Visual C++ V6.0™ and compiled to an executable with minimal additional dependencies other than its data file, so that no installation program was required. As the data requirements for this project were more complex, the data were moved to a database using the free and open source SQLite engine (www.sqlite.org), which is deployed as a single file (sqlite3.dll), maintaining the ease of installation. The program can now calculate and display dose-responses for single applications of products at T1 or T2 (i.e. the fitted curves), mixtures of products at either time and sequences of products or mixtures applied at T1 and T2. Within any mixture or sequence, the dose of a product may be fixed or variable. If all are set to fixed, the program displays a point; otherwise it displays the response curve resulting from varying the doses of the products that are not fixed.

In addition to the curves, the program can display the trial data points used to derive the dose-response curves for each product and the point for the double treatment as either means or individual replicates. The program selects the points that are relevant to the curves being shown. Figure 2.1 shows the program displaying the fitted curve and data for Opus (epoxiconazole) applied at T2 at Andover in 2008. Figure 2.2 shows the results for a sequence of applications of Opus: it displays the two dose-response curves for single applications, the trial points for the double treatment and the predicted response for the same sequence. In this case, the agreement was very good. The data points for the single treatments are not shown, to avoid making the graph too cluttered.

The key design decision was the scale to use for the dose axis for mixtures and sequences. For mixtures at a single time, it uses the same scale as the previous mixture calculator: dose in relative units, where 1.0 means the full dose of a product. This was based on the principle that the curve for a mixture should be as would be expected for a formulated product containing that mixture. As a test case, a mixture of a product with itself adding up to a full dose (e.g., 0.5 Opus + 0.5 Opus) should produce the original dose-response curve for the product. For a mixture, 1.0 means a full dose of the mixture as specified, where the specification is in relative units. For example, if the mixture is 0.5 Bravo (chlorothalonil) + 0.75 Opus, 1.0 means 1.0 l/ha Bravo + 0.75 l/ha Opus, because the full dose of Bravo is 2 l/ha.

For a sequence, the axis shows the total dose, so the double treatments (0.5 + 0.5) are shown at 1.0 on the axis. This makes it easy to compare the effect of the double treatment with a full dose at T1 or T2, because these are shown at the same point on the dose scale.

3.3. Results

Results are reported for approved products Opus, Bravo, Proline (prothioconazole), Aviator Xpro (prothioconazole + bixafen), Seguris (epoxiconazole + isopyrazam) and Brutus (epoxiconazole + metconazole) plus isopyrazam which is approved as a component of Seguris.

Curve fitting

As the dose-response curves were fitted to individual site × season combinations, there were fewer data and hence larger error variation for each curve than in the cross-site and cross-season means. As a result, in some cases it was not possible to fit the model for one or more leaves. Thus there were complete sets for three site × season combinations, with the exception of a poor fit for Bravo at Andover 2008.

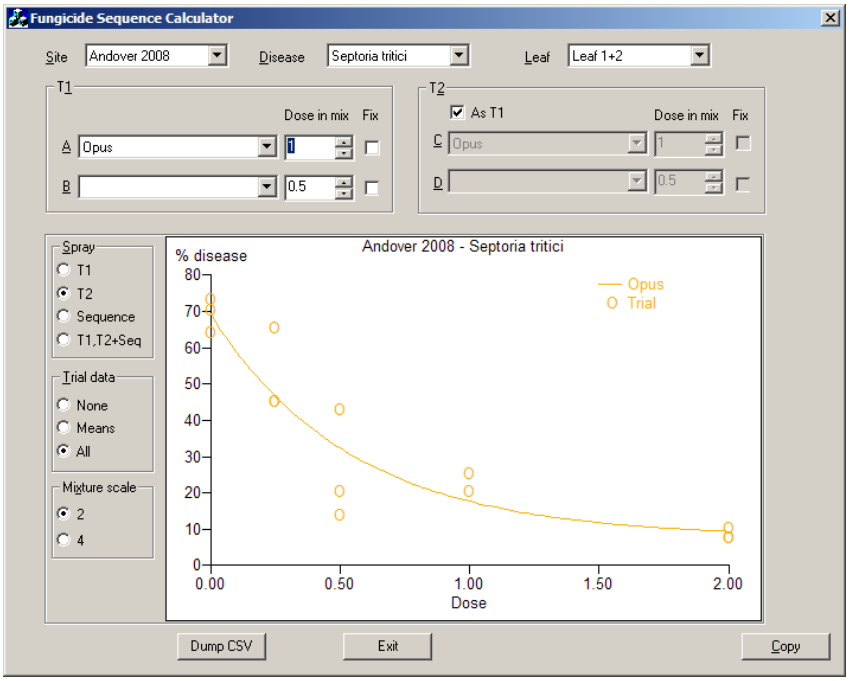

Figure 22. The calculator program showing the curve and trial data for Opus applied at T2

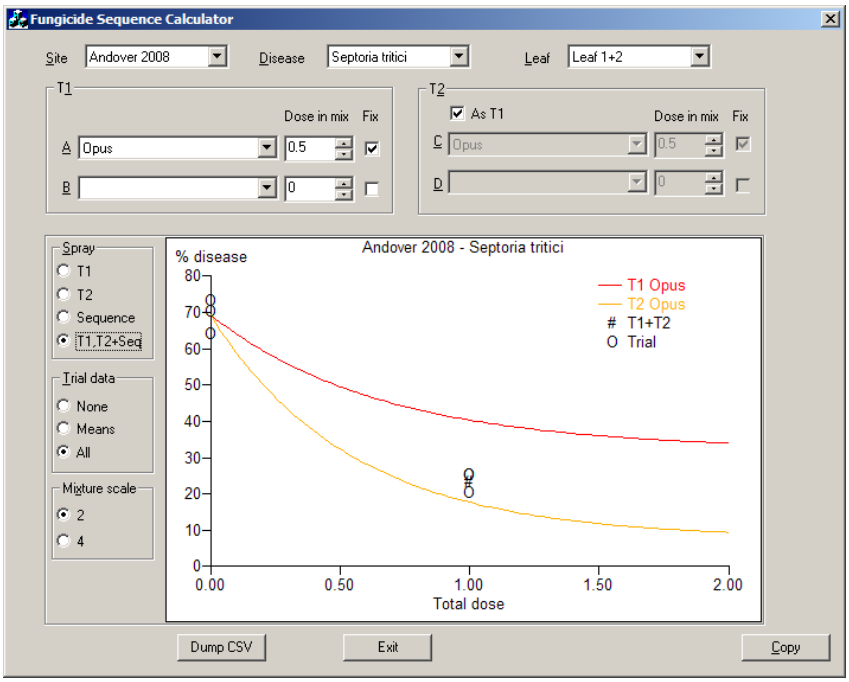

Figure 23. The calculator program showing curves for Opus and the predicted and trial results for the double treatment

3.3.1. Sequences

In the majority of cases, the predicted values were within the observed values (Figure 24), for the double treatments for all products (except Bravo, because of the poor fit of the dose-response curves in several cases) and for the three site × season combinations for which all the results were available. The results are coded, so for example, A8O means Andover, 2008, Opus. It should be noted that each of the predicted points in Figure 2.3 has error variation arising from the data for the single treatments to which the curves were fitted, but these cannot easily be computed. Overall, the multiplicative survival method was successful at predicting the performance of the double treatments.

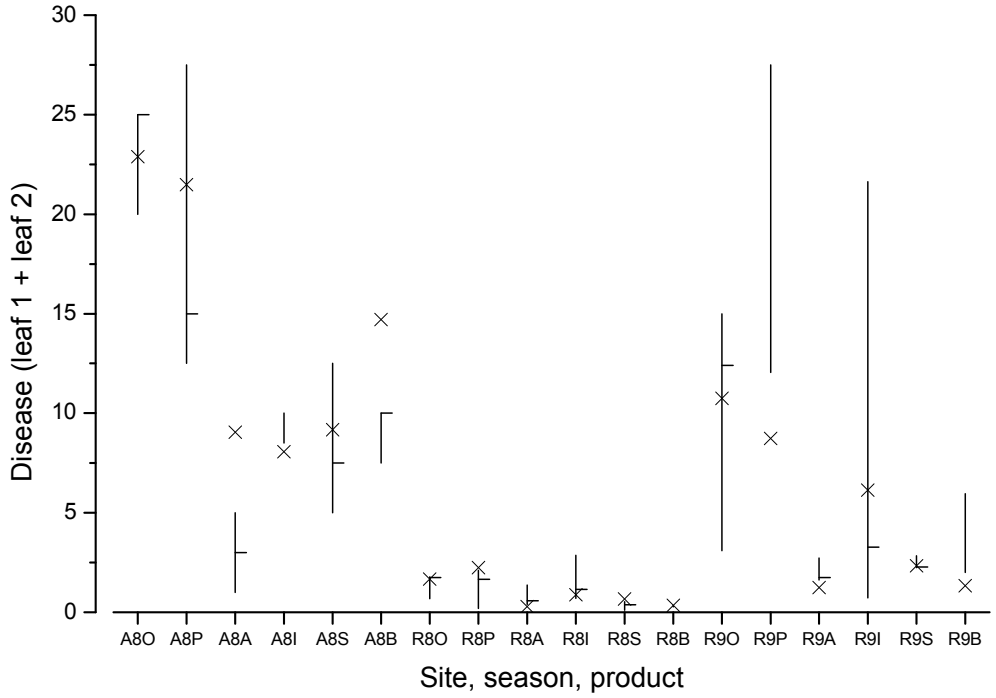

Figure 24. Observed and predicted (×) disease using the double treatments.
Bars show maxima and minima for the 3 replicates, the horizontal mark shows the middle result (if present). A = Andover, R = Rosemaund; 8 = 2008, 9 = 2009; O =Opus, P = Proline, A = Aviator Xpro, I = isopyrazam, S = Sequris, B = Brutus

3.3.2. Mixtures

The mixture model was tested and shown to work for the limited number of mixtures available in RD2004-3026. The inclusion of Seguris, isopyrazam and Opus in the trials provided a further test of the model, as the active ingredients in a full dose of Seguris are equivalent to a full dose of isopyrazam and three quarters dose of Opus. The results showed a range from good agreement for T2 at Andover in 2009 (leaf 2 only) to poor agreement for T1 at Andover in 2008 (Figure 25). In the cases where agreement was poor, the response to Seguris measured in the experiments was counter to expectations (being no better than one or both of its components), whereas the response predicted by the model was logical (with the mixture performing better than the components). The data for these cases generally showed that there were no statistically significant differences between the treatments and that the predicted mixture response was within the variation in the observed data for Seguris. The validity of the model could not be tested further with these data.

3.4. Conclusions

- The sequences model has been tested for all the double treatment cases with a complete data set and shown to give results that are within the experimental variation in the data.
- The mixtures model has been tested by comparing Seguris with a mixture of isopyrazam and Opus. The agreement between predictions and observations varied from good to poor, but was within the error variation in the data from the trials of Seguris from single experiments.

3.5. References

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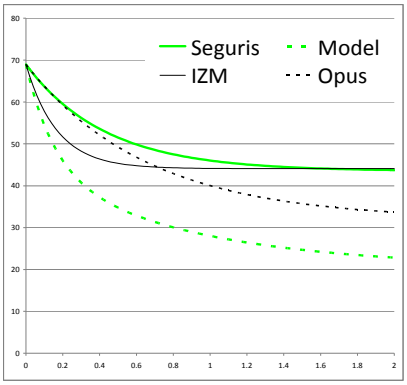
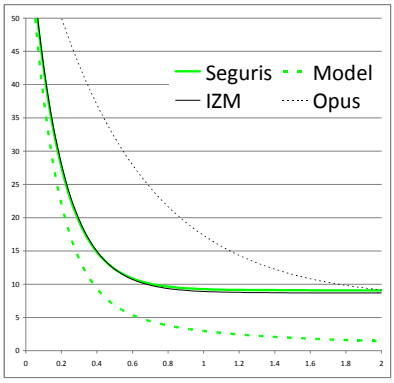


Andover 2008 T1 leaves 1+2



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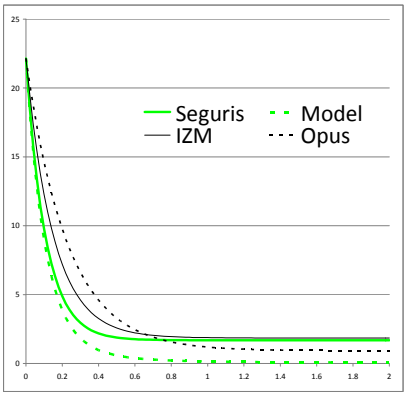
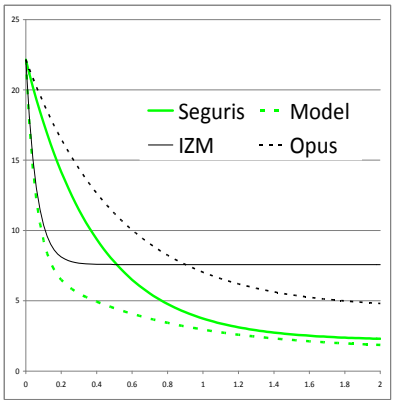
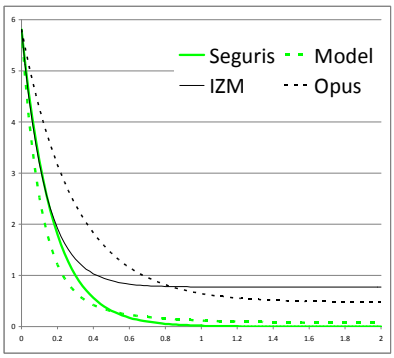


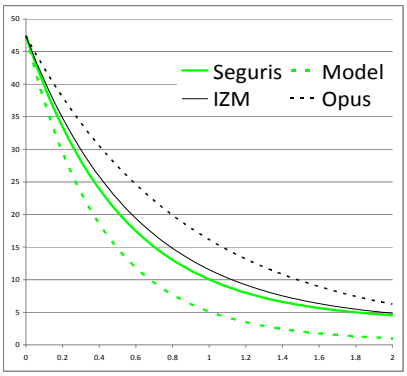
Rosemaund 2008 T1 leaves 1+2



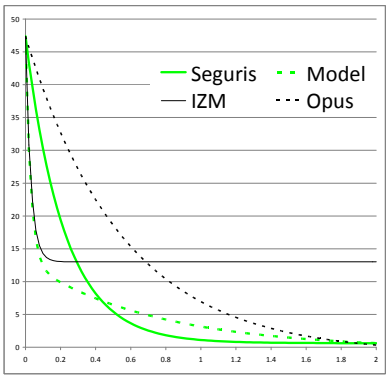
Rosemaund 2008 T2 leaves 1+2



Andover 2009 T2 leaf 2



Rosemaund 2009 T1 leaves 1+2



Rosemaund 2009 T2 leaves 1+2

Figure 25. Comparison of predicted response of IZM + 0.75 Opus mixture with Seguris. On the dose axis, 1 means a full dose of Seguris in both the data and the model, and full doses of IZM and Opus in the individual curves

4. AZOLE SENSITIVITY STUDIES ON POPULATIONS SAMPLED FROM THE FUNGICIDE PERFORMANCE TRIALS DURING 2009 AND 2010.

4.1. Introduction

Azole fungicides have been widely used for over 30 years to control *Septoria tritici* (teleomorph *Mycosphaerella graminicola*). In recent years, shifts in the sensitivity of *Septoria tritici* to azoles have been detected, however these have not reduced the efficacy of all azoles equally. Generally older azoles are more affected and now offer relatively poor control in commercial situations. Changes at specific locations in the azole target protein of the pathogen CYP51 (sterol 14α-demethylase) have been linked to these changes in sensitivity. In comparison with wild-type CYP51, for example, isolates carrying V136A mutations are less sensitive to prochloraz, whereas isolates carrying I381V are generally less sensitive to tebuconazole and metconazole. Isolates carrying A379G, I381V and deletion of amino acid residues Y459 and G460 (ΔY459/G460) combined are more sensitive to prochloraz and higher doses of tebuconazole and epoxiconazole are needed to control these strains *in vitro*.

New mutations and combinations of existing mutations continue to evolve. Samples were collected and assessed from the fungicide performance trials to help explain differences between sites in the performance of specific azoles, to indicate how specific treatments may select for certain mutations, and to provide information on how Septoria tritici populations may be changing over time.

4.2. Materials and methods

4.2.1. In 2009

Due to discoveries made during the HGCA co-sponsored LINK project 'Understanding evolution and selection of azole resistance mechanisms in UK populations of *Mycosphaerella graminicola'* (Fraaije *et al.*, 2011) it was decided to use both DNA diagnostics, able to detect changes in the azole target protein CYP51 (sterol 14α-demethylase) in populations, and *in vitro* azole sensitivity testing of isolates during the harvest 2009 season.

For DNA diagnostics, pyrosequencing assays targeting V136A, A379G and I381V amino acid substitutions in CYP51 were used (see HGCA project report 475 (Fraaije *et al.*, 2011) for methodology). In 2009, 17 treatments were sampled 3 to 4 weeks after the single spray T2 application at each site. Each treatment sample consisted of three pooled samples of 10 infected leaves randomly selected from each from three replicate plots.

4.2.2. *In vitro* sensitivity tests 2009

Strains of *M. graminicola* were isolated from the 2009 samples and tested for their *in vitro* sensitivity to azoles using fungicide amended Czapek Dox liquid medium (see HGCA project report 475 for methodology). Part of this work was co-sponsored by CRD. Results are expressed as EC50 values (effective concentration giving 50% control as μ ml^{-1})

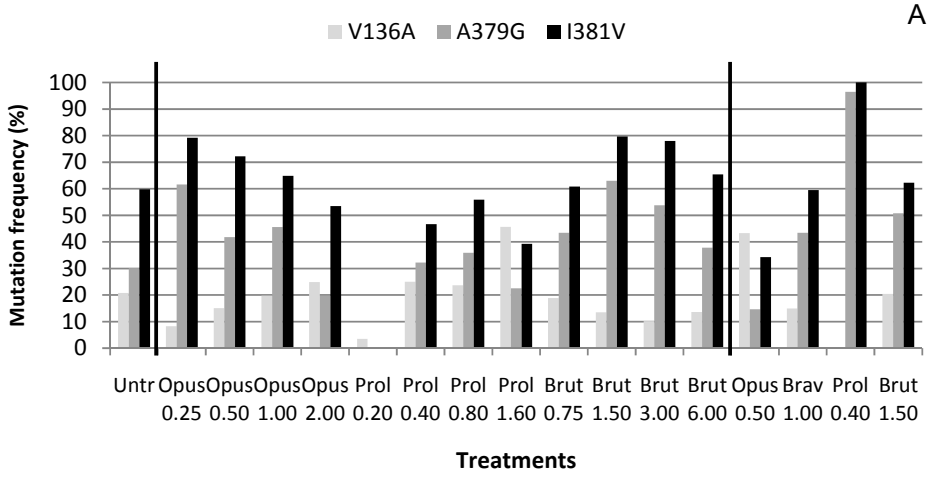
4.2.3. Sensitivity tests 2010

In 2010, it was decided to focus on *in vitro* sensitivity testing and further characterisation (e.g. *CYP51* gene sequencing and *in planta* fungicide sensitivity testing) of a selection of isolates. This was due to the complexity of CYP51 variants, including strains carrying both V136A and I381V, which meant pyrosequencing assays were not informative enough to monitor further CYP51 evolution and dynamics. Strains were isolated from randomly selected infected leaves sampled after a two-spray programme (T1 and T2) 3-4 weeks after the T2 application. Sabouraud instead of Czapek Dox liquid medium and a lower inoculum was used for fungicide sensitivity testing (see HGCA project report 475).

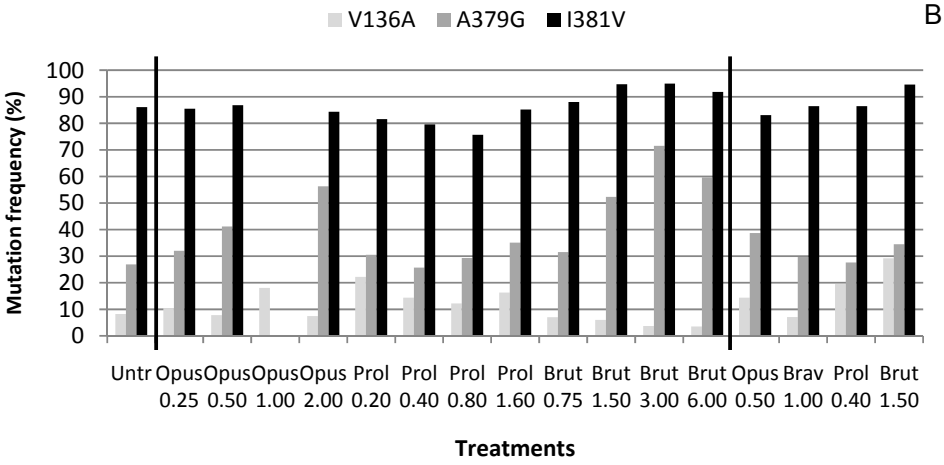
4.3. Results

The initial frequency of I381V in the untreated populations was lowest for the samples from Fife and Carlow. As expected, these samples also had higher frequencies for V136A (Figure 26). Stammler (2008) had previously reported differences in CYP51 variant composition for populations sampled in England, Scotland and Ireland. The frequency of variants carrying Y461S was high in Ireland and Scotland, whereas high frequencies of ΔY459/G460 variants were found in England. Higher V136A and lower I381V frequencies were measured in Fife after single Proline (prothioconazole) sprays. This trend, together with a decrease in A379G frequency was also observed with an increasing rate of Opus and Brutus (containing epoxiconazole). The extremely high frequencies, between 90 and 100 %, measured for A379G and I381V after two sprays of Proline (0.4 rate) (prothioconazole) in Fife was unexpected and may have been due to small populations of the pathogen surviving on the leaves after treatment. At Rosemaund, and to a lesser extent Andover, increased frequencies of A379G and I381V were measured after Opus (epoxiconazole) and Brutus (epoxiconazole + metconazole) applications. Overall, shifts in frequencies were not as strong as previously observed for cytochrome *b* G143A after QoI treatments (Fraaije et al, 2003). However, this is mainly due to smaller shifts in sensitivity (lower resistance factor) associated with different CYP51 variants.

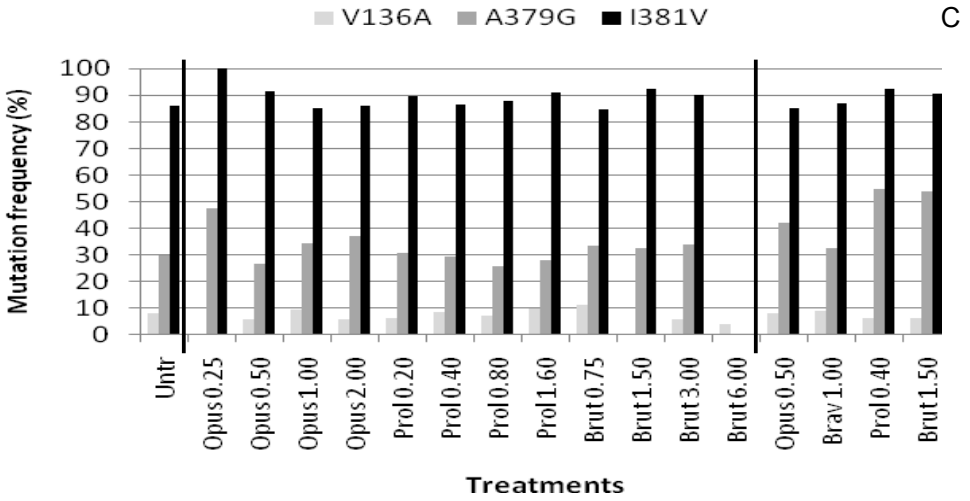
A


V136A
A379G
I381V
Mutation frequency (%)
100
90
80
70
60
50
40
30
20
10
0
Untr
Opus 0.25
Opus 0.50
Opus 1.00
Opus 2.00
Prol 0.20
Prol 0.40
Prol 0.80
Prol 1.60
Brut 0.75
Brut 1.50
Brut 3.00
Brut 6.00
Opus 0.50
Brav 1.00
Prol 0.40
Brut 1.50
Treatments

B


V136A
A379G
I381V
Mutation frequency (%)
100
90
80
70
60
50
40
30
20
10
0
Untr
Opus 0.25
Opus 0.50
Opus 1.00
Opus 2.00
Prol 0.20
Prol 0.40
Prol 0.80
Prol 1.60
Brut 0.75
Brut 1.50
Brut 3.00
Brut 6.00
Opus 0.50
Brav 1.00
Prol 0.40
Brut 1.50
Treatments

C


V136A
A379G
I381V
Mutation frequency (%)
100
90
80
70
60
50
40
30
20
10
0
Untr
Opus0.25
Opus0.50
Opus1.00
Opus2.00
Prol0.20
Prol0.40
Prol0.80
Prol1.60
Brut0.75
Brut1.50
Brut3.00
Brut6.00
Opus0.50
Brav1.00
Prol0.40
Brut1.50
Treatments

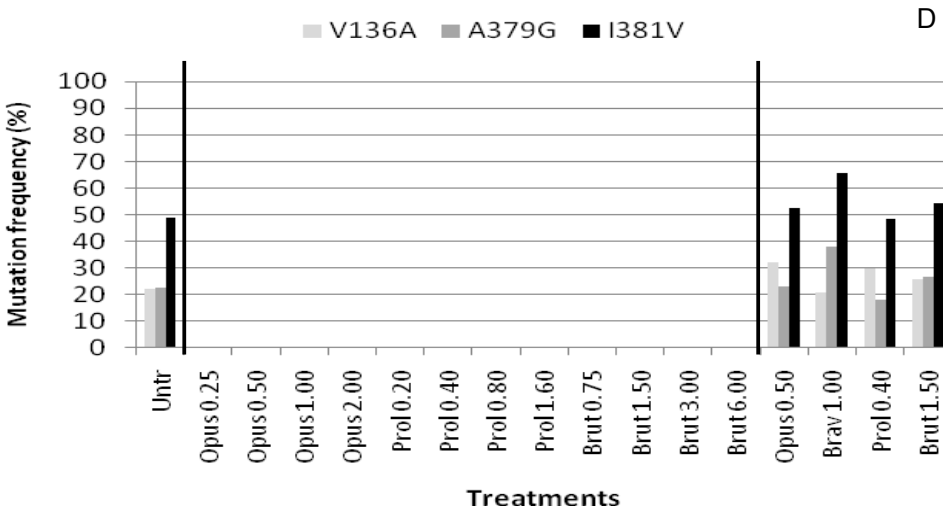
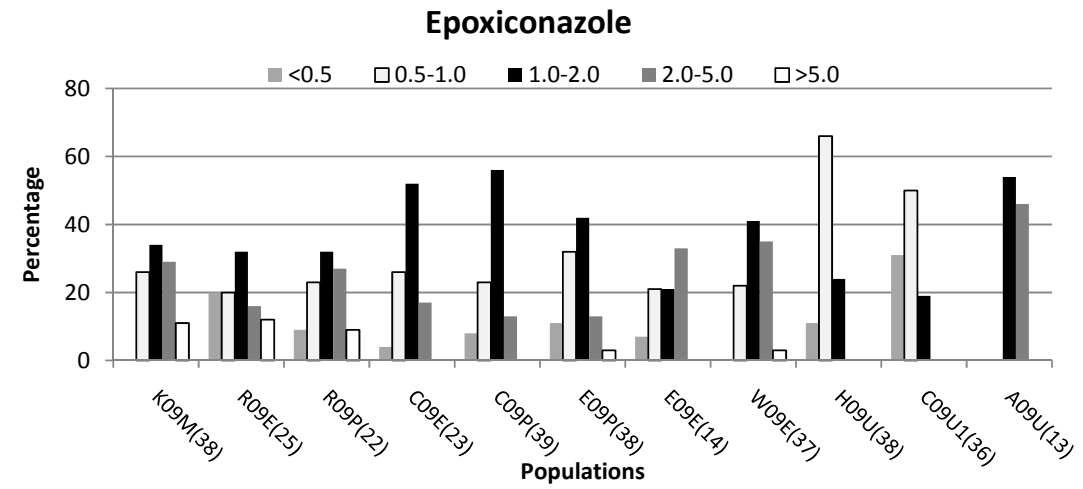
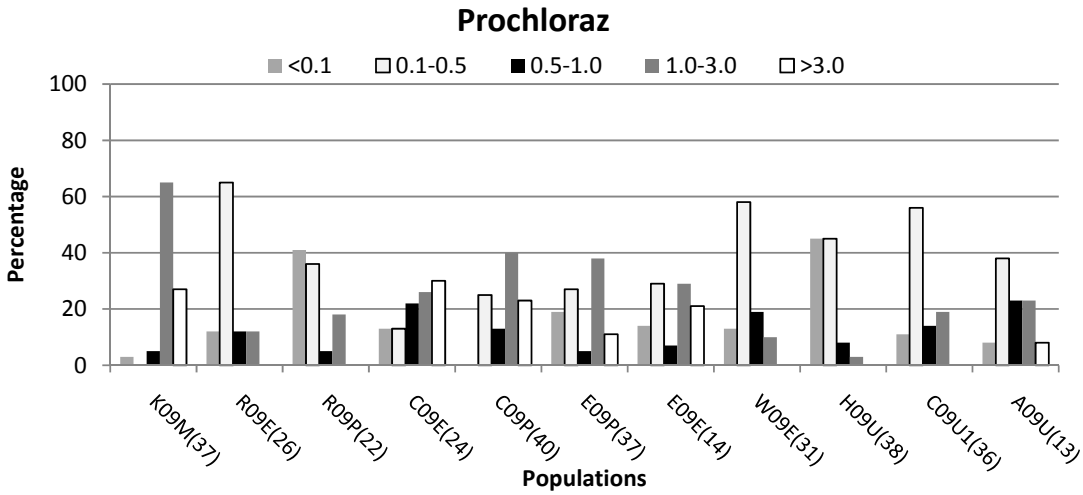


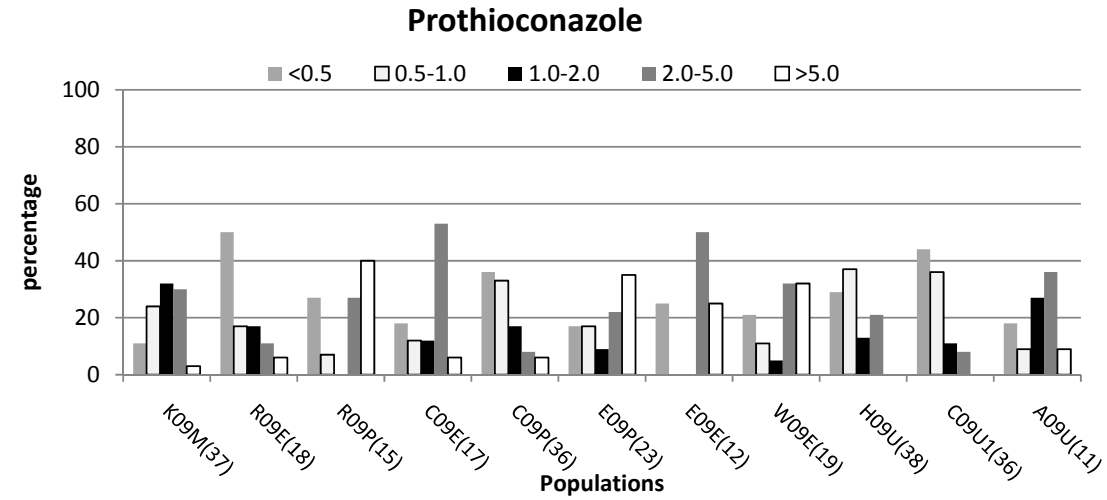
Figure 26. Selection of CYP51 amino acid alterations in *M. graminicola* field populations sampled in Fife, Rosemaund, Andover and Carlow (A to D respectively) three weeks after the final spray during the 2008/09 growing season. Samples without results were not collected (Carlow) or not tested using pyrosequencing. Samples (leaf layer T2 sampled at GS73-79) were collected from untreated plots (first sample in figure), plots treated once (samples 2-13) and plots treated twice with fungicides (samples 14-17, last four treatments). Doses are in litres of commercial product per hectare.

4.3.1. *In vitro* sensitivity 2009

There were clear differences in *in vitro* sensitivity between the untreated populations (Figure 27). The Andover population was least sensitive to epoxiconazole. The Harpenden population was most sensitive to prochloraz. The Carlow population was most sensitive to tebuconazole, followed by the Harpenden and Andover populations. For prothioconazole, the least sensitive strains were detected in the populations from Harpenden and Andover. Of the remaining treated populations tested, the Kent population was least insensitive to prochloraz and prothioconazole overall. Relatively high numbers of tebuconazole and epoxiconazole less sensitive strains were also found from this location. Problems with measuring EC_{50} values for prothioconazole, were encountered for some strains. For example, EC_{50} values could only be determined for 19 out of the 38 strains tested from population W09E. Most of these strains appear to have high levels of prothioconazole resistance *in vitro* based on the curve shape (data not shown), but when the prothioconazole sensitivity was measured *in planta* for a selection of these strains a fully sensitive phenotype was observed (data not shown). Only a few strains could be isolated from the azole-treated Andover populations.


Epoxiconazole
<0.5
0.5-1.0
1.0-2.0
2.0-5.0
>5.0
Percentage
80
60
40
20
0
K09M(38)
R09E(25)
R09P(22)
C09E(23)
C09P(39)
E09P(38)
E09E(14)
W09E(37)
H09U(38)
C09U1(36)
A09U(13)
Populations


Prochloraz
<0.1
0.1-0.5
0.5-1.0
1.0-3.0
>3.0
Percentage
100
80
60
40
20
0
K09M(37)
R09E(26)
R09P(22)
C09E(24)
C09P(40)
E09P(37)
E09E(14)
W09E(31)
H09U(38)
C09U1(36)
A09U(13)
Populations


Prothioconazole
<0.5
0.5-1.0
1.0-2.0
2.0-5.0
>5.0
percentage
100
80
60
40
20
0
K09M(37)
R09E(18)
R09P(15)
C09E(17)
C09P(36)
E09P(23)
E09E(12)
W09E(19)
H09U(38)
C09U1(36)
A09U(11)
Populations

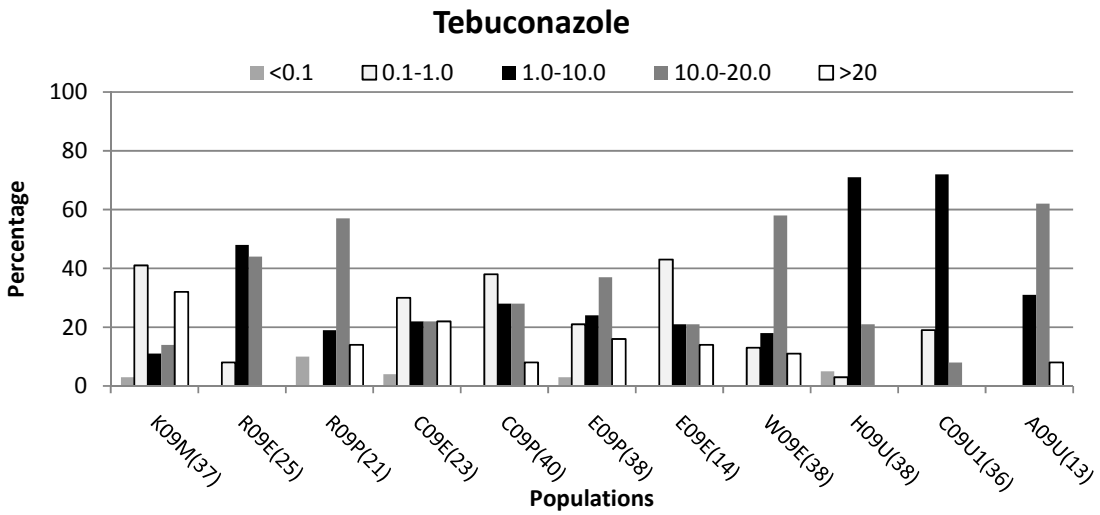
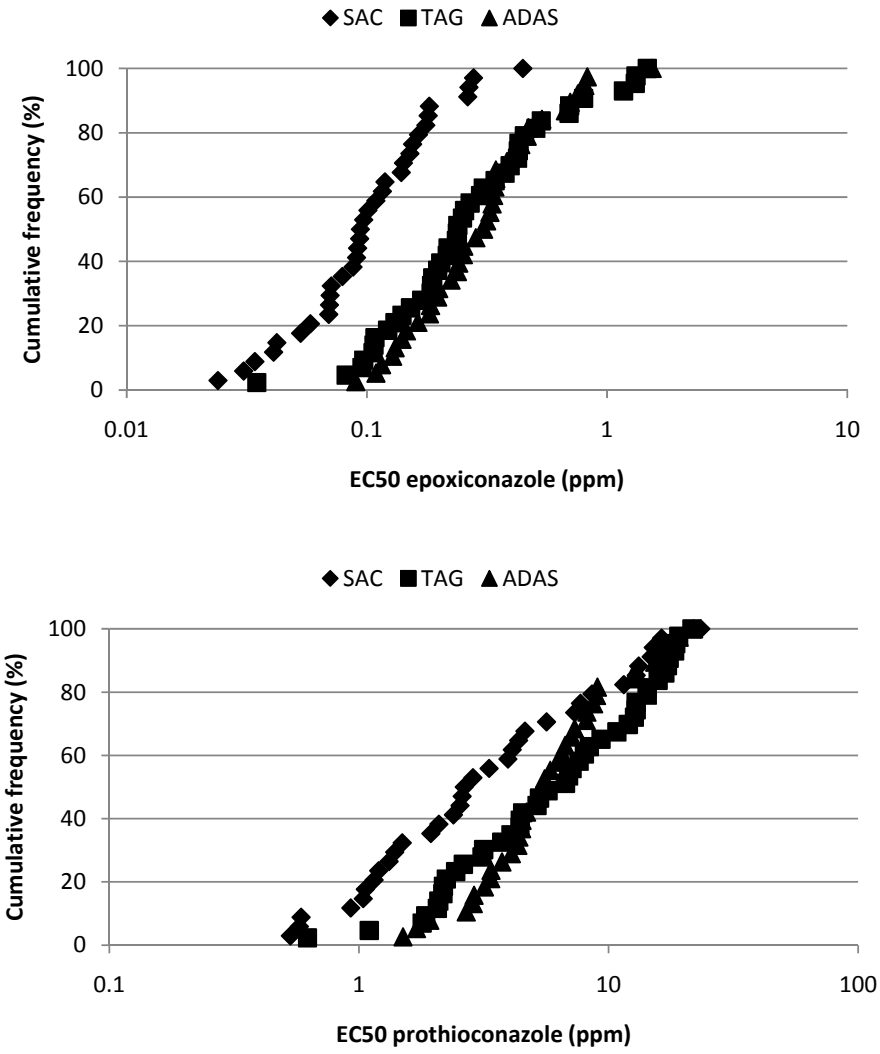


Figure 27. Distribution of azole sensitivities within field populations of *Mycosphaerella graminicola*. Data are presented as ranges of EC_{50} values (µg ml^{-1}). Populations tested: K09M (Kent, cyproconazole, epoxiconazole, prochloraz, propiconazole and prothioconazole-treated), R09E (Rosemaund, epoxiconazole-treated), R09P (Rosemaund, prothioconazole-treated), C09E (Carlow, epoxiconazole-treated), C09P (Carlow, prothioconazole-treated), E09P (Fife, prothioconazole-treated), E09E (Fife, epoxiconazole-treated), W09E (Wellesbourne, epoxiconazole-treated), H09U (Harpenden, untreated), C09U (Carlow, untreated) and A09U (Andover, untreated). Between brackets, the number of strains for each fungicide-population combination for which an EC50 value could be determined using the BMG Labtech OPTIMA Sofware for curve fitting.

In comparison to strains isolated before 2009, several strains had new azole sensitivity phenotypes. Therefore, the *CYP51* gene was sequenced from a selection of these strains. Several new CYP51 variants were found (e.g. L50S, D134G, V136A, I381V & Y461H (Harpenden); L50S, D134G, V136A, Y461S & S524T (Andover); L50S, V136A, S188N, DEL & S524T (Kent) and L50S, V136A, S188N, DEL & S524T (Kent)). The S524T carrying strains were different from the Irish strains reported in 2008 (variant L50S, V136A, Y461S & S524T) and variant L50S, D134G, V136A, Y461S & S524T had lower levels of sensitivity to epoxiconazole and prothioconazole both *in vitro* and *in planta*.

Results obtained for untreated 2010 populations are displayed in Figure 28. The Fife population was most sensitive to epoxiconazole, prothioconazole and tebuconazole. The Andover and Rosemaund populations were similar in their sensitivities to epoxiconazole, prochloraz and prothioconazole, but the Andover population was more sensitive to tebuconazole than the populations at other sites.



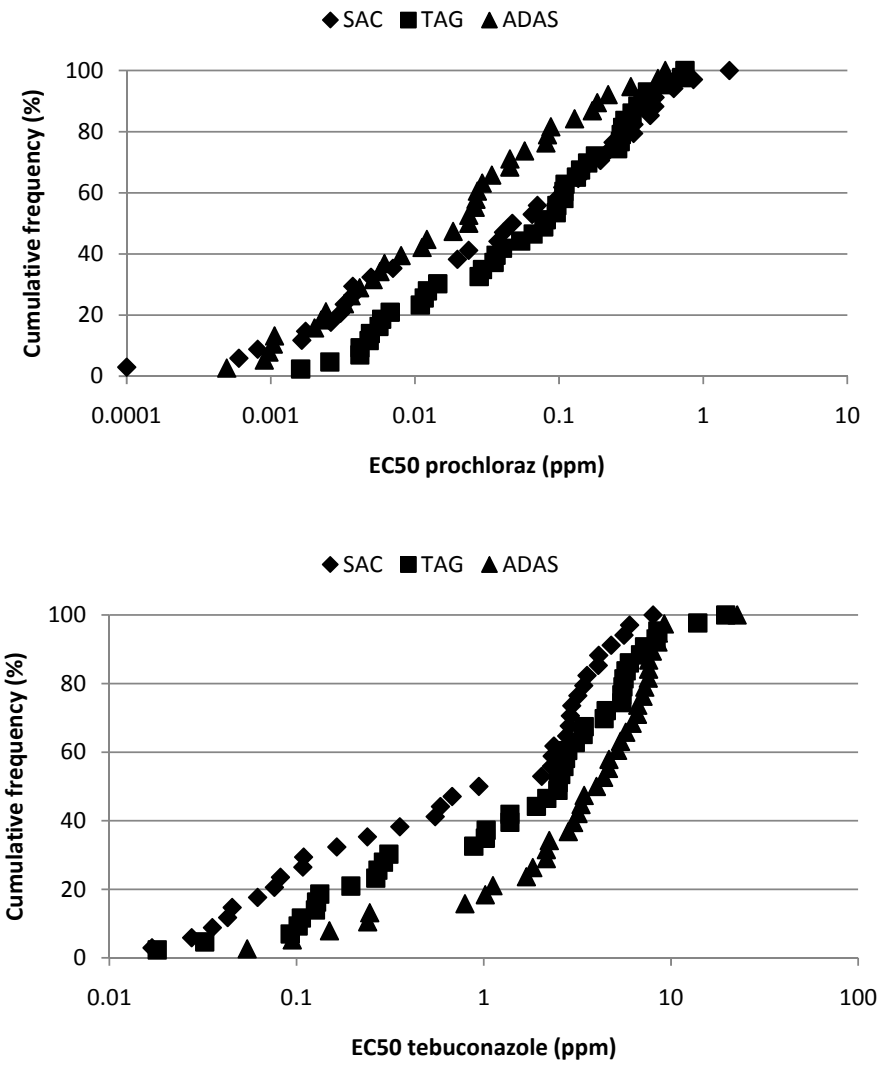


Figure 28. Distribution of azole sensitivities within untreated field populations of *Mycosphaerella graminicola* sampled in 2010. Isolates ranked according to increasing EC_{50} values (cumulative). Locations SAC (Fife, n=34), TAG (Andover, n=43) and ADAS (Rosemaund, n=38).

Populations were also sampled after treatments with epoxiconazole and prothioconazole, and the sensitivity to epoxiconazole was determined (Figure 29). For the Fife and Rosemaund populations, no significant shift towards less sensitive was measured after treatments with epoxiconazole and prothioconazole, but more 'extreme' isolates were detected.

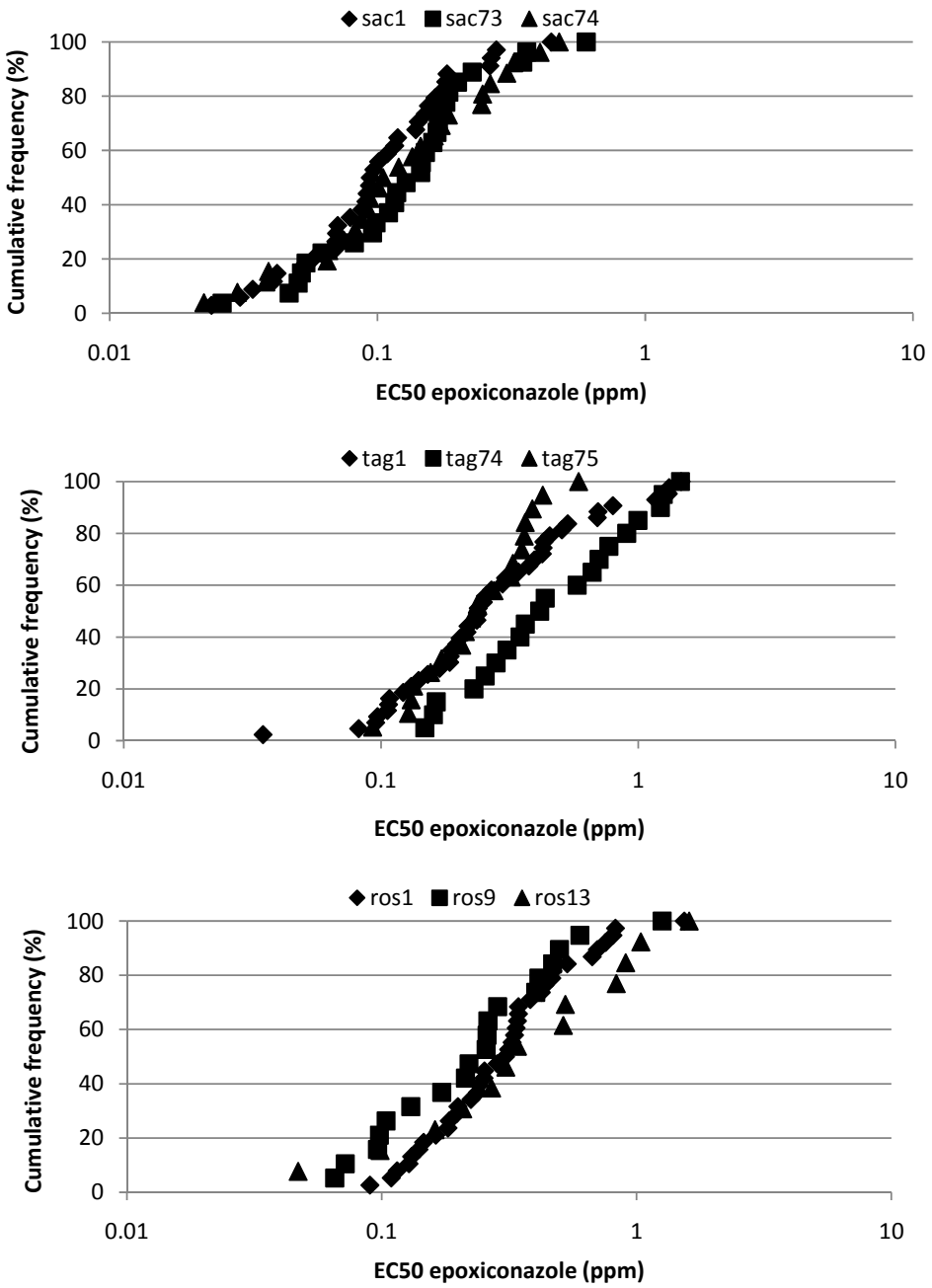


Figure 29. Epoxiconazole sensitivities of untreated and azole-treated 2010 field populations of *Mycosphaerella graminicola*. Isolates ranked according to increasing EC_{50} values (cumulative). Populations from SAC (Fife), untreated (treatment 1, n=34), epoxiconazole treated (73, n=27) and prothioconazole treated (74, n=26). Populations from TAG (Andover), untreated (1, n=43), prothioconazole treated (74, n=20) and epoxiconazole + metconazole treated (75, n=19). Populations from ADAS (Rosemaund), untreated (1, n=38), epoxiconazole treated (9, n=19) and prothioconazole treated (13, n=13).

4.4. Conclusions

Results from 2009 indicated that between 80 and 90% of all Septoria tritici isolates had the I381V mutation at the Andover and Herefordshire sites. This denotes insensitivity to tebuconazole and to a lesser extent metconazole. At the Scottish and Irish sites Fife and Carlow, the frequency was much lower with 60% and 50% of isolates contained I381V mutations respectively, indicating that tebuconazole and metconazole may be less affected in these regions. Different azoles increased the frequency of some mutations in the population; however the products tested here selected for different isolates. This indicates disease control strategies may benefit from the use of mixtures or sequences of different azoles, rather than repeat applications of the same active. However limited data from epoxiconazole sensitivity monitoring post treatment in 2010, suggests prothioconazole application may increase insensitivity to epoxiconazole, so just sequencing these two azoles may be insufficient alone.

Site differences in both 2009 and 2010 in sensitivity to specific azoles were found, however the differences between sites in their relative sensitivities to azoles, were not consistent across the two seasons; indicating a large spatial mobility of populations due to dispersal of airborne ascospores (Fraaije *et al*., 2005).

Sequencing of *CYP51* genes in the least sensitive isolates confirmed the presence of CYP51 variants found in 2009 and new CYP51 variants (Table 3). Some of the new variants (e.g. L50S, V136A, I381V, Y461S & S524T; L50S, S188N, A379G, I381V, Y459D & S524 and L50S, S188N, A379G, I381V, ∆Y459/G460 & S524T) confer increased EC50 values for both epoxiconazole and prothioconazole, but are relatively sensitive to either tebuconazole or prochloraz. Two different ranges of azole sensitivity were measured for variant L50S, S188N, I381V, ∆Y459/G460 & N513K. Further studies revealed that *CYP51* over-expression was detected in strains belonging to the least sensitive phenotype (Cools *et al*., 2012)

Further monitoring the azole sensitivity profiles of field populations, both *in vitro* and *in planta* should continue as the evolution of CYP51 in *M. graminicola* field populations in response to selection by azole fungicide use is an on-going process. This information could be used directly to advise farmers on the most efficient use of azole-based spray programmes. Further development and application of molecular tools such as *CYP51* gene replacement, site-directed mutagenesis and protein modelling will be required to enable a better understanding of fungicide-target interactions and even predict CYP51 evolution in *Mycosphaerella graminicola*. In addition, the role of alternative resistance mechanisms, such as efflux pumps and over-expression of CYP51, should be further investigated as for some CYP51 variants a wider range of sensitivities to azoles was measured than could be explained by changes to the target protein alone.

Table 3. Azole sensititivity profiles of *Mycosphaerella graminicola* CYP51 variants found in the HGCA Fungicide Performance trials during 2009-2010.

CYP51 variant (number of strains)	**Average resistance factor in comparison with wild-type strains[1]**			
	Epox	**Proc**	**Tebu**	**Proth**
Wild-type (4)	-	-	-	-
V136C & Y461H (1)	169	24	48	19
L50S, V136A & Y461H (9)	64	25	4.8	70
L50S, I381V & Y461H (17)	87	4.8	54	27
D107V, I381V, N513K & S524T (2)	271	6.9	43	101
L50S, V136A, Y461S & S524T (5)	203	62	4.2	84
A379G, I381V, ΔY459/G460 & N513K (1)	156	0.5	84	128
L50S, D134G, V136A, Y461S & S524T (1)	209	12	6.5	358
L50S, V136A, I381V, Y461H & S524T (1)	511	43	14	195
L50S, D134G, V136A, I381V & Y461H (6)	316	24	10	186
L50S, V136A, I381V, Y461S & S524T (1)	536	19	11	224
L50S, S188N, I381V, ΔY459/G460 & N513K* (5)	78	3.2	30	59
L50S, S188N, I381V, ΔY459/G460 & N513K* (7)	355	24	211	153
L50S, V136A, S188N, ΔY459/G460 & N513K (5)	78	25	2	57
L50S, V136A, S188N, ΔY459/G460 & S524T (3)	133	74	9.9	94
L50S, S188N, A379G, I381V, Y459D & S524T (1)	560	3.5	208	194
L50S, S188N, A379G, I381V, ΔY459/G460 & N513K (19)	196	1.1	136	45
L50S, S188N, A379G, I381V, ΔY459/G460 & S524T (1)	456	3.3	272	221

[1]Highest EC_{50} values (μg ml^{-1}) for the most insensitive (wild type) CYP51 variants, from which the resistance factors are calculated: epoxiconazole: 1.61; prochloraz: 1.21; tebuconazole: 19.6; prothioconazole: 35.0

*Two azole sensitivity ranges were measured for this variant.

4.5. References

Cools, H.J.; Bayon, C.; Atkins, S.; Lucas, J.A.; Fraaije, B.A. (2012) Over-expression of the sterol 14α-demethylase gene (*MgCYP51*) in *Mycosphaerella graminicola* isolates confers a novel azole fungicide sensitivity phenotype. *Pest Management Science* (in press; available on-line)

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