Project Report No. 491 **April 2012** **£12.25**

Forecasting eyespot development and yield losses in winter wheat

April 2012
Cost £12.25

Project Report No. 491

Forecasting eyespot development and yield losses in winter wheat

by
Fiona Burnett[1], Clare Butler-Ellis[2], Gareth Hughes[1], Stuart Knight[2] and Rumiana Ray[3]

[1]SAC, West Mains Road, Edinburgh EH9 3JG
[2]NIAB TAG, Huntingdon Road, Cambridge CB3 0LE
[3]University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD

This is the final report of a 43 month project (RD-2007-3362) which started in September 2007. The work was funded by a contract for £187,000 from HGCA, with a total project cost of £552,000 including in-kind contributions from British Wheat Breeders (BWB), Bayer CropScience, Syngenta, BASF and Harper Adams University College.

CONTENTS

1. ABSTRACT

The aim of this project was to help growers to predict which crops are at risk from eyespot and are likely to give a cost-effective yield response to treatment. Data on yield, disease and agronomy were collected from field trials located throughout the UK between 2000 and 2010. Two approaches to providing decision guidelines relating to treatment of eyespot were developed.

In the first, the decision-making process is driven by the predicted final level of eyespot disease in the crop in a two phased approach. Using region, previous crop, sowing date, tillage method and soil type, a pre-disease score can be calculated and a decision made on field selection or varietal choice to include eyespot resistance. In the spring fungicide treatment decisions can be made by combining this pre-disease score with an assessment of eyespot incidence in the crop at GS31-32 to place the crop in a risk category ranging from low to high. In the second approach, the decision-making process is driven by the cost of treatment relative to the predicted yield loss due to eyespot disease if the crop were not treated. A revenue calculator was then developed.

No consistent relationship could be established between eyespot levels and yield, with variation between sites. Yield response and eyespot control following fungicide treatment was significant using the whole data set. Significant control and yield benefits were noted for varieties with moderate or good eyespot resistance. Fungicide efficacy varied but up to 50% control was achieved. Alternative spray technologies were evaluated to see if they could improve targeting of the stem base, and hence efficacy. Applications at a later growth stage resulted in significantly less deposit on the lower stem. Angled spray nozzles and small droplet air induction nozzles improved deposition on the stem base.

Inoculated studies showed that initial inoculum level was a significant driver of disease development and subsequent yield loss. Yield loss and response to fungicide treatment were significantly greater when eyespot was associated with whiteheads and lodging. There were differences in how yield losses accrued between the two eyespot species: *Oculimacula yallundae* was associated with the occurrence of eyespot-induced lodging, whilst inoculation with *O. acuformis* resulted in greater number of whiteheads scattered within the crop.

2. SUMMARY

2.1. Introduction

Fungicide treatment for the stem base disease eyespot, caused by *Oculimacula* spp., represents an additional cost compared to the standard sprays that would be applied to the winter wheat crop for the control of the foliar diseases alone that are the main target at stem extension. The primary aim of this project was to help growers predict which crops are at risk of eyespot and are likely to give a cost effective yield response to specific eyespot treatment. A previous model allowed growers to determine the need for fungicide treatment in the spring but the introduction of varieties with the Pch1 gene conferring improved resistance to eyespot means that growers need to be able to make an initial judgement of eyespot risk in the autumn.

Further aims of the project were to better understand how the two fungal species, *O. yallundae* and *O. acuformis*, cause yield loss and to evaluate the efficacy of fungicides and varietal resistance in eyespot control. An additional objective was to investigate spray technologies to determine if treatment efficacy could be improved.

2.2. Methods

Data on yield, disease and agronomy were collected from field trials located throughout the UK between 2004 and 2010, and combined with data from a previous eyespot project running from 2000 to 2003 to give a data set of over 700 untreated scenarios.

2.3. Results and discussion

Two approaches to providing decision guidelines relating to treatment of eyespot were developed.

- In the first, the decision-making process is driven by the predicted final level of eyespot disease in the crop. In this case, no explicit link between the level of eyespot disease in the crop and yield loss is made. Eyespot risk has been categorised into (1) pre-disease (conditional) risk and (2) risk attributable to the level of disease at a GS31-32 disease assessment. Decision guidelines are then based on a combination of these two risk categories, depending on the decision-maker's attitude to risk (Tables 1 and 2).

- In the second approach, the decision-making process is driven by the cost of treatment relative to the predicted loss from the value of the yield reduction attributable to eyespot disease in the crop if it were not treated. In this case, an explicit link is required between the level of eyespot disease in the crop and yield loss. It is therefore problematic that no suitable overall generic relationship between yield loss and the level of eyespot disease has

been described on the basis either of the present work or of other related work reported in the literature. Instead, we have described the calculation of an economic threshold based on an estimate of the rate of yield loss to eyespot disease to be provided by a decision-maker in relation to their specific circumstances. We have illustrated this with examples using yield loss data from sub sets of the project data and previous yield loss estimates in the literature.

2.3.1. Risk prediction for eyespot

Using a logistic regression model region, soil type, previous crop, tillage method and sowing date were identified as factors that had importance in final disease outcome. These were weighted by influence and arranged in Summary Table 1 so that an autumn assessment of risk could be made, ideally before drilling. At this point a crop could accumulate a maximum risk score of 25 points which would place it at the highest risk of eyespot (Summary Table 2). Growers could then choose to select a different field or to drill a variety with eyespot resistance based on the *Pch1* resistance gene such as Grafton or Battalion, or a variety with moderate resistance thought to be based on the *Pch2* gene, such as Einstein. Trial work shows that such varieties can reduce eyespot final severity by 30% relative to a susceptible variety such as Robigus.

Summary Table 1. Pre-disease risk factors

Factor	Level	Odds ratio	Log_{10}	Risk points
Region	East	1	0	0
	North	1.149	0.0603	1
	West	1.788	0.2524	5
Soil type	Light	1	0	0
	Medium	1.071	0.0298	1
	Heavy	1.559	0.1928	4
Previous crop	Non-host	1	0	0
	Other cereal	2.245	0.3512	7
	Wheat	2.420	0.3838	8
Tillage	Minimum Till	1	0	0
	Plough	2.044	0.3105	6
*Sowing date**	Late	1	0	0
	Early	1.336	0.1258	2

* Early = before or including 6 Oct; Late = after 6 Oct

Summary Table 2. Pre-disease risk categories (conditional risk)

Pre-disease risk points	Verbal description of pre-disease risk category
1-4	Low risk (L)
5-9	Low-medium risk (LM)
10-14	Medium risk (M)
15-19	Medium-high risk (MH)
≥20	High risk (H)

* Advisory rather than prescriptive

A field, once drilled, will come out of the winter with this pre-disease score determined. A decision then needs to be made about the need to treat with a fungicide with eyespot efficacy. Summary Table 3 describes the risk of eyespot developing in that crop by combining the pre-disease score in Summary Table 1 with the level of disease visible in the crop, from which a risk category can be determined.

Summary Table 3. Eyespot disease risk categories*

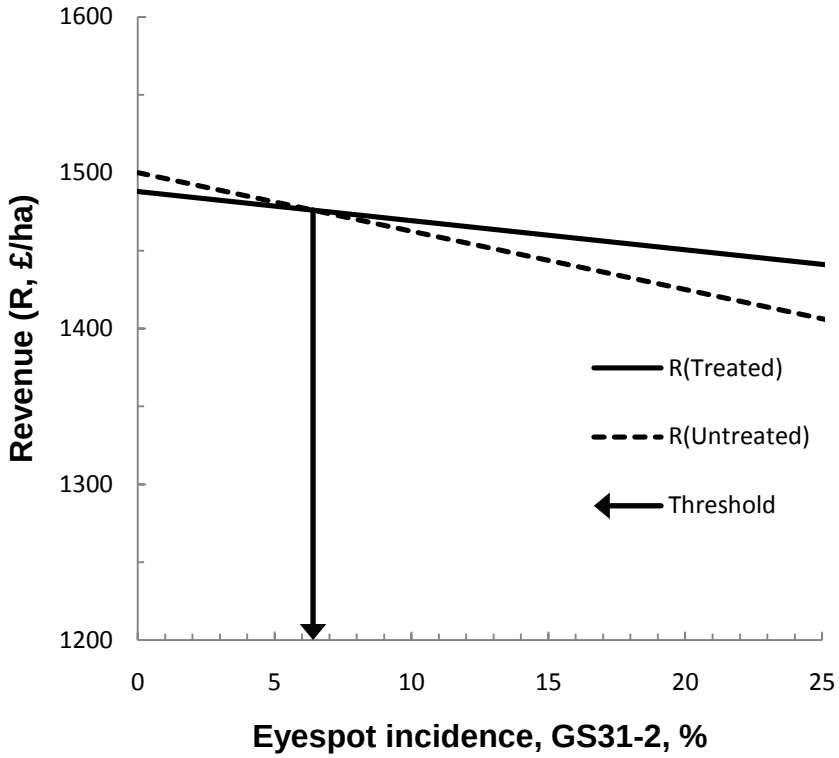
Pre-disease risk points (conditional risk)	Eyespot disease assessment % incidence at GS 31-32				
	1-4	5-9	10-14	15-19	≥20
1-4	L	LM	M	MH	H
5-9	LM	M	M	MH	H
10-14	M	M	MH	MH	H
15-19	MH	MH	MH	H	H
≥20	H	H	H	H	H

*Verbal description of category: Low risk (L), Low-medium risk (LM), Medium risk (M), Medium-high risk (MH), High risk (H).

The decision to treat or not is then made by the operator. They may choose to do this based on past experience where eyespot is always or seldom a problem to them in which case they would be described as risk sensitive or risk tolerant respectively. Or they may choose to do this based on the predicted yield loss and revenue calculator example illustrated in the full report, of which one example is given below in Summary Figure 1. The yield loss estimate in this case was observed in inoculated trials.

2.3.2. Calculation of economic threshold

The report describes the likely benefits of eyespot control measures based on a simple overall average relationship between yield loss and disease. We have used the potential yield of the crop, the value of that yield, the estimated yield loss, and the disease incidence to calculate a threshold for treatment and then used the cost of treatment and the proportional reduction in disease intensity to calculate the revenue from a treated crop, as illustrated in Summary Figure 1.



Summary Figure 1. An example of a graphical plot of revenue reduction against disease illustrating the economic treatment threshold at 6.4% eyespot incidence at GS31-32.

The rate of revenue reduction is steeper in an untreated crop than in a treated crop. The economic threshold is the point where the revenue reduction resulting from the disease is equal to the revenue reduction incurred for treatment. In this example, calculations are based on the crop yield being = 10t/ha, the value of grain = 150£/t, the cost of treatment = £12/ha, the efficacy of the fungicide = 0.5 (50%), and the yield loss = 0.025t/ha/% eyespot incidence at GS31-32 (these efficacy and yield loss data are taken from data sets within the main report). From this worked example the economic threshold value is calculated at 6.4% eyespot incidence at GS31-32. The larger data set would suggest that losses to eyespot are often somewhat lower than this. The lowest estimate was 0.005 t/ha per percentage eyespot, taking the whole data set, which would set the economic threshold at 30% eyespot incidence at GS31-32, if all other parameters are kept the same.

The lack of any consistent significant correlation between eyespot levels and yield in the data set is an important finding of this work and suggests that responses to eyespot occur on a far more local scale than the factors that were evaluated in the project could predict. Factors such as region, weather, soil type, sowing date and previous crop that were useful in predicting final eyespot severity did not assist in predicting the likelihood of a yield response. In order to provide guidance to decision makers a table is included in the main report where different yield loss estimates can be selected for use in the calculation to determine the economic threshold for treatment.

A work package within the report aimed to determine the effects of quantity of primary inoculum sources on the development of *Oculimacula* spp. and final yield loss. Results indicate that initial inoculum level plays a significant role in the initiation of yield damaging eyespot epidemics. In practice this would equate to the disease inoculum left behind by the previous crop.

Artificially inoculated experiments using *O. acuformis* or *O. yallundae* were carried out in 2008 and 2009 to quantify the yield losses attributable to each species. Experiments evaluated the effects of three inoculation rates, the fungicide cyprodinil and the growth regulator trinexapac-ethyl applied at stem elongation GS32 on disease development and pathogen DNA. Yield, specific weight and thousand grain weight at harvest were also investigated.

In these inoculated trials the highest inoculum rate reduced yield by 10%. Reductions in thousand grain weight were greater for eyespot caused by *O. acuformis* than *O. yallundae*. Eyespot disease caused by *O. yallundae* was associated with greater occurrence of lodging whilst *O. acuformis* caused increased number of whiteheads in 2008 when the disease was more severe. Cyprodinil application reduced visual eyespot disease index, *Oculimacula* DNA, lodging and the number of whiteheads significantly resulting in a 12% yield response. The growth regulator trinexapac-ethyl application failed to directly affect disease index, pathogen DNA or lodging, but reduced the number of whiteheads by 36% in 2008.

PCR analysis of stem base samples from the 50 field sites evaluated in the development of the risk model, showed that *O. yallundae* was the dominant strain – with 10 times the quantity of DNA recovered compared to *O. acuformis*. Most sites had mixed infections. This is a reversal of the situation in the previous HGCA project 347, when *O. acuformis* was the dominant strain in trials throughout the UK. Although yield was highly variable between trials and no significant relationship with eyespot levels could be established, there were significant yield benefits to fungicide treatment in the data set. Yield response to fungicide treatment at GS31-32 was significant for boscalid + epoxiconazole, cyprodinil, epoxiconazole and prothioconazole, and significant levels of eyespot control were also noted. The levels of eyespot control observed with epoxiconazle had not been predicted from previous work and the swing towards *O. yallundae*, and away from *O. acuformis,*

could be one explanation why epoxiconazole was more effective as the azole group of chemistry has more activity on this species.

This study would concur with previous work that the most effective eyespot fungicides offer around 50% disease control at best. Difficulties in targeting the stem base are one reason why control is not higher and a work package within the project looked at spray technologies to see if they could improve targeting of the stem base. Applications at the later growth stage of GS 37 resulted in significantly less deposit on the lower stem than at the earlier growth stage of GS31-32. It was not possible to improve targeting at this later growth stages with conventional spray nozzles. Increasing spray volumes above 100 l/ha did not improve penetration into a dense crop. Results suggest that 80^{o} spray angles and the use of a small droplet air induction nozzle gave the highest achievable levels of lower stem deposits in a cereal crop.

An evaluation of predicted climatic changes and expected eyespot distribution did not suggest any large shift in current risk. Climate change predictions would suggest that because of an increasing likelihood of dry springs, eyespot severity and distribution might be expected to decline in the UK in the 2020 and 2050 time frames, but weather predictions beyond this time frame suggest an slightly increased risk so it is important that resistance to eyespot remains a target in wheat breeding programmes.

3. TECHNICAL DETAIL

3.1. General introduction

The project aim was to reduce the uncertainty surrounding yield responses to eyespot treatments and to provide growers with a method of judging likely yield loss from eyespot, allowing treatment decisions to be made. Treatment for eyespot involves either additional or increased dosages of fungicides applied to target the stem base and / or the use of varietal resistance in high eyespot risk situations - if they can be confidently identified prior to sowing.

A complex of diseases infect the stem base in wheat of which eyespot is the most common and the most damaging. There are two species of fungal pathogen that cause eyespot which are common in the UK, *Oculimacula yallundae* (formerly known as *Tapesia yallundae*) and *Oculimacula acuformis* (formerly known as *Tapesia acuformis*) (Crous *et al*., 2003). *O.yallundae* is still commonly referred to as 'W type' eyespot and *O. acuformis* as 'R type' eyespot, referring back to a previous name change when they were both thought to be pathotypes of *Pseudocercosporella herpotrichoides*. The W and R type nomenclature refers to their relative pathogenicity. W type is highly pathogenic on wheat, but less so on barley and on rye, while the R type is equally pathogenic on wheat, barley and rye (Scott *et al*., 1975).

The symptoms of eyespot at the end of the season are of oval, eye-shaped lesions on the stem base, with a diffuse brown margin. The disease blocks the movement of water and nutrients upwards in the plant leading to reduced yield and smaller grains (Ray *et al*., 2006). In severe infections the stem becomes brittle and can lead to lodging. Stiffer-strawed varieties and improved use of growth regulators mean that naturally induced lodging is now much less common. In the early stages of infection symptoms are less clear and may appear as small, honey brown smudges on the outer leaf sheaths. Symptoms are often confounded by the presence of other stem base diseases like footrot caused by *Fusarium* spp. and sharp eyespot casued by *Rhizoctonia cerealis*.

The two Oculimacula species fluctuate in occurrence and distribution. Early field work on eyespot control was carried out on the W type (*O. yallundae*) which predominated at the time. *O. acuformis* (R type) then became the dominant species in the UK by the mid 1980s up to the late 1990s and early part of the 2000s (King and Griffin, 1985; Nicholson and Turner, 2000 and Burnett and Hughes, 2004). There are differences in the infection processes for the two eyespot species. *Oculimacula acuformis* has a slower initial phase of growth than *O. yallundae,* which grows faster after spore germination (Daniels, 1993a). *O. acuformis* will invade all cell parts after it has penetrated the host whereas the *O. yallundae* will only infect the cell wall. After the formation of infection plaques *O. acuformis* plaques are more compact and symmetrical in comparison to the *O. yallundae* probably due to its slower growth. *Oculimacula acuformis* isolates develop more slowly

on leaf sheaths and on stems than *O. yallundae* isolates (Goulds and Fitt, 1990). They are therefore less likely to show visual browning and lesions early in the season when compared to the *O. yallundae*.

3.1.1. Agronomic influences

The severity of disease development as a result of infection by eyespot is determined by agronomic as well as environmental factors. Conidia are spread to the host plant by rain splash from trash in the soil, so levels of trash are potentially important. The mycelium then penetrates the coleoptiles or leaf sheaths of the host plant. Infection is localised at the stem base; it seldom infects above the second node and does not colonise leaf or root tissue. The infection can proceed through several leaf layers to eventually penetrate the stem. Under natural conditions, *Oculimacula* spp. sporulate to produce abundant conidia on infected crop debris, which is considered the main source of primary inoculum. There is a peak in sporulation in March/April/May followed by a decline as the temperature increases but with some late conidia produced on infected stems in June/July (Rowe and Powelson, 1973; Fitt *et al*. 1988). However, whilst strong evidence exists that the quantity of inoculum causing disease on seedlings and young plants has a significant effect upon disease incidence (Wan *et al*., 2005), the role of quantity of primary inoculum sources initiating disease epidemics and yield loss in field has remained unclear.

The development of the disease is favoured in the UK by mild, wet weather in winter and cool damp weather in spring. Eyespot is most severe in early-sown crops and can be reduced in high risk fields by late sowing and crop rotation (Cook, 1993).

There is also evidence that tillage method can influence eyespot levels. For example minimum tillage has been observed to reduce eyespot levels in crops, when compared to crops established through ploughing (Jalaluddin and Jenkyn, 1996). This finding was confirmed in HGCA Project 347 (Burnett and Hughes, 2004) when ploughing was identified as a factor increasing eyespot risk, compared to minimum tillage. There is no understanding of the mechanism for this effect, which may be due to increased populations of bacterial or fungal antagonists, or to infected straw retaining a greater degree of infectivity when ploughed down compared to being left on the surface. Possibly remaining trash could act as a physical barrier to spore splash and movement.

3.1.2. Varietal resistance

There are two genes conferring eyespot resistance that have been incorporated into UK varieties, commonly known as *Pch1* and *Pch2*. The *Pch2* gene is sometimes called Capelle-Desprez resistance after the early wheat variety into which it was bred. Much of the UK cereal production relies on varieties thought to contain this gene with, at best, moderate eyespot resistance and yield

losses to eyespot can still be high even in varieties where it is present. An alternative and more robust form of resistance, the *Pch1* gene derived from *Aegilops ventricosa*, has more recently been incorporated into breeding programmes and was first available in an HGCA Recommended List variety as Hyperion. More recently varieties such as Grafton have become available. HGCA-funded work (Project Report No 216) has shown that the use of varieties with some eyespot resistance can obviate the need for fungicide treatment in some situations.

3.1.3. Influence of break crops and surrounding crops

Eyespot is thought to be predominately trash-borne and infects new crops by conidial spores splashing up from straw debris. It can therefore be reduced to some extent by rotation. However, there is also a sexual, air-borne phase in the disease cycle which means that surrounding crops may also be influential in increasing disease risk. Advisory cases from throughout the UK suggest that, given the numbers of first wheat crops infected, the sexual air-borne stage of the eyespot pathogen may be much more prevalent than it was when the original guidelines on eyespot and its control were devised. In addition researchers are also no longer confident about what represents a true break crop.

It is now clear that grasses, such as annual meadow grass and couch, commonly carry types of the eyespot fungus that will infect wheat and barley (Hocart and McNaughton, 1994). Eyespot levels can be very high in cereal crops following oilseed rape, despite the fact that this is a recommended break crop. This may be related to the incidence of cereal volunteers. There may be an additional influence resulting from common agronomic practices in these break crops. It is not clear what triggers the sexual stage of the fungus but in general fungi only go into their sexual phase when they are stressed. It is possible that the use of graminicides on volunteers in oilseed rape or set aside or as desiccants on cereals triggers the sexual stage of the eyespot pathogen as a survival mechanism once the host plant dies.

3.1.4. Fungicide treatment for eyespot

Eyespot is conventionally controlled in winter wheat crops with a fungicide spray at early stem extension between growth stages Zadoks 30 to 32 (Anon, 1987; Burnett *et al*., 1997), sometimes applied as a split treatment. Several active ingredients with eyespot activity are available. These include older fungicides such as prochloraz and cyprodinil, and more recent introductions such as prothioconazole, metrafenone and boscalid. Work carried out for HGCA Project Report 150 showed that both cyprodinil and prochloraz were more effective at controlling *O. yallundae* than *O. acuformis* type. Fungicides from the azole group such as prochloraz have been reported to control *O. yallundae* more effectively than *O. acuformis* (Bierman *et al*., 2002) and cyprodinil also showed improved control of *O. acuformis* in work carried out in France (Migeon *et al*., 1995). Metrafenone

is applied for mildew control and commercial work at SAC (Burnett, pers.com) indicates that at high doses it reduces eyespot incidence and severity. Prothioconazole and boscalid (applied in formulated mixture with epoxiconazole) give greater control and it is these two fungicides that are applied most often in commercial practice. Control of both eyespot species seems to be comparable, with no indication that *O. acuformis* is more poorly controlled. This may reflect the fact that they were developed and launched when this was the dominant species in the UK.

Levels of control from eyespot are typically not as good as the control levels expected by growers for control of foliar diseases. Typical levels of eyespot control in HGCA project report 347 (Burnett and Hughes, 2004) seldom exceeded 50%. One reason for this is the difficulty in targeting the stem base with fungicide deposits using conventional spray nozzles. New advances in application techniques may improve targeting and as a consequence improve eyespot control from fungicides.

3.1.5. Yield losses from eyespot

No consistent yield loss has been associated with eyespot infection in the literature and its impact on crop lodging is unclear. Sutherland and Oxley (1993) found that early fungicide use at GS 31 did not always result in an increase in yield. Clarkson (1981) found a correlation between eyespot severity and individual plant yield loss. The yield loss model by Clarkson (1981) was commonly used to estimate losses from eyespot as follows:

% yield loss = $0.1\chi_1 + 0.36\chi_2$

where χ_1 = % incidence of moderate eyespot

and χ_2 = % incidence of severe eyespot.

Trials carried out by SAC in the course of an HGCA-funded project looking at the biology and control of eyespot (Project No. 0015/1/91) found that there was a significant association between eyespot levels and yield. Although lodging was also shown to be associated with yield loss, the correlation was not as strong as that between eyespot and yield. There was also a significant correlation between eyespot and lodging (Burnett and Oxley, 1996).

The work of Scott and Hollins (1978) related to a period when *O. yallundae* eyespot was prevalent. It has been hypothesised that because *O. acuformis* tends to infect later it is not damaging to yield but the work of Burnett and Oxley (1996) related to a site which was predominantly R type. Work at Harper Adams from 2000-2003 (Ray *et al*., 2004) showed a significant negative correlation between R type infection and yield loss such that percentage yield loss = $-0.02\chi + 11.97$ for the variety Consort, where χ was the % eyespot incidence at growth stage 69.

In the absence of lodging, Glynne and Salt (1958) reported winter wheat grain yield losses of 33%. In the presence of lodging, yield loss due to eyespot has been shown to increase significantly. For example, Glynne *et al*. (1945) and Jørgensen (1964) recorded yield loss of 40% and 44%, respectively. Scott and Hollins (1974) carried out inoculated experiments where wheat plants were supported or left to lodge and demonstrated by regression analysis that yield loss was significantly greater in the unsupported plots than in the supported plots due to eyespot induced lodging.

Predicting eyespot development and yield losses has been complicated due to discrepancies in inducing eyespot disease of different severity, dependence of the disease on favourable temperature (Rapilly *et al*., 1979) and rainfall (Matusinsky *et al*., 2009) and furthermore intrinsic differences in the progression of eyespot disease when caused by the two fungal species (Wan *et al*., 2005). In addition, the relationship between yield loss and the disease has been difficult to quantify because of the lack of correlation between early (GS31/32 stem elongation, first/second node detectable, Zadoks *et al*., 1974) disease before treatment decisions are made and disease severity or yield at harvest (Scott and Hollins, 1978; Goulds and Fitt, 1991).

In the majority of the early experimental work carried out in the 1970s and 1980s to determine yield loss relationships for eyespot disease, the precise fungal species causing the disease is uncertain. Thus it is unclear if yield loss is similar for disease epidemics caused by each individual *Oculimacula* species. More recently, Ray *et al*. (2006) reported wheat grain losses by each individual species quantified at 11% for eyespot caused by *O. acuformis* and 6% for *O. yallundae*, however this study was based on single-tiller measurements that may not be representative of true field populations of wheat. Pathogen population shifts have also occurred between 1990s and 2000 (West *et al*., 1998). *Oculimacula acuformis* was considered the predominant pathogen causing the disease in the UK (Burnett and Hughes, 2004) and there is evidence that the species is capable of causing significant yield losses in winter wheat (Ray *et al*., 2004).

Yield response to an effective chemical control can provide a useful estimate of potential yield loss due to untreated disease. Winter wheat is commonly treated with fungicide application at GS 31-32 (April/May) for optimum eyespot control before lesions have become established on the stem (Burnett, 1999). Discrepancies however have been encountered due to overestimation of response to treatment particularly when fungicides exhibited broad spectrum activity often controlling foliar diseases as well as eyespot (Ray *et al*., 2004).

Plant growth regulator products have been commonly used to control naturally occurring lodging in cereals via reductions of the centre of gravity resulting from decreased plant height (Crook and Ennos, 1995). However, the activity of plant growth regulators under field conditions against

Oculimacula spp., eyespot severity or disease-induced lodging in wheat remains unknown and indeed there are no published studies in the literature.

3.1.6. Predicting eyespot risk

As treatment decisions have to be made early in the season if eyespot is to be targeted, disease risk assessment and prediction has been the aim of many research projects, with the objective of determining a threshold level of eyespot early enough in the season to identify crops where control of eyespot would be cost effective. Some schemes have relied on weather data, but this does not allow for the loss of lesions that either die out or are shed with the outer leaves and never penetrate the stem (Polley and Clarkson, 1978). The threshold scheme for identifying crops at risk of eyespot was based on assessing the number of stems infected at the start of stem extension and recommending treatment if an incidence of more than 20% of lesions penetrating to the stem is found (Anon, 1987; Jones, 1994).

Eyespot assessment in the spring, however, has long been recognised as an unreliable indicator of subsequent disease progress (Scott and Hollins, 1978). Hughes *et al*., 1999 demonstrated the fallibility of this threshold method and concluded that while it would identify correctly those crops that passed the threshold at stem extension as being those that would benefit from treatment it would miss all those that had not passed the threshold but would go on to develop serious infections. HGCA-funded work confirmed the poor predictive capability of a threshold approach to treatment (Burnett *et al*., 2000). Leaving risk assessment and treatment decisions to the spring also does not allow for a judgement to be made the previous autumn about the need to select a resistant variety for high risk situations.

This threshold was developed when *O. yallundae* of eyespot predominated whereas *O. acuformis* then became more common (King and Griffin, 1985; Nicholson and Turner, 2000). The fungicides most commonly used on wheat in the decade subsequent to King and Griffin's study were in the azole (demethylation inhibitor) group which act differentially on the two types, and are far more effective in controlling *O. yallundae*. This may be one reason why *O. acuformis* came to predominate throughout the UK. *O. acuformis* often infects later and then increases fast which may make it more difficult to devise a disease threshold criterion for use as a decision guideline at GS31-32. *O. yallundae* tends to cause more stem browning and therefore is easier to assess using a visual threshold. HGCA Project Report 150 found that in one season there was a significant correlation between *O. yallundae* levels at stem extension and the final levels at the end of the season, indicating how thresholds may have been more effective when this was the dominant type of eyespot in the UK.

Diagnostics are a useful tool for accurate identification of the pathogens in the stem base complex (Nicholson *et al*., 1997). The advances in PCR diagnostics mean that the progress of the two eyespot species can be followed accurately throughout the season and the two eyespot pathogens can be differentiated for the first time. Diagnostics have not, however, helped in determining a threshold for eyespot treatment and it is clear from previous work (Burnett and Hughes, 2004) that eyespot is very often absent, or present at only low levels at stem extension, but can develop into a serious disease by the end of the season. An analysis of the amount of pathogen DNA in untreated plots in SAC trials between 1995 and 1998 (HGCA Project Nos. 0015/1/91 and 0050/01/97) showed no correlation between eyespot DNA levels at any point in the season before GS 65 and the final levels that developed by the end of the season.

HGCA project 347 (Burnett and Hughes, 2004) developed an accumulated risk score which allowed growers to use factors such as tillage method, sowing date, expected spring rainfall, previous crop, soil type and eyespot incidence at stem extension to judge the risk of economically-damaging eyespot developing. This risk assessment was more accurate than a threshold approach but was designed to assess the need for fungicide treatment in the spring. With the introduction of varieties of wheat carrying the Pch1 eyespot resistance gene, an approach that enabled growers to judge risk prior to drilling was required. Other changes such as the introduction of alternative fungicides to cyprodinil, and shifts in the incidence of the eyespot species have also occurred since that approach was developed. Identifying crops at risk from eyespot requires further study. At present taking account of other risk factors such as sowing date and previous cropping would seem to be a more successful approach to identifying crops that would benefit from an eyespot spray, than would the use of thresholds.

3.1.7. Aims

The project aim was to provide growers with a method of judging likely yield loss from eyespot, allowing accurate treatment decisions. The aim of the project was arranged in four independent work packages, which were strongly related in terms of relevance.

1. To update the existing eyespot risk model to encompass developments in varietal resistance and recent fungicide advances – reported in section 3.2 (varietal and fungicide control) and section 3.6 (model development).
2. To define the risks relating to yield loss from eyespot – reported in section 3.3.
3. To determine whether improved spray application technologies would allow later but more accurate treatments for eyespot – reported in section 3.4.
4. To use the weather parameters conducive to eyespot infection and development to model likely eyespot spread and incidence under climate change scenarios – reported in section 3.5.

3.2. Eyespot control through fungicide application and varietal resistance

3.2.1. Aim

The aim of this work package was to inform the eyespot model that would be developed with information on fungicides and varieties, in terms of eyespot control and yield response.

3.2.2. Materials and Methods

Data were collected from multiple sources. The use of the historical data sets provided large amounts of information in a very cost-efficient manner. The data sets included treated and untreated trial data from SAC, Harper Adams University College, University of Nottingham and NIAB TAG, funded at these institutions by agrochemical companies. The previous HGCA eyespot model data set (project report 347) was an important component of the current project as yield response to treatment could be mapped over a wide range of geographical situations, soil types etc. 300 paired sites made up this data base, running the years 2000 - 2003. Data were mined from research partner data sets for the years 2003 – 2006 in order to provide continuity with the start date of this project in 2007. These data included fungicide trials and variety trials. Thereafter, until project completion in the 2010 season, data were collected in real time from commercial fungicide trials placed with the research partners by agrochemical companies. The data gathered included yield, yield response to treatment (comparing treatments with eyespot efficacy to treatments designed to offer only foliar disease control), location, soil type, sowing date, variety, eyespot disease throughout the season (using both visual assessment and pathogen DNA quantification by real-time PCR) and weather data.

Field trial design

Field trials were carried out by the research partners according to the treatments required by the commercial sponsors but core treatments were included. These are shown in Table 1. A full list of trial sites is shown in Appendix 1.There were 50 field trial sites in total in the new data set. The varieties trialled are shown in Table 2.

Table 1. Core treatments in fungicide trials.

Product name	Manufacturer	Active ingredient (a.i.)	Amount of a.i.	Full commercial dose rate
Opus 0.5	BASF	epoxiconazole	125 g/l	1.0 l/ha
Proline*	Bayer CropScience UK	prothioconazole	250 g/l	0.8 l/ha
Tracker	BASF	boscalid + epoxiconazole	233 g/l +67 g/l	1.5 l/ha
Unix	Syngenta UK	cyprodinil	75% w/w	1.0 kg/ha

*Now replaced on market with Proline 275 at 275 g a.i. /l

Table 2. Varieties evaluated in 2007 and 2008 seasons (drilled previous autumn)

2007 site Perthshire Varieties	2008 site Dundee Varieties
Hyperion	Hyperion
Robigus	Alchemy
Einstein	Duxford

The variety trials were designed and treated as per the other fungicide trials.

A randomised block design was used in all trials, with four replicates per treatment. The trials were mainly drilled (preferable) or superimposed in a predominantly cereal rotation. Trials were over-sprayed at GS39 and GS55-69 to minimise the effects of foliar disease on yield. These over-sprays varied by site but were selected to offer robust control against the main foliar disease threats perceived at each of the sites.

Information was recorded for each site on environment-based factors either as discrete variable (D) or continuous variable (C): Region (D), Tillage method (D), Sowing date (C), Straw removal (D), Soil type (D), Soil pH (C), Soil P (C), Soil K (C), Soil Mg (C), Previous crop (D), Mean temperature during September/October/November (C), Mean temperature during December / January / February (C), Mean temperature during March / April / May (C), Total rainfall during September / October / November (C), Total rainfall during December / January / February (C), Total rainfall during March/April/May (C).

Assessments were made as follows:-

GS31-32	Eyespot visual assessment over trial site, one PCR assessment over trial site
Pre GS 39	Before flag sprays applied, foliar disease assessment all plots
GS45	Eyespot visual assessment
GS70-80	Eyespot visual assessment, lodging and whiteheads if present
Harvest	Lodging and whiteheads, yield

Application Details

Treatments were applied with a hand held CP3 Knapsack sprayer or Azo plot sprayer in approximately 200 L of water per ha.

Sampling methods

At GS25 to GS 32, 25 plants per plot were sampled and the stem bases assessed for eyespot which was recorded as % incidence. At later growth stages 25 tillers per plot were sampled and the stem base diseases scored. Eyespot was recorded as 0 = no symptoms, 1= lesions affecting less than 50% of the stem circumference, 2 = lesions affecting over 50% of the stem circumference and 3 = lesions affecting over 50% of the stem circumference and tissue softened so that lodging would readily occur.

A % stem base index was then be calculated for each disease :- (((no of score 1) + (no of score 2 x 2) + (no of score 3 x 3))/ no of stems) x (100 / 3).

3.2.3. Results

Eyespot infection by season

Eyespot levels varied between years in the project as shown in Table 3 below:-

Table 3. Mean eyespot levels by harvest season.

Harvest Year	Eyespot GS31-32 % index	Eyespot GS37-45 % index	Eyespot GS70-85 % index	GS70-85 *O. acuformis* DNA pg/ng of total DNA	GS70-85 *O. yallundae* DNA pg/ng of total DNA
2004	73.68	82.81	72.86	16.4	51.3
2005	67.55	58.39	51.33	36.8	94.6
2006	70.46	76.38	66.83	15.4	64.0
2007	75.89	53.76	58.3	6.83	57.2
2008	37.68	43.86	32.48	5.79	60.6
2009	34.41	27.65	32.08	0.88	10.4
2010	28.83	42.21	26.42	2.26	17.6

Levels of visual eyespot were significantly lower (P = <0.001) in 2008, 2009 and 2010. The dominant eyespot species was *O. yallundae* but most sites had mixed infections of *O. yallundae* and *O. acuformis*. Over the trial sites mean DNA of *O. yallundae* was 37.3 pg/ng of total DNA compared to *O. acuformis* of 3.69 pg/ng of total DNA.

Eyespot control from fungicides

There were significant reductions in eyespot disease and yield increases to fungicide treatments applied at GS31-32, shown in Figure 1 and Figure 2.

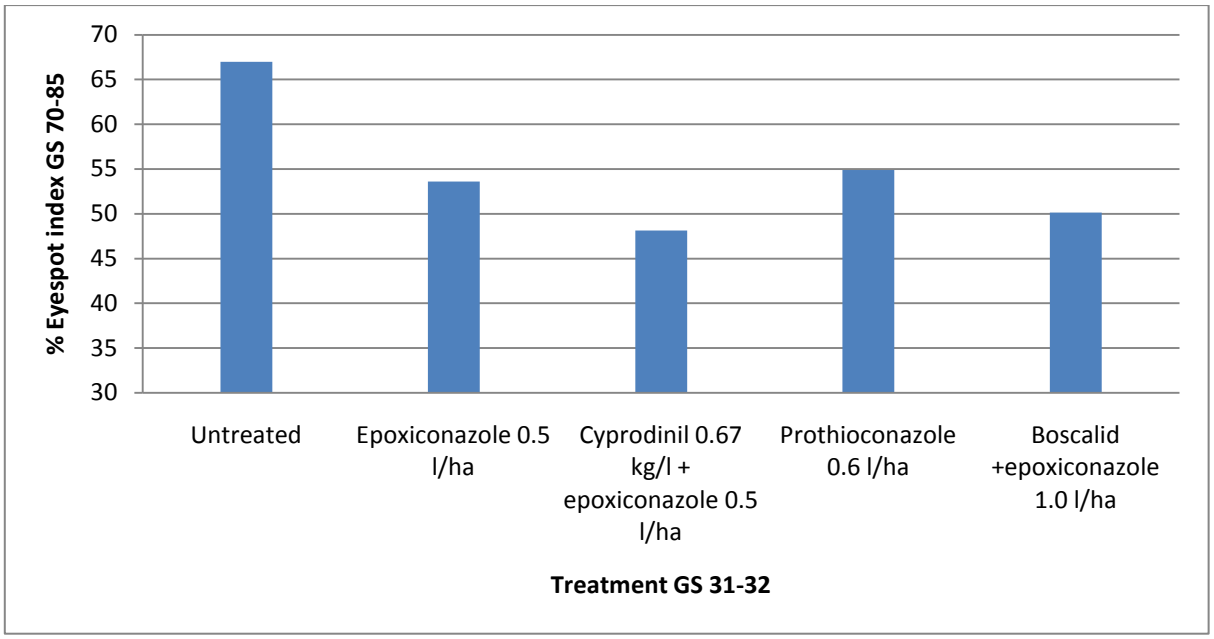


Figure 1. Mean Eyespot levels at GS 70-85 in fungicide trials 2000 – 2010 (P = <0.001, LSD =8.294).

All fungicides significantly reduced eyespot compared to the untreated controls and cyprodinil at 0.67 kg/ha + epoxiconazole 0.5 l/ha was significantly better than prothioconazole. There were no other significant differences between fungicides in terms of end of season eyespot control. High levels of control were noted from epoxiconazole and may reflect the high levels of *O. yallundae* in the trial seasons. Despite the small difference in disease control, there were significant yield benefits to treatment with prothioconazole, cyprodinil + epoxiconazole and boscalid + epoxiconazole in comparison to the straight epoxiconazole treatment, shown in Figure 2.

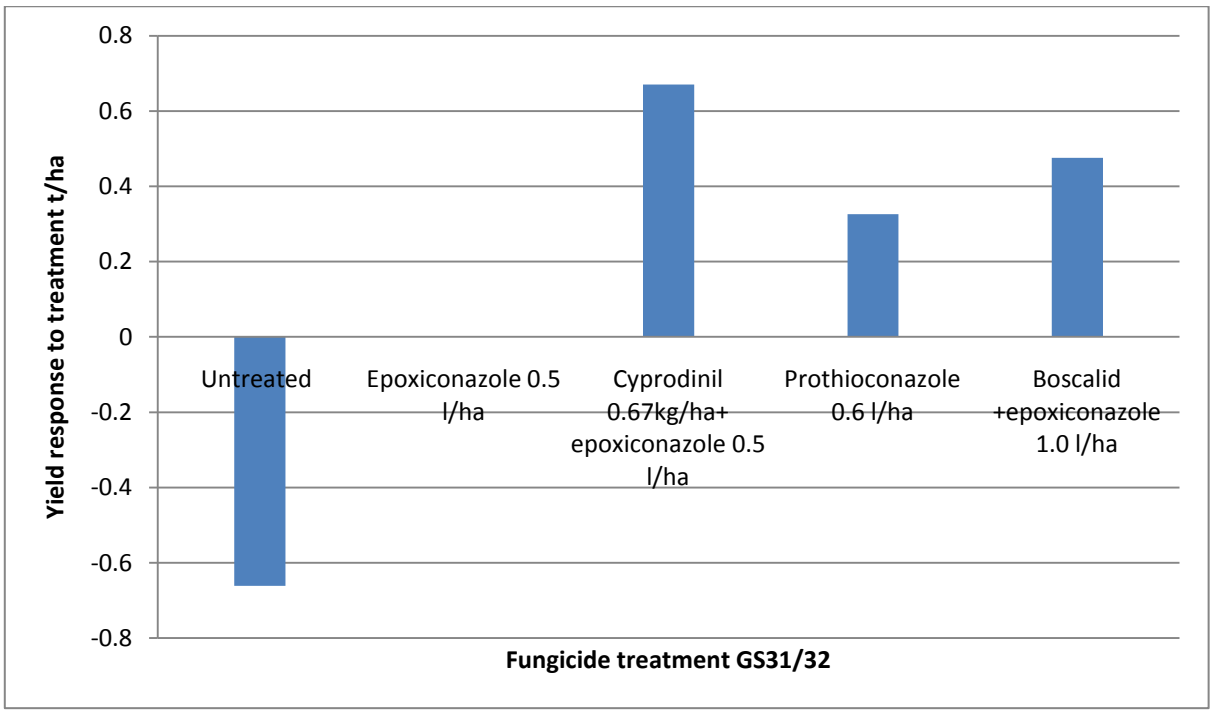


Figure 2. Mean yield response to GS31-32 treatments compared to epoxiconazole treatment as t/ha at 85% dry matter (*P* = 0.001, LSD = 0.283)

A strong correlation between the mean disease control in Figure 1 and the mean yield response in Figure 2 was found such that y = -0.0678x + 3.8745
R^2 = 0.9156. Mean yield also correlated significantly with eyespot index at GS70-85:- y = -0.0419x + 11.614, r^2 = 0.9002.

Varietal control of eyespot

There were significant differences in eyespot levels and yield in the varieties trialled. Hyperion and Einstein had significantly less eyespot at the end of the 2007 season than Robigus (LSD = 5.69, *P* = 0.05). Eyespot was significantly reduced by fungicide treatment (LSD = 6.57, *P* = 0.05). The addition of the eyespot active fungicides applied at GS31-32, prothioconazole and boscalid + epoxiconazole, achieved additional consistent benefits for Hyperion and Einstein, compared to epoxiconazole only treatment as shown by the lack of significant interaction between variety and fungicide (*P* = 0.483). There was no significant control of eyespot on Robigus in the 2007 trial as a consequence of either epoxiconazole or prothioconazole treatment, but there was a significant reduction resulting from treatment with boscalid + epoxiconazole, shown below in Figure 3.

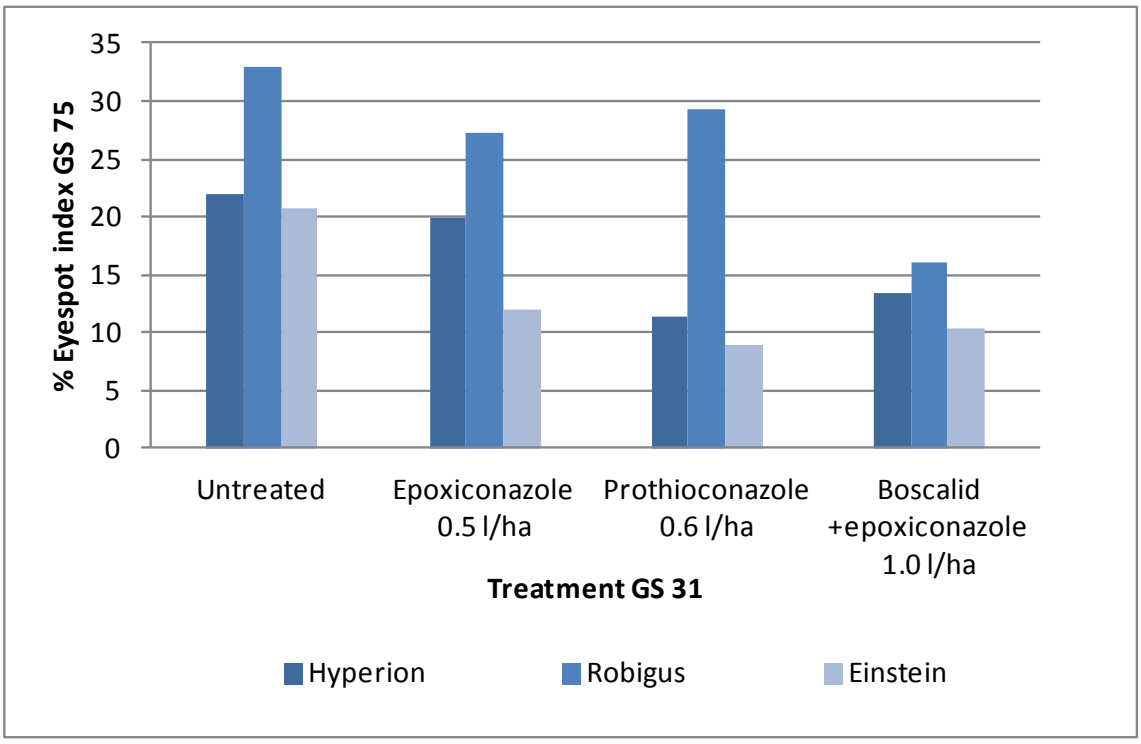


Figure 3. Eyespot % disease index at GS75, following treatment at GS31

There were significant improvements to yield by variety (LSD = 0.325, *P* = 0.05) but yield differences by fungicide or by variety x fungicide interaction were not significant (*P* = 0.437 and 0.993 respectively), shown in Figure 4.

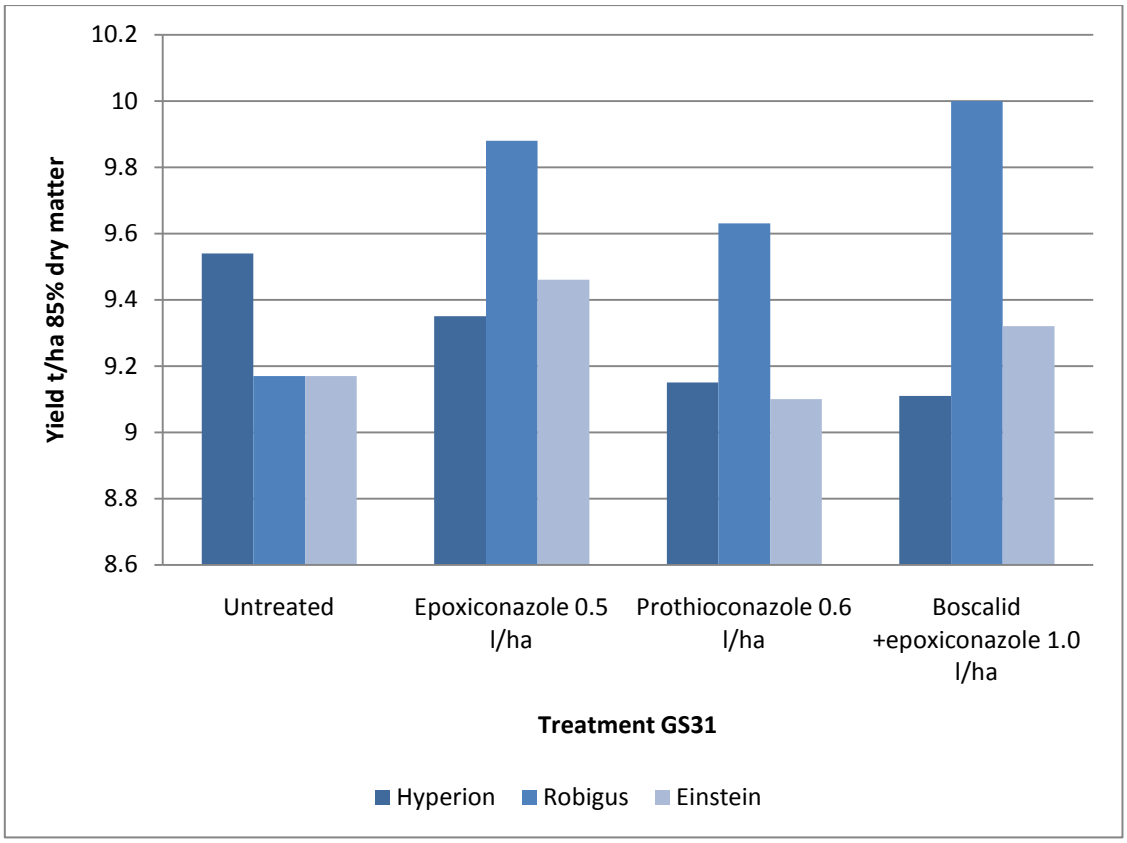


Figure 4. Yield t/ha at 85% dry matter following fungicide treatment at GS31.

The variety trial in the 2008 season had much lower levels of eyespot although there were still significant differences by the end of the season in response to fungicide treatment (LSD = 2.695, *P* = 0.05). There was significantly more eyespot disease in Duxford compared to the other two varieties, Hyperion and Alchemy, in all fungicide treatments (LSD = 4.669, *P* = 0.05). The variety x fungicide interaction was not significant (*P* = 0.746). Levels of eyespot were significantly reduced on Duxford following treatment at GS 31 with prothioconazole or boscalid + epoxiconazole compared to treatment with epoxiconazole, shown in Figure 5.

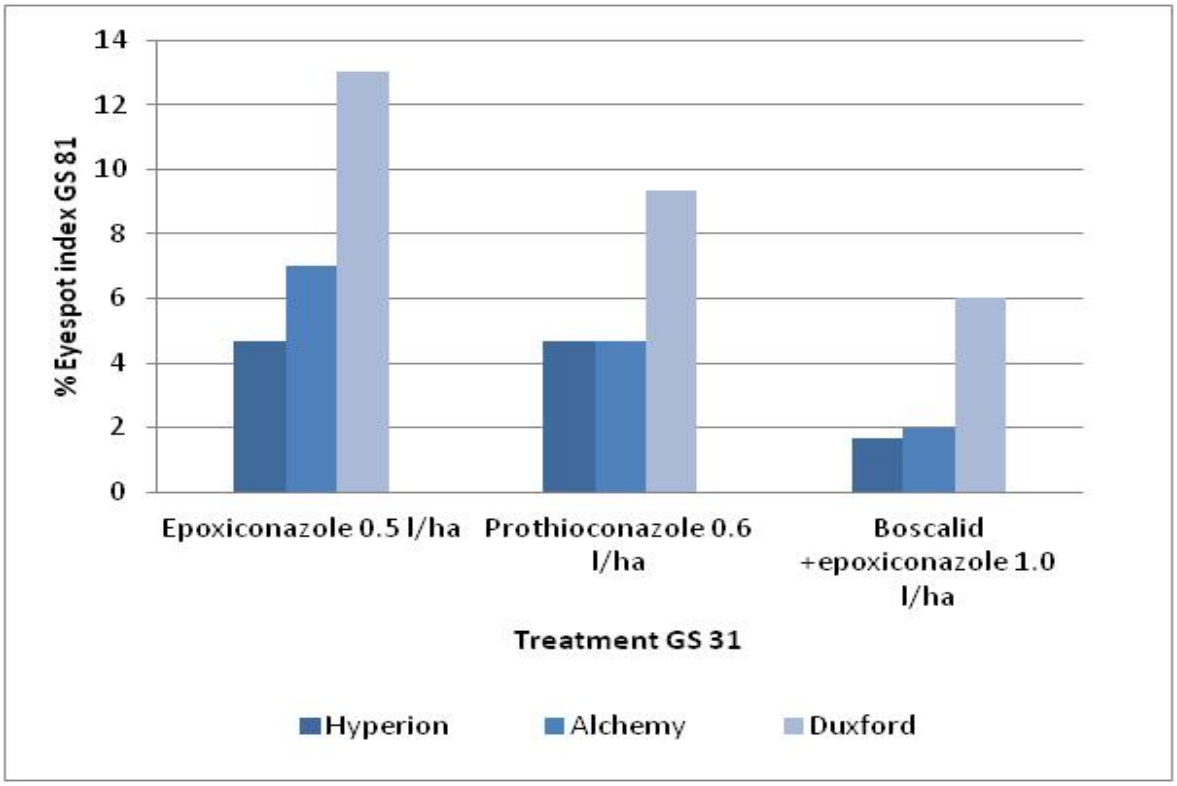


Figure 5. Eyespot % disease index at GS81, following treatment at GS31.

There were no significant differences in yield in this trial although there was a trend towards yield improvement following prothioconazole treatment on the varieties Hyperion and Alchemy, and no evidence of yield benefit to the boscalid + epoxiconazole treatment on these varieties. However on the variety Duxford boscalid + epoxiconazole achieved higher yields than prothioconazole. Without eyespot treatment, Duxford was the highest yielding variety, but Hyperion treated with prothioconazole yielded comparably, shown in Figure 6. (Fungicide treatment *P* = 0.615, Variety *P* = 0.292, Fungicide x variety interaction *P* = 0.278).

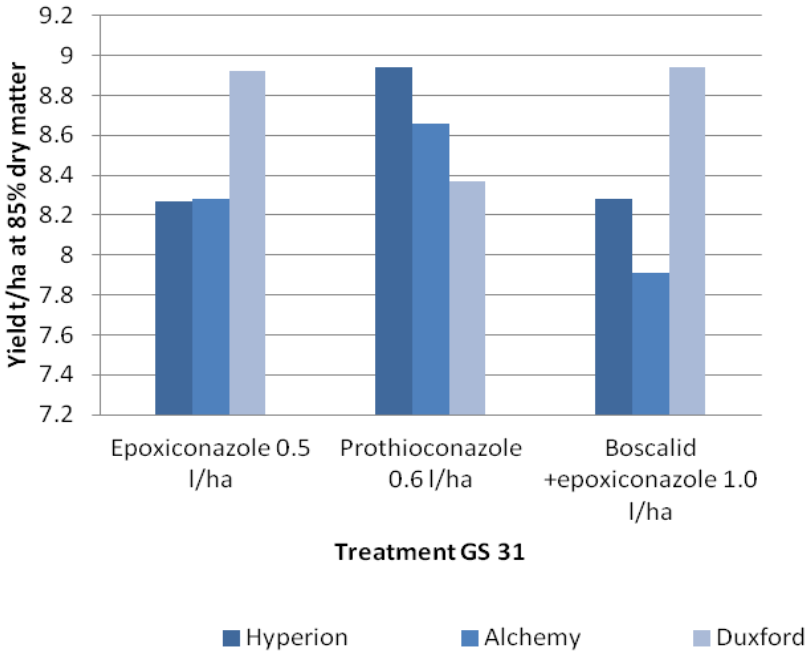


Figure 6. Yield t/ha at 85% dry matter following fungicide treatment at GS31.

Yield losses to eyespot over the trial series

There was no significant relationship between eyespot levels and yield over the data set. The trend line that could be drawn through the data is shown in Figure 8 and would equate to 0.005 t/ha yield loss for every one percent eyespot disease severity, or a tenth of a tonne for every 20 percent disease index. Using meaned disease control data and meaned yield response data (the means shown in figures 1 and 2) then a relationship was found ($y = -0.0638x + 3.6604$, $r^2 = 0.9247$), which equates to a yield response of 0.6 t/ha per 10% eyespot in the crop at the end of the season.

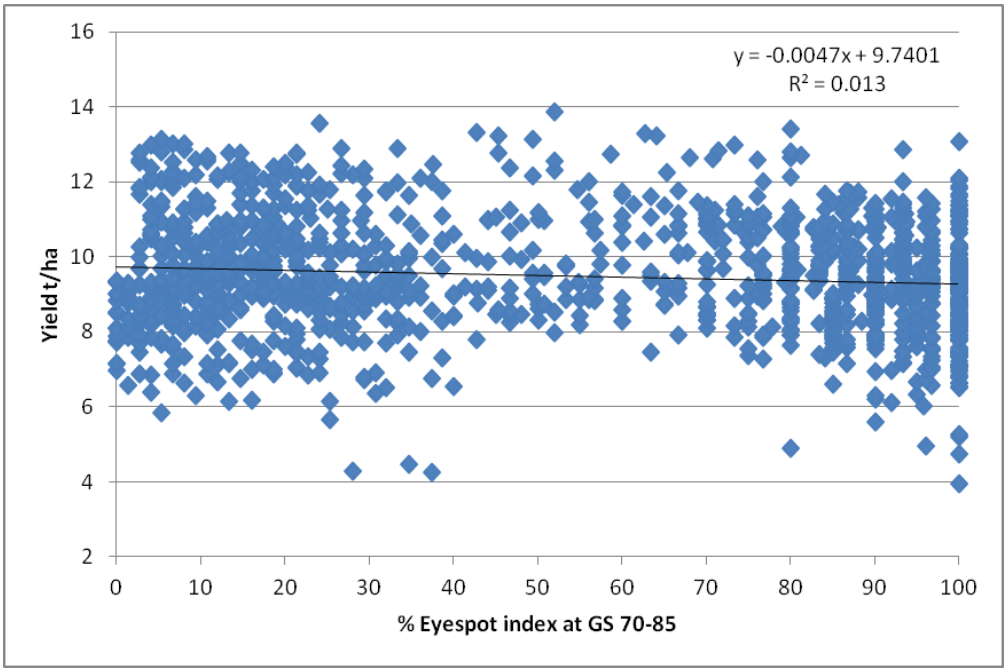


Figure 7. Yield t/ha at 85% dry matter plotted against late season eyespot severity across all sites and seasons.

Similarly there was no significant correlation between pathogen DNA at the end of the season and yield, shown in Figure 8.

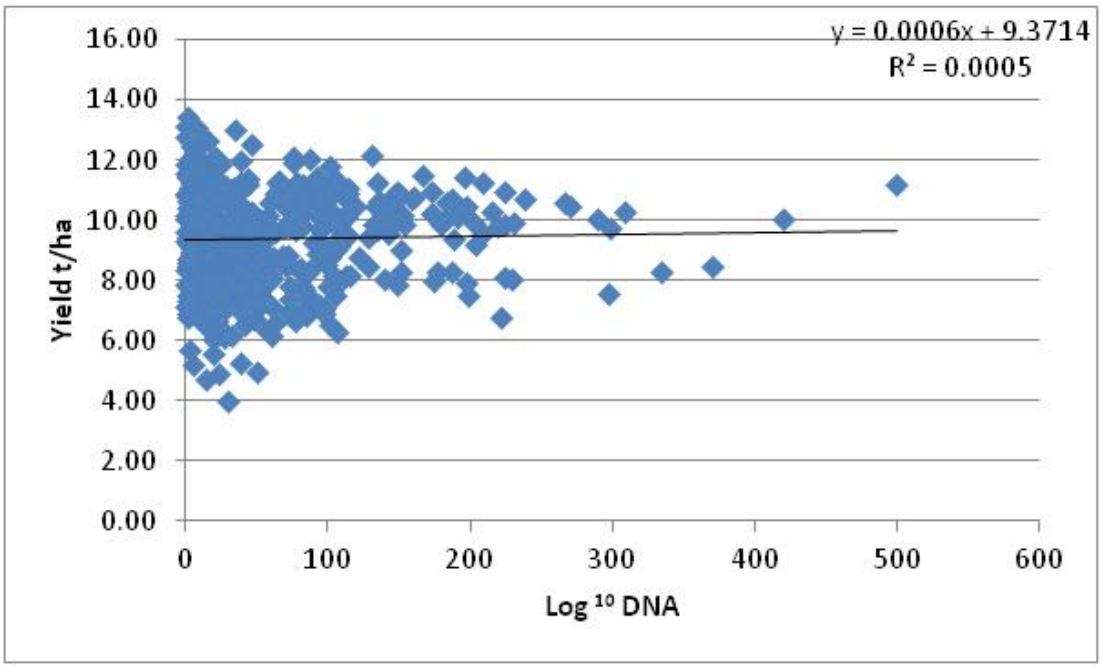


Figure 8. Total pathogen DNA (pg/ng) plotted against yield t/ha at 85% dry matter.

Yield varied widely between sites because of factors unrelated to eyespot so a mean response to eyespot fungicide (prothioconazole, cyprodinil or boscalid + epoxiconazole) compared to epoxiconazole was calculated and is plotted in Figure 9. Epoxiconazole is a typical standard application to wheat crops at GS31-32 and is expected to give control of foliar diseases but is not applied for eyespot control. However as shown in Figure 1 it gave comparable control of eyespot to the fungicides with label claims for eyespot efficacy. There was no significant response to treatment in relation to eyespot severity (Figures 9 and 10) at the end of the season in the data series. There was a trend towards higher yielding crops responding better to the eyespot fungicides, shown in Figure 11, but this was not significant.

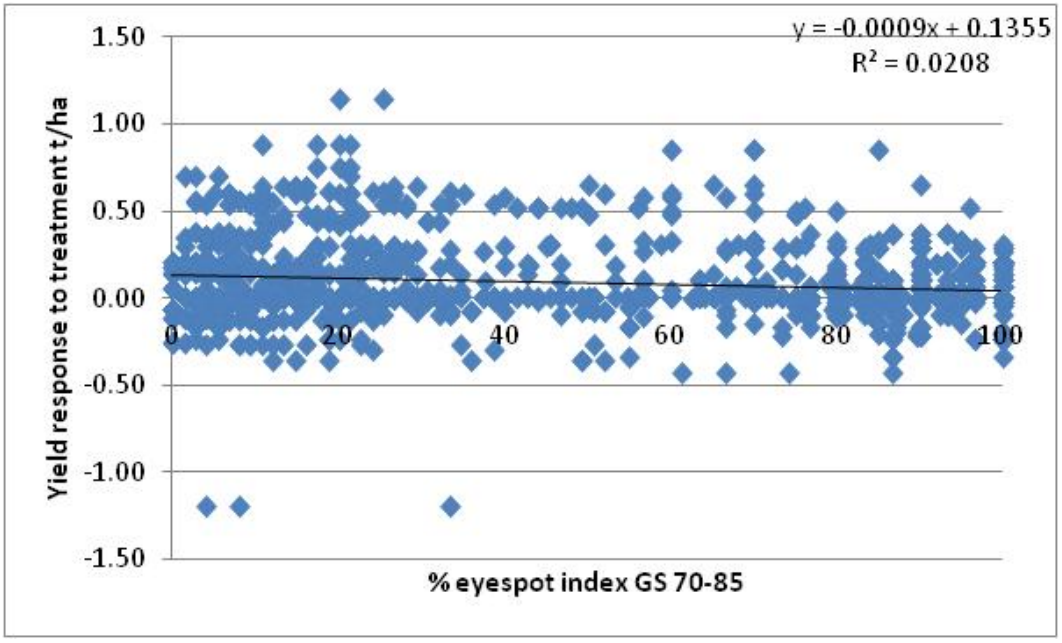


Figure 9. Yield response (t/ha) from fungicides with eyespot efficacy compared to epoxiconazole treatment applied at GS31-32.

There was also no significant correlation between pathogen DNA and yield response, as shown in Figure 10.

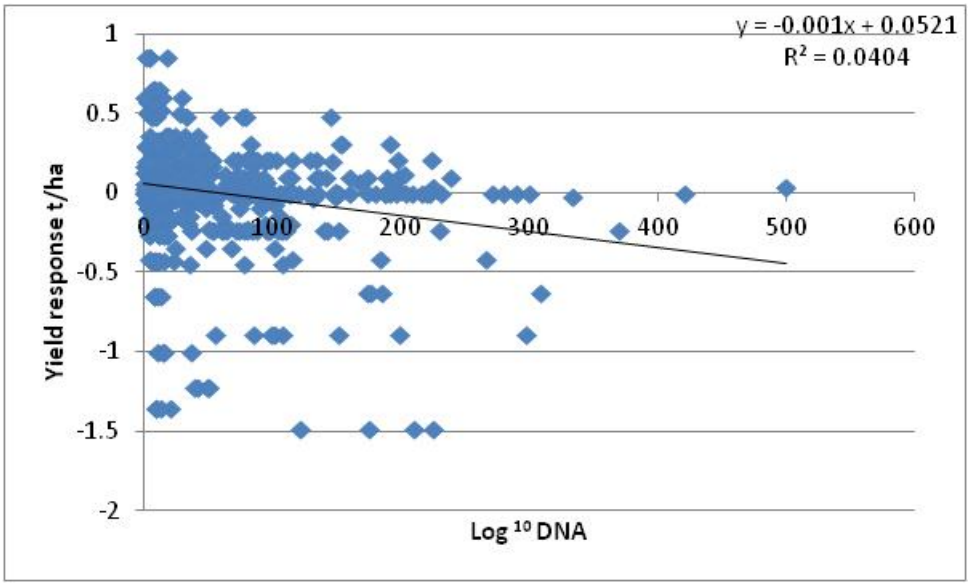


Figure 10. Pathogen DNA (Log_{10} pg/ng) plotted against yield (t/ha at 85% dry matter) in response to fungicides.

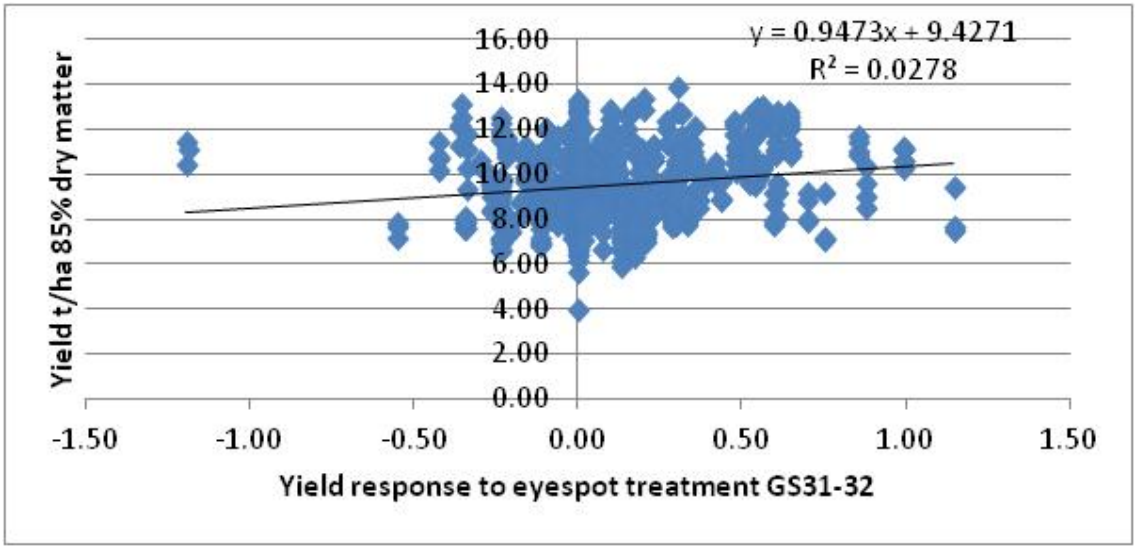


Figure 11. Yield response to eyespot treatment plotted against yield t/ha at 85% dry matter, across the data series.

The environmental variables recorded Region (D), Tillage method (D), Straw removal (D), Soil type (D), Soil pH (C), Soil P (C), Soil K (C), Soil Mg (C), Previous crop (D), Mean temperature during September/October/November (C), Mean temperature during December / January / February (C), Mean temperature during March / April / May (C), Total rainfall during September / October / November (C), Total rainfall during December / January / February (C) or Total rainfall during March/April/May (C) did not significantly influence either eyespot severity or yield as individial factors.

There was a significant association between eyespot levels at GS31-32 and eyespot severity at the end of the season, shown in Figure 12.

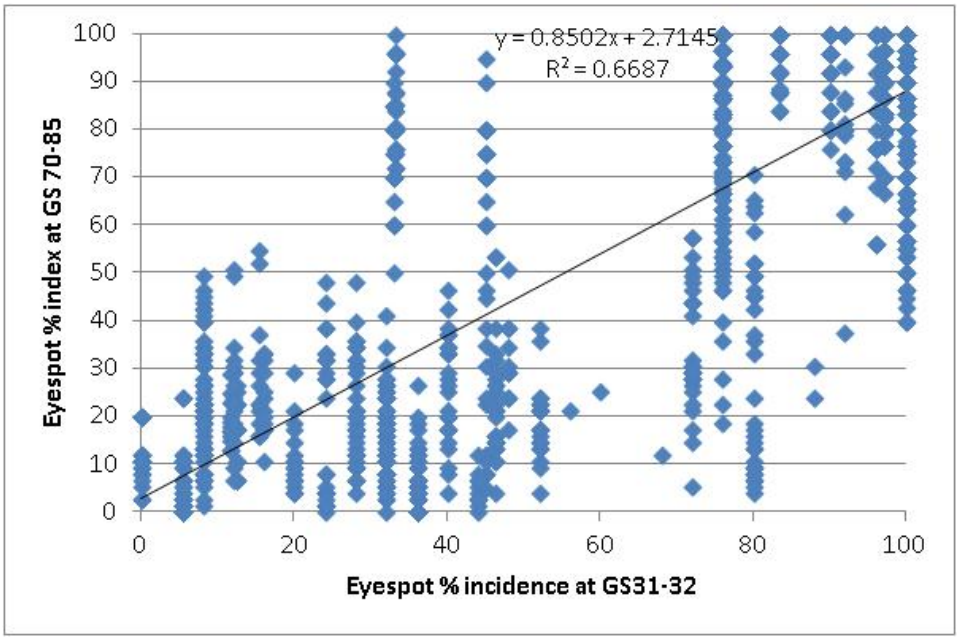


Figure 12. Eyespot incidence at GS 31-32 as a predictor of eyespot severity at GS 70-85

3.2.4. Discussion

There were several findings from this section of the work that have implications on how we consider eyespot management in crops. The change in the relative occurrence of the two eyespot species between the early years of the data base, starting in 2000, when *O. acuformis* was the dominant species to the recent years where *O. yallundae* was the dominant species. The high level of control with epoxiconazole would support the hypothesis that *O. yallundae* is now the more common species at the sites tested. Sites were situated throughout the UK and ranged from Kent and Sussex up to Perth and Dundee, numbering 50 in total, so that it is likely this distribution between the two species is representative of commercial crops in the UK. In previous work (Burnett and Hughes, 2004) there was no significant correlation between eyespot incidence at stem extension, when spray decisions are made, and the disease severity that developed at the end of the season. This may relate to differences between the two species. *O. yallundae* produces more visible browning early in the season than does *O. acuformis* (Daniels, 1993a). In the mid 1980s *O. yallundae* also dominated and a simple threshold approach to treatment was reasonably effective (Anon, 1987) in the visible browning produced. By the mid 1990s when *O. acuformis* was the more common species this threshold approach was ineffective as visible browning in the early season was less common. Shifts in dominance between the two fungicides are likely to continue.

A correlation between disease incidence at stem extension and severity at the end of the season can assist with spray decisions at that time but cannot be useful in the previous autumn when decisions have to be taken about varietal choice, field choice and sowing date all of which can be used to reduce eyespot risk (Burnett and Hughes, 2004).

There were significant differences in eyespot levels in the two variety trials undertaken. Hyperion carries the Pch1 resistance gene which conveys a greater degree of resistance than the Pch2 resistance gene which is widely assumed to be present in many widely grown varieties such as Einstein. Robigus is weak-strawed and is acknowledged to be weaker in terms of eyespot susceptibility. Alchemy showed reasonable eyespot tolerance comparable to Hyperion and Duxford was significantly weaker. In comparison to the weaker varieties Hyperion gave approximately 30% control in the first season's trials where moderate eyespot levels developed, and proportionally better control in the following season when eyespot levels were very low. Although variety × fungicide interactions were not significant in the trials there was a trend of increased eyespot control and improved yield where both varietal resistance and fungicides were deployed together. It suggests that a dual approach to control has potential, in terms of efficacy and such an approach would be a strategy to preserve both the efficacy of fungicides against resistance and to avoid over-exposure and breakage of the Pch1 varietal resistance mechanism.

Fungicides gave significant control compared to untreated plots and best efficacy (with cyprodinil) offered approximately 30% reductions in eyespot severity. The level of control seen from epoxiconazole was unexpected, and confounded the experimental design where this treatment had been selected as a core treatment in trials in order to compare a foliar-only spray with a treatment with eyespot activity such as cyprodinil, boscalid or prothioconazole. *O. yallundae* is more susceptible to treatment with azole fungicides, which could explain this finding. There were yield responses though to the fungicides with on-label claims for eyespot control, which could suggest that yield loss as a consequence of eyespot infection was reduced using these treatments despite the lack of a simple correlation between disease levels and yield. Cyprodinil performed well both in terms of eyespot control and in terms of yield. This was not noted in early work when boscalid and prothioconazole were launched and offered better control of eyespot than cyprodinil (SAC trial unpublished data). Cyprodinil is not widely used for eyespot control for reasons of expense as it represent a specific addition to a spray programme, but technically it remains an effective eyespot treatment.

The lack of any consistent significant link between eyespot severity and yield was unexpected. Using meaned data from the fungicide trials large yield losses could be ascribed to eyespot infection – on a ten tonne crop the mean yield loss would equate to 0.4 tonne per 10% eyespot index and the mean response to treatment would equate to 0.6 tonnes per 10% eyespot reduction. But this is an oversimplification and within the data set many sites did not show a yield response at all from treatment or gave a negative response to treatment. There were many examples of sites within the data series and examples within the literature of yield loss being significantly associated with eyespot severity, and estimates of loss range from Scott and Hollins (1978) at almost 40% of potential yield to the previous HGCA eyespot report (347) at 10%. It is interesting that the Scott and Hollins figure is equivalent to the value found in this project from the meaned fungicide trial data. The mechanism of yield loss to eyespot is poorly understood and it is likely that surrounding plants can compensate to a degree for infected stems. It also follows that final wheat yield is likely to be driven by other agronomic and weather variables and that eyespot disease is probably problematic on a specific field basis, but a relatively minor factor against these main drivers of yield.

3.3. The effect of primary inoculum quantity on eyespot disease and yield loss

3.3.1. Aim

Defining yield loss from eyespot

The aim of this work package was to determine the effect of quantity of primary inoculum source, fungicide application and plant growth regulator (PGR) on disease severity and yield loss due to eyespot caused by *O. acuformis* or *O. yallundae* in winter wheat.

3.3.2. Materials and methods

Visual assessment and TaqMan PCR were used to follow the progress of eyespot disease and causal pathogens, respectively, at key growth stages of wheat artificially inoculated with different quantities of oat grains colonised by each *Oculimacula* spp. Application of cyprodinil at 502 g a.i/ha and trinexapac-ethyl at 100 g a.i. / ha (commercial name Moddus, Syngenta UK) at GS 32 were used to provide range of disease severity and lodging occurring in field. Yield losses in terms of grain yield (t/ha), thousand grain weight (TGW, g) and specific weight (SW, kg/hl) were measured at the end of each year of experimentation.

Field experimental conditions

Artificially inoculated field experiments were carried out at Harper Adams University College, Newport, UK in 2008 and at The University of Nottingham, Sutton Bonington Campus, UK in 2009. On each site, winter wheat plots (2 x 10 m) of cv. Robigus were established following grass with more than five years break from cereal production in order to minimise the risk of naturally occurring eyespot. The seed was treated with prothioconazole (commercial name Redigo, Bayer CropScience UK). Sowing dates and rates for both experiments are listed in Table 4.

Table 4. Sowing date, sowing rate, isolates of *Oculimacula acuformis* (Oa) and *O. yallundae* (Oy) used for inoculum production and pathogen DNA quantified in 4 g of inoculated oats for experiments in 2007 and 2008. Values shown in parentheses are the quantified background pathogen DNA for *Oculimacula* spp.

Sowing date	Sowing rate seeds m^2	Isolates used for inoculum production Oa	Oy	Pathogen DNA Pg/ng of total DNA Oa (Oy)	Oy(Oa)
11.10.2007	300	109/12	93/5	247 (0.04)	120 (0.07)
03.10.2008	300	159/2	166/3	232 (0.01)	375 (0.10)
		130/4	136/8		
		163/6	109/13		
		162/3	165/3		

The experimental design was factorial block with four factors (fungal species, inoculation rate, fungicide and plant growth regulator) with multiple levels (Table 5). There were four replicates of each treatment combination. Single spore isolates of known pathogenicity were used for artificial inoculation and were obtained from culture collection at Harper Adams University College, UK (Table 4).

Table 5. Experimental factors and their levels in 2007 and 2008.

Factor/ Level	I. Species	II. Cyprodinil g a.i./ha	III. Trinexapac-ethyl g a.i./ha	IV. Inoculum g /m
1	*O. acuformis*	0	0	0
2	*O. yallundae*	502	100	1.5
3				15

Inoculum was produced and prepared as detailed previously by Ray *et al*. (2006). *Oculimacula* spp. DNA and any background pathogen DNA were quantified in the oats used for inoculation using TaqMan assay as indicated in the next section of materials and methods.

Inoculation was carried out at GS 13 (seedling growth, three leaves unfolded, Zadoks *et al*., 1974) of the crop at each site by manually spreading oat grain colonised by a mixture of five isolates of *O. acuformis* or *O. yallundae* to each plot (Table 4). Fungicide and PGR applications were made at GS 32 (stem elongation, second node detectable, Zadoks *et al*., 1974). To minimise any movement of inoculum between plots, a 0.5 m strip of crop was sown on the side of each plot. Fungicide over-spray applications were made to all plots at GS 30, 32, 39 and 59 (Zadoks *et al*., 1974) to minimise the occurrence of stem-base (foot rot and sharp eyespot), foliar (rusts, septoria, mildew) and ear (fusarium head blight) diseases which may have affected the final yield of the crop. Fungicides for these overall sprays were selected with minimum activity against *Oculimacula* spp. At GS 30 (stem elongation, ear at 1 cm) , both sites received azoxystrobin (100 g.a.i./ l) and chlorothalonil (500 g.a.i./ l) as Amistar Opti, Syngenta UK and epoxiconazole (125 g.a.i./ l) as Opus, BASF UK applied at field rates of 2 l/ha and 0.75 l/ha, respectively. At GS 32 and GS 39 (stem elongation, flag leaf blade all visible), an overall application of epoxiconazole as Opus at field rates of 0.5 l/ha and 0.75 l/ha, respectively, were made. At GS 59 (complete ear emergence above flag leaf ligule) of the crop, metconazole (60 ga.i./ l) was applied as Caramba, BASF UK in addition to fenpropimorph (750 g.a.i./ l) as Corbel, BASF UK at field rates of 1.0 l/ha and 0.75 l/ha, respectively. The rest of husbandry operations were made according to standard agronomy practice.

Following harvest, grain yield (15% moisture content, t/ha), thousand grain weight (TGW, g) and specific weight (SW,kg /hl) were determined.

Disease assessments and pathogen DNA quantification

Visual eyespot assessment as detailed previously by Ray *et al*. (2004) was carried out on 30 plants per plot collected at GS 32, before fungicide or PGR applications were made, followed by assessments at GS 39 and GS 73/75 (early to medium milk development). At the latter two growth stages, the eyespot assessment was made on the main shoot only, the rest of secondary tillers were discarded and were not processed further for DNA extraction. Eyespot severity was classified as slight, moderate or severe based on the number of shoots infected, and the amount of girdling, leaf sheath penetration and stem softening. Disease index (DI, %) representing disease intensity (based on incidence and severity of the disease) was calculated using the following formula: DI = ((number of plants with slight symptoms) + (2 x number of plants with moderate symptoms) + (3 x number of plants with severe symptoms)) / (3 x total number of assessed plants) x 100. Immediately following assessment, pathogen DNA was extracted from plant material as described by Ray *et al*. (2004) and quantified using TaqMan probe quantitative Real-time assay (Walsh *et al*., 2005).

Statistical analysis

All data were analysed using analysis of variance (ANOVA) using Genstat® Version 11 for Windows (Lawes Agricultural Trust, UK). Before performing ANOVA, data on the counts of whitehead numbers per plot occurring in 2008 was transformed using square root function. Pathogen DNA (pg/ng of total DNA) and DI (%) data were transformed using complementary log log and angular transformations, respectively, in order to normalise residual distributions. All transformed data were checked for homogeneity before further ANOVA. Different experimental years (2008 and 2009) were treated as experimental factors and were included as such in ANOVA for visual, DNA, yield, TGW and SW data.

3.3.3. Results

Effect of primary inoculum of O. yallundae or O. acuformis on disease index in winter wheat

DNA of *O. acuformis* was present in inoculated oats at similar quantities during both years of experimentation (Table 4). There was a 3-fold difference in DNA of *O. yallundae* detected between years of experimentation. However, overall inoculum load used for both experiments was greater than 100 pg/ng of total DNA with very small quantities of background DNA detected.

At GS 32 before fungicide application, there were significant differences for disease index between *Oculimacula* species, inoculation rates and years of experimentation (Table 6).

Table 6. Effect of quantity of primary inoculum source of *Oculimacula* species on eyespot disease index assessed at GS 32of cv. Robigus in 2008 and 2009. Angular transformed data analysed, back-transformed means are shown in parentheses.

Angular Disease Index (%) at GS 32								
Species / Inoculation g/m^2	*Oculimacula acuformis*			*Oculimacula yallundae*			Year	
	none	1.5	15	none	1.5	15	P	LSD
2008	28.5 (22.7)	32.1 (26.9)	38.1 (38.1)	30.8 (26.3)	45.7 (51.2)	52.5 (62.9)	0.003	6.355
2009	23.4 (15.8)	20.8 (12.6)	23.5 (15.9)	20.8 (12.6)	22.2 (14.3)	26.5 (20.0)		
Inoculation								
P	<0.001							
LSD	2.181							
Species								
P	<0.001							
LSD	1.781							

There were no significant interactions between the experimental factors indicating that the main treatment effects were consistent for both years of experimentation. Disease index was greater at stem extension in 2008 than in 2009. Plants inoculated with *O. yallundae* exhibited significantly more severe disease symptoms than those infected with *O. acuformis*. There were also significant differences between disease index of plants inoculated with different rates of infected oats. Plots inoculated at 1.5 g/m^2 and 15 g/m^2 showed 32% and 46% higher disease index compared to the control. Whilst precautions were taken (0.5 m of crop rows drilled on each plot side) to minimise any crossover of inoculum, the presence of low levels of disease in the control plots suggested that some conidia movement occurred.

The dispersal range of *Oculimacula* spp. has been shown to be short, in the range of 1-2 m from inoculum source (Rowe and Powelson, 1973; Fitt and Nijman, 1983). However, under field conditions where inoculum has been applied to the ground early in crop development it has been difficult to completely prevent such occurrences.

Analysis of variance revealed a significant interaction between experimental year, species and inoculation rate for a disease index at GS 39 (Table 7).

Table 7. Effect of quantity of primary inoculum source of *Oculimacula* species on eyespot disease index assessed at GS 39 of cv. Robigus in 2008 and 2009. Angular transformed data analysed, back-transformed means are shown in parentheses.

Angular Disease Index (%) at GS 39						
Species /	*Oculimacula acuformis*			*Oculimacula yallundae*		
Inoculation g/m^2	none	1.5	15	none	1.5	15
2008	28.4 (22.5)	25.8 (18.9)	27.9 (21.9)	27.7 (21.6)	39.2 (39.9)	43.0 (46.4)
2009	24.4 (17.1)	24.7 (17.4)	22.5 (14.6)	22.4 (14.5)	25.2 (18.1)	27.1 (20.8)
Year*Species*Inoculation						
P			0.006			
LSD			3.704			

Whilst a clear increase in disease index in response to inoculation rate was observed in plots inoculated with *O. yallundae*, there was a slight decrease in disease index for plots inoculated at the higher rates with *O. acuformis*. Differences in disease index between inoculation rates were also significant only for 2008.

At GS 73/75, there were no significant interactions between experimental factors (Table 8). Final disease severity was similar for both *Oculimacula* species. However, disease index at GS 73/75 was 50% lower in 2009 compared to 2008. There was a significant and consistent disease index response to primary inoculum rate across years of experimentation. Disease index of plots inoculated at 15 g/m^2 was 17% and 29% higher than the disease index of plots inoculated at 1.5 g/m^2 and the control respectively.

Table 8. Effect of quantity of primary inoculum source of *Oculimacula* species on eyespot disease index assessed at GS 73/75 of cv. Robigus in 2008 and 2009. Angular transformed data analysed, back-transformed means are shown in parentheses.

Angular Disease Index (%) at GS 73/75								
Species /	*Oculimacula acuformis*			*Oculimacula yallundae*			Year	
Inoculation g/m^2	none	1.5	15	none	1.5	15	P	LSD
2008	40.8 (42.7)	43.8 (47.8)	46.7 (53.0)	41.3 (43.5)	51.3 (61.0)	54.3 (65.9)	<0.001	2.43
2009	28.6 (22.9)	26.7 (20.1)	38.7 (39.1)	28.6 (22.9)	24.6 (17.3)	36.6 (35.6)		
Inoculation								
P	<0.001							
LSD	2.442							

Effect of primary inoculum of O. yallundae or O. acuformis on pathogen DNA in winter wheat

Significant interactions between experimental year, species and inoculation rate were observed for DNA of *Oculimacula* spp. at GS 32 (Table 9). In 2008, significantly more DNA of *O. yallundae* was quantified under all three inoculation rates, which was in contrast to 2009 when DNA of *O. acuformis* was found in higher concentrations.

Table 9. Effect of quantity of primary inoculum source of *Oculimacula* species on pathogen DNA quantified at GS 32 of cv. Robigus in 2008 and 2009. Complementary log log transformed data analysed, back-transformed means are shown in parentheses.

Complementary log log pathogen DNA (pg/ng of total DNA) at GS 32						
Species /	*Oculimacula acuformis*			*Oculimacula yallundae*		
Inoculation g/m^2	none	1.5	15	none	1.5	15
2008	-7.8 (0.04)	-6.3 (0.19)	-5.2 (0.54)	-6.1 (0.22)	-4.1 (1.59)	-4.0 (1.88)
2009	-8.4 (0.02)	-7.6 (0.05)	-7.0 (0.09)	-11.1 (0.00)	-8.5 (0.02)	-7.2 (0.08)
Year*Species*Inoculation						
P	0.001					
LSD	1.298					

There were no significant interactions between year, species and inoculation revealed by analysis of variance at GS 39 (Table 10) or at GS 73/75 (Table 11). The main effect of year of experimentation was significant for all DNA assessments, showing that in 2008 there was higher pathogen DNA concentration compared to 2009. DNA concentration of *O. yallundae* for individual inoculation rates was always higher than the DNA of *O. acuformis*. Overall, DNA of *O. acuformis* at GS 39 was 5-fold and at GS 73/75 was 3-fold lower than the DNA *of O. yallundae*.

Slight differences in DNA accumulation for *Oculimacula* spp. in relation to primary inoculum rates were observed for both GS 39 (Table 10) and GS 73/75 (Table 11). Pathogen DNA concentrations were always significantly higher at inoculum rate of 15 g/m^2 compared to the control, but in 2008 differences for pathogen DNA of *O. acuformis* and *O. yallundae* quantified at GS 39 or GS 73/75, respectively, following inoculation at 1.5 g/m^2 and 15 g/m^2 were not significantly different. Similarly, in 2009, for both GS 39 and GS 73/75, there were no significant differences for DNA of *O. yallundae* accumulated in the control and under inoculation at 1.5 g/m.

Table 10. Effect of quantity of primary inoculum source of *Oculimacula* species on pathogen DNA quantified at GS 39 of cv. Robigus in 2008 and 2009. Complementary log log transformed data analysed, back-transformed means are shown in parentheses.

Complementary log log pathogen DNA (pg/ng of total DNA) at GS 39								
Species /	*Oculimacula acuformis*			*Oculimacula yallundae*			Year	
Inoculation g/m^2	none	1.5	15	none	1.5	15	P	LSD
2008	-7.0 (0.09)	-5.6 (0.35)	-5.7 (0.33)	-5.0 (0.65)	-2.7 (6.68)	-2.2 (10.20)	<0.001	0.472
2009	-8.8 (0.01)	-8.2 (0.03)	-7.8 (0.04)	-9.1 (0.01)	-9.0 (0.01)	-6.1 (0.23)		
Species								
P	<0.001							
LSD	0.365							
Inoculation								
P	<0.001							
LSD	0.447							

Table 11. Effect of quantity of primary inoculum source of *Oculimacula* species on pathogen DNA quantified at GS 73/75 of cv. Robigus in 2008 and 2009. Complementary log log transformed data analysed, back-transformed means are shown in parentheses.

Complementary log log pathogen DNA (pg/ng of total DNA) at GS 73/75								
Species /	*Oculimacula acuformis*			*Oculimacula yallundae*			Year	
Inoculation g/m^2	none	1.5	15	none	1.5	15	P	LSD
2008	-5.3 (0.52)	-4.1 (1.71)	-3.9 (2.06)	-4.2 (1.53)	-2.5 (8.03)	-2.5 (7.58)	<0.001	0.224
2009	-6.3 (0.19)	-5.9 (0.27)	-3.7 (2.39)	-5.4 (0.46)	-5.5 (0.41)	-2.7 (6.69)		
Species								
P	<0.001							
LSD	0.406							
Inoculation								
P	<0.001							
LSD	0.497							

Effect of inoculation with O. yallundae or O. acuformis, fungicide and PGR application at GS32 on eyespot disease index (DI) and pathogen DNA in winter wheat

Analysis of variance revealed significant interaction between year, species and fungicide for disease index at GS 32 (Table 12). At GS 32 before fungicide application, plants inoculated with *O. acuformis* in 2008 and 2009 and with *O. yallundae* in 2009, in plots due to be treated with cyprodinil, had higher disease index albeit not significantly different than the control plots.

Table 12. Effect of inoculation with *Oculimacula* species and cyprodinil application at GS 32 on eyespot disease index at GS 32 of cv. Robigus in 2008 and 2009. Angular transformed data analysed, back-transformed means are shown in parentheses.

Angular disease index (%) at GS 32				
Species	*Oculimacula acuformis*		*Oculimacula yallundae*	
Cyprodinil g a.i/ha	0	502	0	502
2008	31.3 (26.9)	34.6 (32.2)	44.2 (48.5)	41.8 (44.5)
2009	22.5 (14.6)	22.6 (14.8)	22.3 (14.4)	24.0 (16.6)
Year*Species*Fungicide				
P	0.049			
LSD	6.32			

At GS 39, cyprodinil application consistently reduced eyespot disease index caused by either *O. acuformis* or *O. yallundae* (Table 13). Reductions of 19% of eyespot index by fungicide application were observed across experimental years.

Table 13. Effect of inoculation with *Oculimacula* species and cyprodinil application at GS 32 on eyespot disease index at GS 39 of cv. Robigus in 2008 and 2009. Angular transformed data analysed, back-transformed means are shown in parentheses.

Angular disease index (%) at GS 39						
Species	*Oculimacula acuformis*		*Oculimacula yallundae*			
Cyprodinil g a.i/ha	0	502	0	502	Year P	Year LSD
2008	30.0 (25.0)	24.7 (17.4)	38.5 (38.7)	34.8 (32.5)	<0.001	2.23
2009	25.1 (18.0)	22.6 (14.8)	27.7 (21.6)	22.1 (14.1)		
Species P	<0.001					
Species LSD	1.483					
Fungicide P	<0.001					
Fungicide LSD	1.483					

Table 14. Effect of inoculation with *Oculimacula* species and cyprodinil application at GS 32 on eyespot disease index at GS 73/75 of cv. Robigus in 2008 and 2009. Angular transformed data analysed, back-transformed means are shown in parentheses.

Angular disease index (%) at GS 73/75				
Species	*Oculimacula acuformis*		*Oculimacula yallundae*	
Cyprodinil g a.i/ha	0	502	0	502
2008	55.9 (68.5)	31.7 (27.6)	57.1 (70.5)	40.8 (42.7)
2009	34.4 (31.8)	28.3 (22.4)	33.4 (30.3)	26.4 (19.8)
Year*Species*Fungicide P	0.032			
Year*Species*Fungicide LSD	3.898			

At GS 73/75, there were significant interactions between year of experimentation, fungicide and species (Table 14). The disease developed more in 2008 than 2009. Fungicide application at GS 32 was particularly effective in reducing disease index caused by *O. acuformis* providing reductions of 43% compared to 28% when the disease was caused by *O. yallundae* (Table 14). In contrast, in 2009, there were small differences in fungicide efficacy against eyespot caused by either *Oculimacula* spp. (Table 14). Disease index was reduced by 21% and 18%, for plots inoculated with *O. acuformis* and *O. yallundae*, respectively.

There were no observed differences for pathogen DNA between plots due to be treated with fungicide or left untreated, however there was significantly more pathogen DNA present in experimental plots in 2008 than in 2009 (Table 15). The effect of fungicide application at GS 32 on pathogen DNA measured at GS 39 was not consistent for fungal species and years of experimentation (Table 16). Cyprodinil reduced DNA of *O. acuformis* measured at GS 39 significantly for both years of experimentation (Table 16). However, whilst similar effect was observed in 2009 for DNA of *O. yallundae* quantified at GS 39, cyprodinil failed to achieve reductions of *O. yallundae* DNA in 2008 (Table 16).

Table 15. Effect of inoculation with *Oculimacula* species and cyprodinil application at GS 32 on pathogen DNA at GS 32 of cv. Robigus in 2008 and 2009. Complementary log log transformed data analysed, back-transformed means are shown in parentheses.

Complementary log log pathogen DNA (pg ng^{-1} of total DNA) at GS 32

Species	*Oculimacula acuformis*		*Oculimacula yallundae*			
					Year	
Cyprodinil g a.i/ha	0	502	0	502	P	LSD
2008	-6.5 (0.16)	-6.4 (0.17)	-5.0 (0.66)	-4.5 (1.14)	0.004	1.282
2009	-7.4 (0.06)	-7.9 (0.04)	-8.5 (0.02)	-9.3 (0.01)		

Table 16. Effect of inoculation with *Oculimacula* species and cyprodinil application at GS 32 on pathogen DNA at GS 39 of cv. Robigus in 2008 and 2009. Complementary log log transformed data analysed, back-transformed means are shown in parentheses.

Complementary log log pathogen DNA (pg/ng of total DNA) at GS 39				
Species	*Oculimacula acuformis*		*Oculimacula yallundae*	
Cyprodinil g a.i/ha	0	502	0	502
2008	-5.3 (0.48)	-6.9(0.10)	-3.5 (2.87)	-3.1 (4.51)
2009	-7.8 (0.04)	-8.8 (0.02)	-7.7 (0.04)	-8.4 (0.02)
Year*Species*Fungicide				
P		0.021		
LSD		0.724		

The efficacy of fungicide application in reducing the DNA of *Oculimacula* spp. was evident at GS 73/75 (Table 17). However, small discrepancies existed between years of experimentation and efficacy against individual fungal species. Cyprodinil reduced DNA of *O. acuformis* and *O. yallundae* significantly in 2008 by 45% and 30%, respectively (Table 17). In 2009, cyprodinil was more effective against *O. yallundae* than *O. acuformis* (Table 17).

Table 17. Effect of inoculation with *Oculimacula* species and cyprodinil application at GS 32 on pathogen DNA at GS 73/75 of cv. Robigus in 2008 and 2009. Complementary log log transformed data analysed, back-transformed means are shown in parentheses.

Complementary log log pathogen DNA (pg/ng of total DNA) at GS 73/75				
Species	*Oculimacula acuformis*		*Oculimacula yallundae*	
Cyprodinil g a.i/ha	0	502	0	502
2008	-3.1 (4.24)	-5.7 (0.35)	-2.5 (7.81)	-3.6 (2.67)
2009	-4.9 (0.73)	-5.7 (0.33)	-4.0 (1.85)	-5.0 (0.65)
Year*Species*Fungicide				
P		0.043		
LSD		0.721		

Application of trinexapac-ethyl at GS 32 failed to reveal any significant direct effects on either eyespot disease index or pathogen DNA measured during both years of experimentation (data not shown).

Effect of primary inoculum of O. yallundae and O. acuformis, fungicide and PGR application at GS 32 on whiteheads and lodging due to eyespot

Whiteheads and lodging associated with eyespot disease occurred only in 2008. Analysis of variance revealed significant interactions between fungal species and inoculation for lodging assessed at GS 73 (Figure 13).

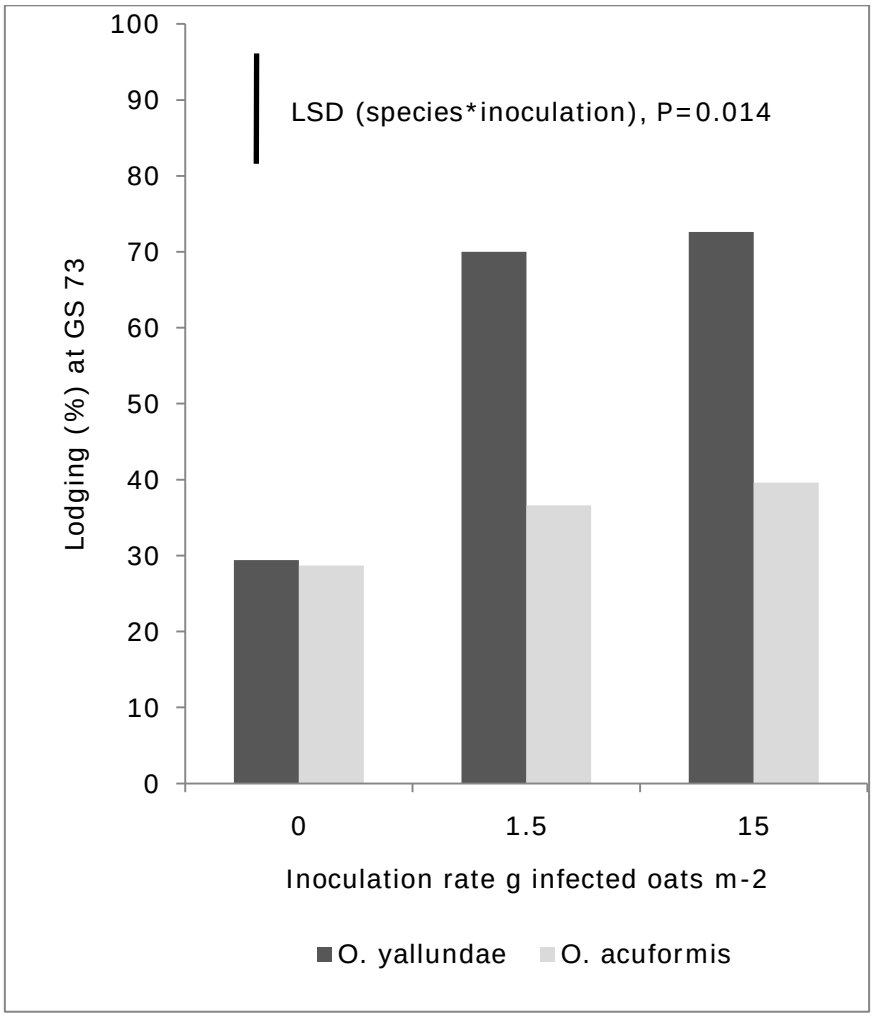


Figure 13. Effect of quantity of primary inoculum source of *O. yallundae* and O *acuformis* on lodging at GS73 on cv Robigus in 2008.

Lodging was primarily associated with artificial inoculation by *O. yallundae* in 2008 although there were no significant differences between rates of 1.5 and 15 g/m^2 of primary inoculum (Figure 13). Furthermore, cyprodinil reduced lodging significantly in inoculated plots, with reductions of more than 50% achieved under high inoculation rate of 15 g/m^2 (Figure 14).

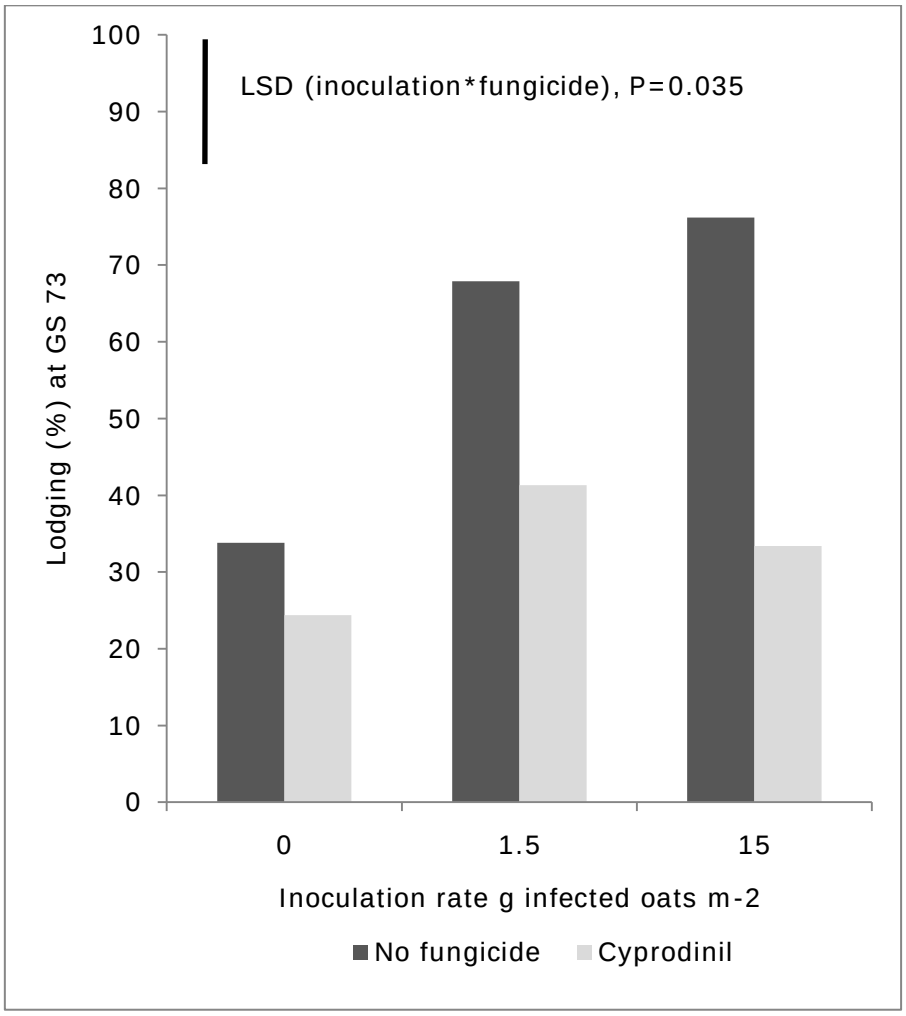


Figure 14. Effect of quantity of primary inoculum source of O. yallundae and O. acuformis and cyprodinil application at 502 g a.i/ha at GS 32 on lodging at GS 73 of cv. Robigus in 2008.

In contrast to lodging, whiteheads occurrence was associated primarily with eyespot due to inoculation with infected oats at 15 g/m^2 with *O. acuformis* (Figure 15). Cyprodinil was effective in reducing whitehead occurrence in *O. acuformis* inoculated plots but failed to show the same effect in *O. yallundae* inoculated plots at the high rate of inoculation.

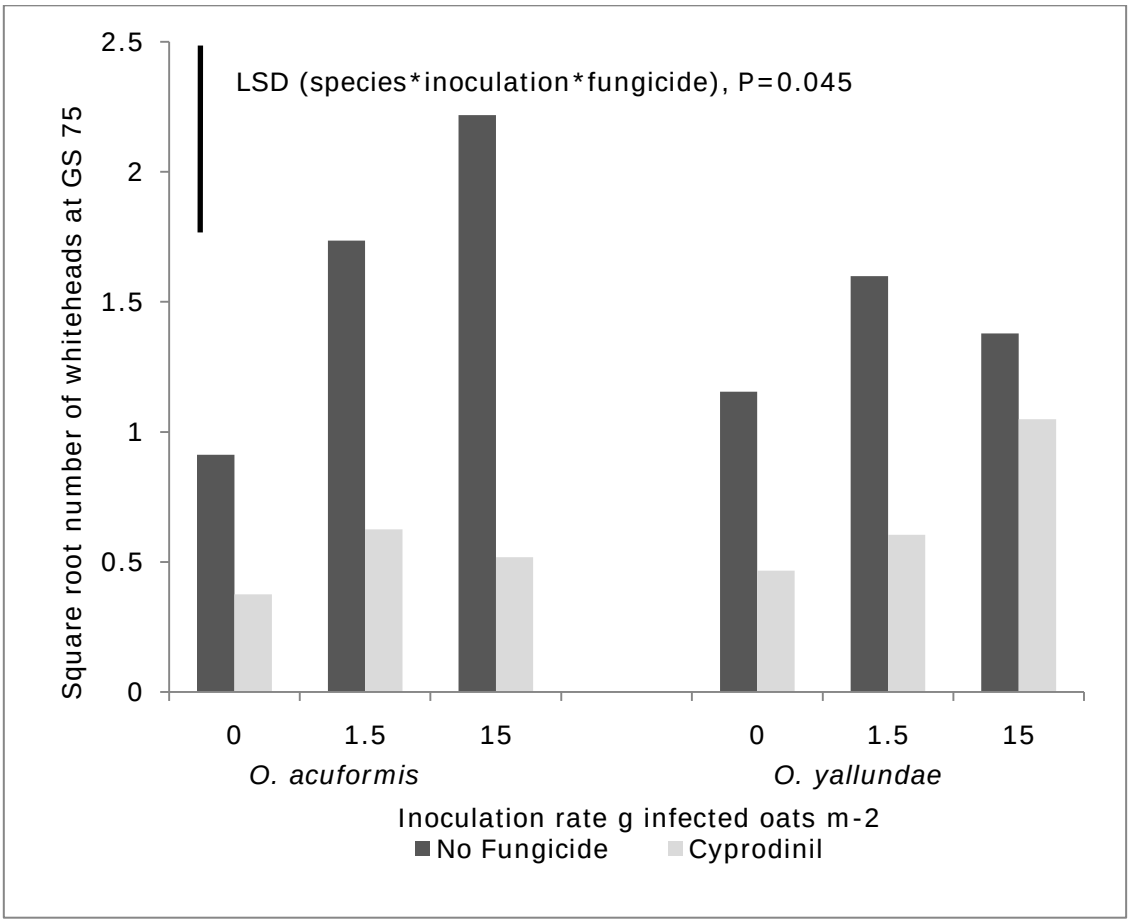


Figure 15. Effect of quantity of primary inoculum source of *Oculimacula* species and cyprodinil application at 502 g a.i./ha at GS 32, on square root of number of whiteheads at GS 75 of Robigus in 2008.

Application of trinexapac-ethyl at GS 32 consistently reduced the appearance of whiteheads by 36% in inoculated plots with either *Oculimacula* spp. (Figure 16).

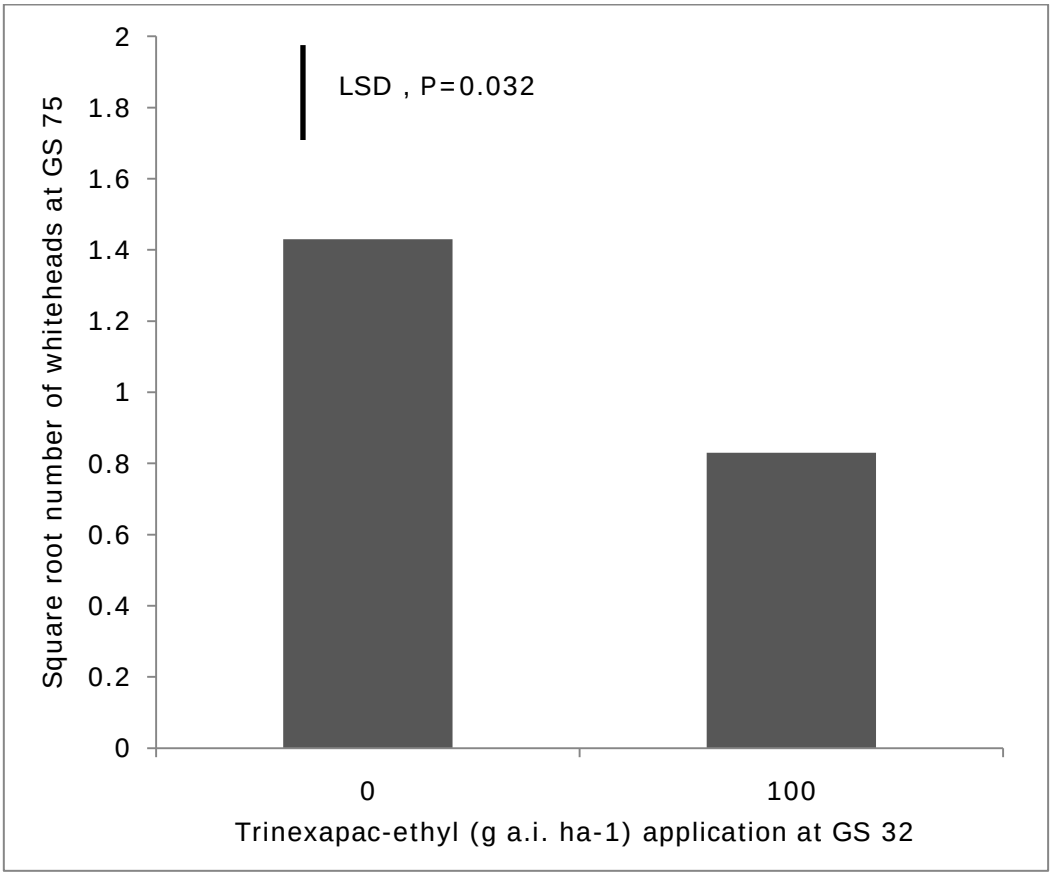


Figure 16. Effect of trinexapac-ethyl application at GS 32 on square root number of whiteheads at GS 75 in Robigus in 2008.

Effect of primary inoculum of O. yallundae and O. acuformis, fungicide and PGR application at GS 32 on yield, thousand grain weight (TGW) and specific weight (SW) of winter wheat

There were no significant interactions between experimental year, species and inoculation indicating that the effect of quantity of primary inoculum source of either *Oculimacula* spp. on yield was consistent and there were no differences between species (Figure 17).The yield response to inoculation rate was evident for both years of experimentation and eyespot occurring under high primary inoculum of either *Oculimacula* spp. reduced yield by 10% compared to control (Figure 18).

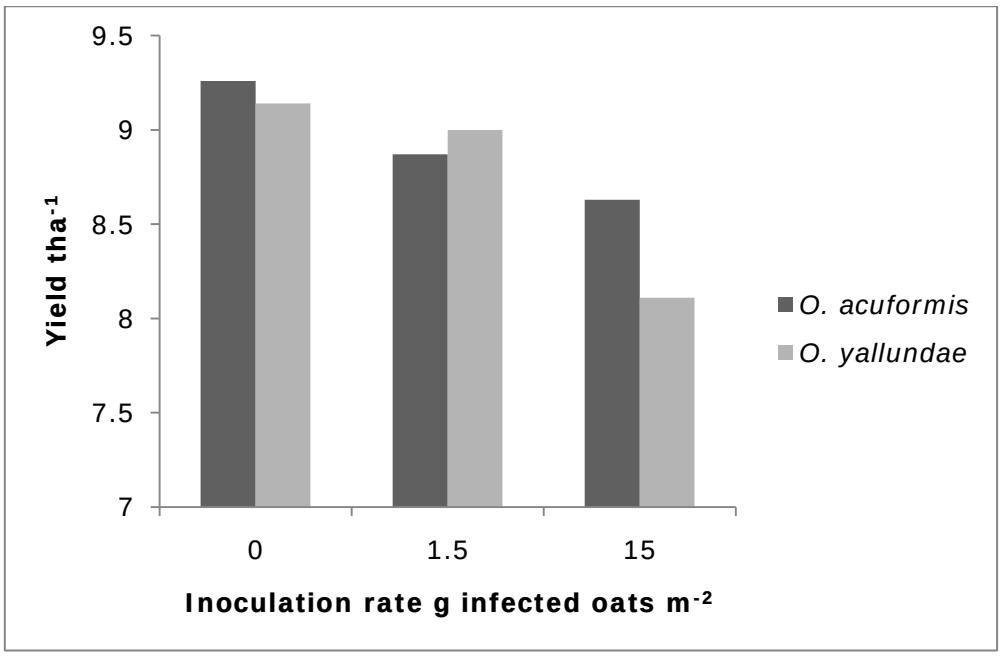


Figure 17. Effect of quantity of primary inoculum source of *O. acuformis* or *O. yallundae* on yield in Robigus in 2008 and 2009.

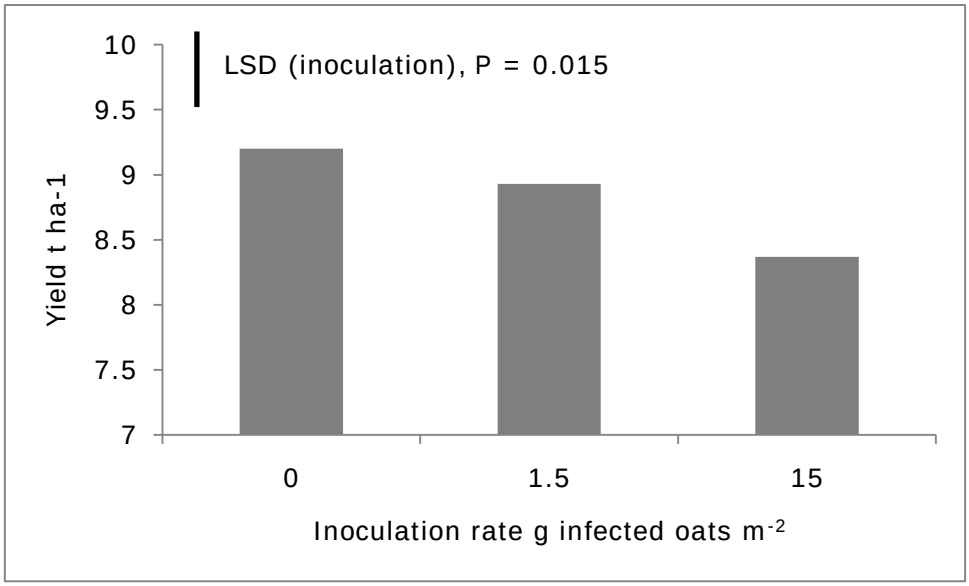


Figure 18. Effect of quantity of primary inoculum source of *Oculimacula* spp.on yield in Robigus in 2008 and 2009.

Plots inoculated at the lower rate of 1.5 g of *Oculimacula* infected oats/m^2 also measured lower yields but the difference was not significantly different from the control (Figure 17).

Analysis of variance revealed that during both years of experimentation eyespot caused by *O. acuformis* consistently reduced TGW significantly more than disease caused by *O. yallundae* (Figure 19).

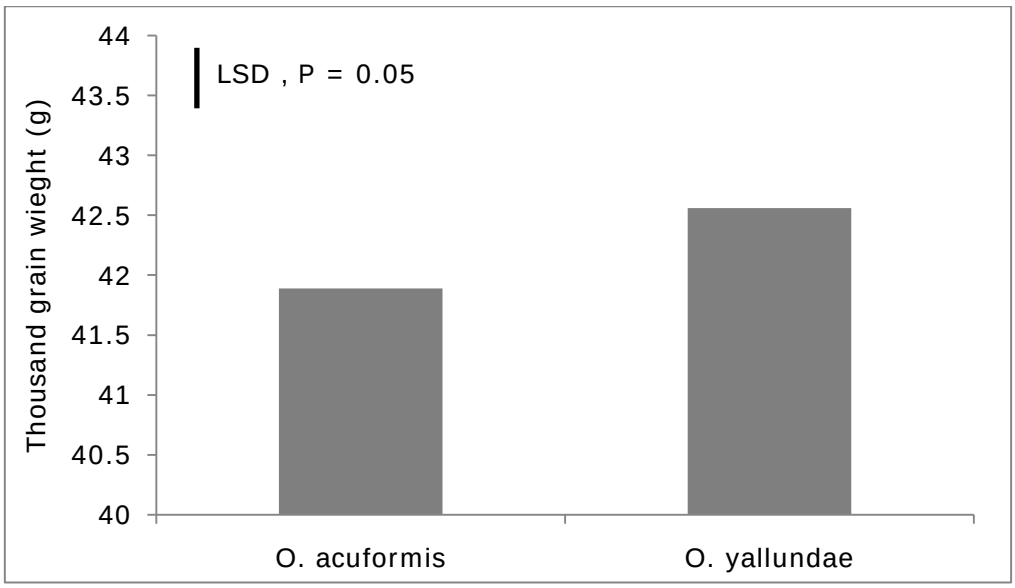


Figure 19. Effect of eyespot caused by *O. acuformis* or *O. yallundae* on thousand grain weight of Robigus in 2008 and 2009.

The effect of trinexapac-ethyl application on SW was inconsistent across inoculation rates and years of experimentation (Table 18). Application of trinexapac-ethyl to plots inoculated at the high inoculation rate of 15 g of *Oculimacula* infected oats /m^2 resulted in significant increase of SW compared to the control in 2008 (Table 18). A similar effect was also observed in 2009 but the difference was not significantly different from the control.

There was a significant interaction between fungicide application and year of experimentation for measured yield, TGW and SW (Table 19). Application of cyprodinil increased yield, TGW and SW significantly compared to the control in 2008, however differences between fungicide-treated plots and untreated in 2009 were small and not significant.

Table 18. Effect of primary inoculum of *Oculimacula* spp. and application of trinexapac-ethyl at GS 32 on specific weight of cv. Robigus grown in 2008 and 2009.

		Trinexapac-ethyl	
		g a.i./ha	
	Inoculation		
Year	g m^{-2}	0	100
2008	0	72.18	73.73
	1.5	72.01	72.01
	15	70.99	72.16
2009	0	78.94	79.05
	1.5	78.12	79.06
	15	78.78	79.03
Year*inoculation*PGR			
P		0.033	
LSD		1.324	

Table 19. Effect of cyprodinil application at GS 32 on yield (t/ha), specific weight (SW, kg/hl) and thousand grain weight (TGW, g) of cv. Robigus grown in 2008 and 2009.

Year	Cyprodinil g a.i./ha	Yield t /ha	Specific Weight kg/hl	Thousand Grain Weight g
2008	0	6.97	71.19	36.59
	502	8.85	73.17	38.43
2009	0	9.55	78.89	46.96
	502	9.97	78.78	46.93
Year*Fungicide				
P		0.002	<0.001	0.007
LSD		0.819	1.204	0.945

3.3.4. Discussion

This is the first study to clearly demonstrate the effect of quantity of primary inoculum source of individual *Oculimacula* spp. on yield loss due to eyespot in winter wheat. Yield loss during both years of experimentation occurred consistently under the high inoculation rate irrespective of causal organism. Results from this study suggest that eyespot is indeed a monocyclic disease and final yield loss is significantly related to large amounts of uniformly distributed, primary inoculum sources. Rowe and Powelson (1973) and Fitt and White (1988) considered that eyespot epidemics caused by *Oculimacula* spp. was dependent upon large amounts of primary inoculum delivered from short-range (1-2m) dispersal of rain-splashed conidia in the spring (March/April/May) and epidemics then developed as a function of accumulated temperature, fitting Van der Plank's "simple interest" model (Van der Plank, 1963). Furthermore, disease progress during crop development and growth was later shown to be affected by thermal time in relation to crop development and by epidemiological differences between the individual *Oculimacula* species (Wan *et al*., 2005). There is considerable evidence demonstrating that *O. yallundae* is able to infect and penetrate plant leaf sheaths at the seedling stage more rapidly than *O. acuformis*, however *O. acuformis* progresses through stem tissues more rapidly than *O. yallundae* (Goulds and Fitt, 1990; 1991; Wan *et al*., 2005). In agreement, results from this study also show consistent differences between species in disease development early in the crop growing season with disease indices being significantly higher up to GS 39 in plots inoculated with *O. yallundae* than in plots inoculated with *O. acuformis*. Early epidemiological differences between species typically become less apparent late in the season (Goulds and Fitt, 1988; Ray *et al*., 2006). Indeed, in this study there were no significant differences between eyespot disease index caused by individual *Oculimacula* spp. assessed at GS 73/75. Although inoculation was successful for both years of experimentation and provided a range of eyespot disease indexes in both *Oculimacula* infected plots, it was evident that eyespot was more severe in 2008 and contributed to greater overall yield losses because of lodging and whiteheads.

Previous studies using PCR to quantify pathogen DNA have shown that visually similar eyespot symptoms at the end of the season were commonly associated with markedly different DNA concentrations of the two fungal species (Ray *et al*., 2006). In this study, at GS 73/75 for both years of experimentation, DNA of *O. yallundae* was significantly higher than DNA of *O. acuformis* although there were no differences in eyespot disease index or overall final grain yield loss. Furthermore, this study has shown that in 2008, severe eyespot caused by individual *Oculimacula* species resulted in different disease symptoms late in the growing season. Inoculation with *O. yallundae* caused increased occurrence of eyespot-induced lodging, whilst inoculation with *O. acuformis* resulted in a greater number of whiteheads scattered within the crop. Previous research has demonstrated that moderate lesions, where lesions girdle half of the stem interference with no stem softening caused by *O. yallundae* and severe lesions, where stem softening has occurred

caused by either *Oculimacula* spp. significantly reduce the lodging resistance of wheat by reducing stem bending strength (Ray *et al*., 2006). Thus damaging, defined as moderate and severe, eyespot lesions initiated by the faster developing *O. yallundae* are more likely to induce lodging than lesions caused by *O. acuformis*. Interestingly, at the time when *O. yallundae* was considered the predominant pathogen (pre-1990s) there were more frequent reports of lodging associated with eyespot in published literature (Glynne, 1944; Jørgensen, 1964; Scott and Hollins, 1978).

Plants inoculated with *O. acuformis* exhibited significantly higher number of whiteheads at GS 73/75 in response to increased initial inoculum rate compared to *O. yallundae*. The high number of whiteheads in plots inoculated with *O. acuformis* could explain the significant reductions of TGW compared to *O. yallundae*. It is likely that the partially empty, bleached heads yielded a greater number of shriveled grains with reduced weight. The mechanisms of lodging and whiteheads caused by individual *Oculimacula* spp. require further investigation and it is likely that intrinsic differences between the species in their effect on plant physiology are greater than previously considered. *Oculimacula* spp. have been previously differentiated in the infection, penetration process and enzyme activity at cellular level in wheat seedlings (Daniels *et al*., 1991; Lucas *et al*., 2000), it is unclear if differences between species are present and persistent during disease progression at an adult host stage, in turn influencing the development of specific disease symptoms.

Overall yield loss caused by severe eyespot disease occurring under the high rate of initial inoculum was significant irrespective of year of experimentation or causal *Oculimacula* species. However, under natural infection conditions, the relationship between initial inoculum and yield loss is likely to be more complex and dependent upon quantity and/or quality of inoculum in addition to environmental conditions favouring dispersal. Whilst crop debris is considered the main source of primary inoculum in field, interactions with soil type and cultivation methods have been shown to affect disease development and progression of eyespot epidemics (de Boer *et al*., 1993; Jenkyn *et al*., 2010). Furthermore, pathogen interactions with other fungal competitors, part of the stem-base pathogen complex, for example *Fusarium* spp. or *Microdochium* spp. are also likely to influence the establishment of *Oculimacula* spp. on stems and the outcome of eyespot epidemics under natural infection conditions. These competitors were removed during our inoculated experiments using selective fungicide treatments in order to reduce any influence on eyespot disease, however in practice it is likely that pathogen interactions will play a role in determining disease predominance on stems.

Fungicide application of cyprodinil at GS 32 was effective in reducing both eyespot DI and pathogen DNA of *Oculimacula* spp. These results are consistent with previous reports on the efficacy of cyprodinil against eyespot disease under natural infection conditions (Burnett, 1999;

Bateman *et al*., 2000; Ray *et al*., 2004). Reductions of more than 50% of *Oculimacula* DNA were observed at GS 73 in inoculated plots for both years of experimentation. However, the yield response to fungicide treatment was significantly greater in 2008 when eyespot induced whiteheads and lodging. Cyprodinil application resulted in 5% and 3% increases of TGW and SW, respectively in 2008 but not in 2009. These increases were possibly related to the direct effects of cyprodinil on eyespot DI, pathogen DNA and lodging and whiteheads. Cyprodinil application reduced the occurrence of lodging by more than 50% in plots inoculated with either *Oculimacula* spp. However, the fungicide was more effective in reducing whiteheads caused by *O. acuformis* than by *O. yallundae* under high inoculum rate.

Application of trinexapac-ethyl failed to directly affect DI, pathogen DNA, yield or reduce the occurrence of lodging. Trinexapac-ethyl application increased SW but inconsistently across years and inoculum rates with the effect being most pronounced at the high inoculum rate of *Oculimacula* spp. in 2008. Interestingly, although PGR failed to directly affect disease or pathogen DNA, trinexapac-ethyl reduced the number of whiteheads by more than 40% in 2008. It is possible that this has occurred via modification of the stem properties of the crop by the plant growth regulator application. Trinexapac-ethyl has been shown to increase stem diameter and stem wall thickness although the effects on the latter were less consistent (Zagonel *et al*., 2002; Matysiak, 2006) thus some delay of progression and/or blockage of vascular tissues by *Oculimacula* spp. could have occurred in PGR treated plants resulting in lower numbers of whiteheads.

This study has provided new information of the effect of quantity of primary inoculum source on yield loss caused by eyespot indicating that large primary inoculum of either *Oculimacula* spp. contributes significantly to yield loss. *Oculimacula yallundae* was associated more with lodging whilst *O. acuformis* caused greater number of whiteheads. *Oculimacula acuformis* reduced thousand grain weight more than *O. yallundae* and this effect was consistent across experiments. There were no differences between species on their effect on yield (t ha^{-1}) although yield loss increased significantly in the year when whiteheads and lodging occurred. Fungicide application was effective in reducing lodging and eyespot via direct reduction in disease index and pathogen DNA. In contrast to fungicide efficacy, trinexapac-ethyl failed to control lodging or directly affect disease index or pathogen DNA. However, there was evidence that trinexapac-ethyl was effective in reducing the occurrence of whiteheads irrespective of causal organism. Further investigation of the role of quantity and/or viability of inoculum in eyespot disease severity will allow for improved understanding of eyespot disease epidemiology.

3.4. Application technology field trials

3.4.1. Aim

The aim was to investigate factors affecting spray distribution in a wheat crop canopy and their possible impact on efficacy of targeting eyespot at the stem base, using a limited range of application variables, and to determine the potential for delaying eyespot treatment to later in the season when decision thresholds might be more accurate.

3.4.2. Materials and methods

A single trial was carried out by Silsoe Spray Applications Unit in the first year of the project, to investigate the distribution in the canopy of a fungicide and its efficacy in targeting eyespot at the stem base with a limited range of application variables and at two different timings (one conventional and one at a later growth stage).

Field trial design

Six treatments were tested at each timing (Table 20). A range of volumes between 100 and 400 l/ha were included, because it is a common label recommendation to *increase* volumes in order to improve penetration into the crop. This increase in volume was achieved by using larger nozzle sizes (greater outputs for a given pressure) and slower forward speeds. The highest volume tested (400 l/ha) was not intended to represent current practice, but was included to ensure that any volume effect could be identified, given the likely variability in results. The remaining two treatments were 100 l/ha with two different nozzle designs: the spray produced by the Amistar/Guardian Air nozzle, which, because of its backward angle designed to compensate for forward speed, would be expected to have a more vertical trajectory than other nozzles; the 80^{o} flat fan nozzle would also produce a spray with a more vertical trajectory than standard 110° nozzles. These designs would therefore be expected to improve penetration into the crop, although that might not necessarily translate into greater deposits on the lower stem.

Table 20. Treatments evaluated in trial. Timing A: GS31-32 (approx. mid April), Timing B: GS37 (approx. mid May).

Treat No.	Timing	Volume l/ha	Nozzle Type	Nozzle Size mm	Speed km/h	Boom height m
1	A	100	Conventional	025	12	0.6
2	A	200	Conventional	05	12	0.6
3	A	200	Conventional	025	6	0.6
4	A	400	Conventional	05	6	0.6
5	A	100	Amistar/Guardian	025	12	0.6
6	A	100	Conventional	025 80° angle	12	0.6
7	B	100	Conventional	025	12	0.6
8	B	200	Conventional	05	12	0.6
9	B	200	Conventional	025	6	0.6
10	B	400	Conventional	05	6	0.6
11	B	100	Amistar	025	12	0.6
12	B	100	Conventional	025 80° angle	12	0.6

All nozzles 110° angle unless otherwise indicated for treatments 6 and 12

Plots were 6 m wide by 8 m long, with 8 m gaps between, and partially randomised (treatments have to remain on the same side of the boom for practical reasons, and were matched so as to spray two treatments simultaneously, one on each side). Plots were grouped according to timing of the application. There were five replicate plots per treatment.

Each plot was sprayed with a solution containing a non-ionic surfactant at 0.1% by volume, and 2 g/l of a tracer dye (Green S). Samples of the tank mix were taken at intervals through the applications. Following the spray application, 30 plants were taken randomly from each plot, excluding the area 1.0 m from the edge. The lower section of the stem was cut off (70 mm for the early timing, 120 mm for the later timing) and the lower leaves removed. These, with the rest of the plant, were discarded. Ten lower stems were bulked together into a plastic bag, creating three samples per plot. Each bag of samples was stored in dark conditions until taken back to the laboratory for analysis.

Each sample was weighed, then 10 ml of deionised water was placed in the bag and was shaken to remove the deposited dye. The rinsate was decanted into test tubes and the concentration compared with that of the tank samples using spectrophotometry to determine the quantity of original spray liquid deposited on the lower stems.

Three replicate samples were taken from untreated areas of the crop before the applications, to provide background readings.

Applications were made on 22nd April (GS31- 32) and 14th May (GS37) 2008.

3.4.3. Results

The quantity of spray liquid deposited on the lower stem is shown in Figure 20, with the data normalised for applied volume. Figure 21 shows the relative deposit, when compared with what would be considered a 'standard' treatment, i.e. a conventional flat fan nozzle applying 100 l/ha at 12 km/h.

The early timing resulted in significantly more spray liquid per gram of plant material being deposited on the lower stem than at the later timing, by a factor of approximately 6 for the 110° flat fan nozzle, but only by a factor of around 3 for the Guardian Air and the 80° flat fan. Over all treatments, the early timing resulted in higher deposits by a factor of four.

At the early timing, the deposit from the Guardian Air nozzle was significantly greater than all other treatments and there were no other significant differences. The Guardian Air nozzle put approximately 30% more deposit on the lower stem than the other treatments (Figure 20).

Increasing the volume from 100 l/ha to 400 l/ha had no effect on lower stem deposits.

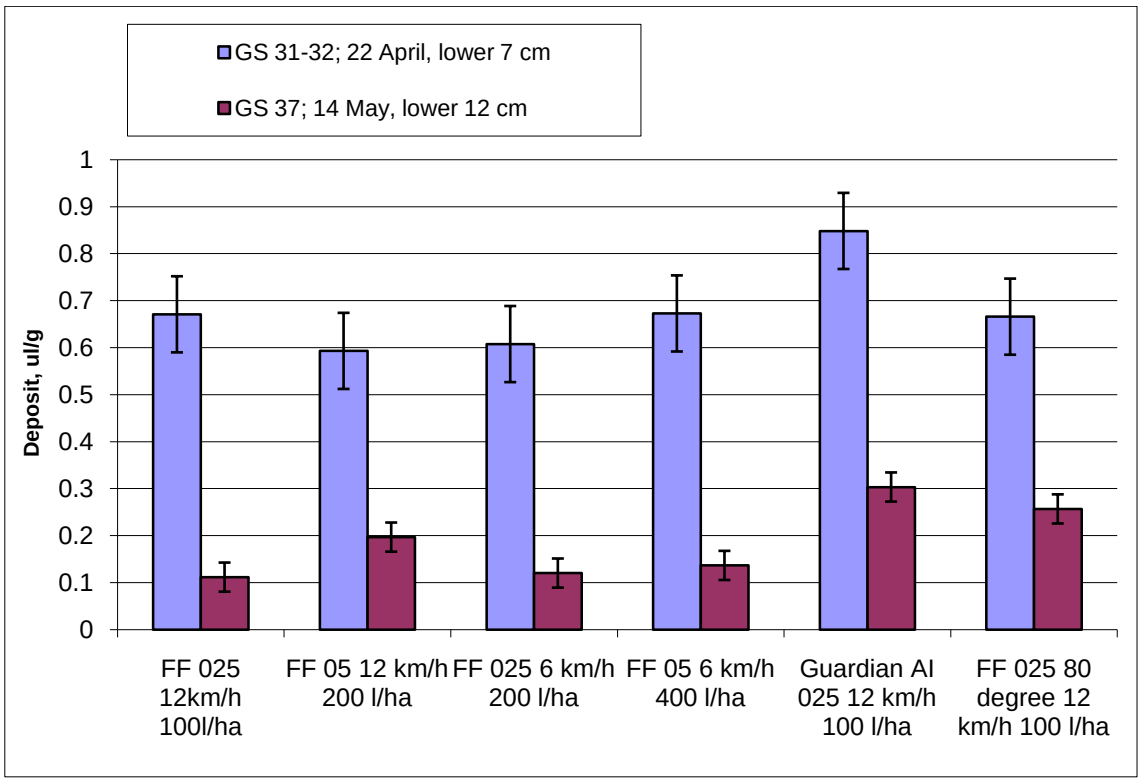


Figure 20. Deposit on lower stem of winter wheat plants (FF= flat fan) (*Error bars = LSD/2)*

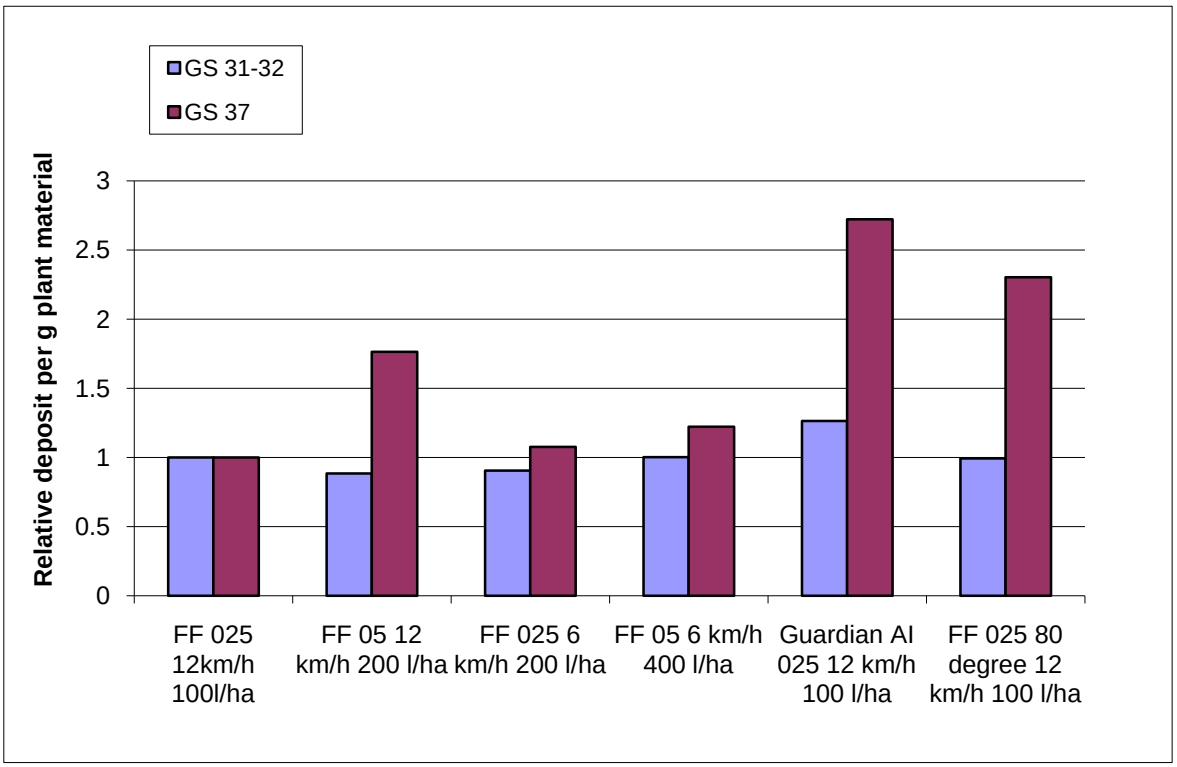


Figure 21. Deposit on lower stems of winter wheat for different applications, relative to standard (FF 025 100 l/ha).

At the later timing, the Guardian Air nozzle and the 80° degree flat fan nozzles both achieved significantly higher deposits on the lower stem, as did the 05 flat fan at 12 km/h.

The Guardian Air nozzle deposit was approximately 2.7 times that of the standard flat fan nozzle; the 80° degree nozzle 2.3 and the 05 at 12 km/h 1.8 times that of the standard nozzle.

3.4.4. Conclusions

Applications at the later growth stage resulted in significantly less tracer deposit on the lower stem per gram plant material than at the earlier growth stage – on average 0.19 µl/g compared with 0.68 µl/g.

The Guardian nozzle is the best choice for getting chemical down to the lower part of the stem - by far - at both growth stages; however, the differences were greater than were expected and therefore further work is needed to confirm this result before clear recommendations can be given to growers.

The best treatment at the later growth stage only put on half the quantity per gram by comparison with the worst treatment at the earlier growth stage - if quantity is more important than timing in the performance of the application, then the early growth stage remains the best time to spray.

The later growth stage was more sensitive to application variables such as water volume, nozzle type and speed than the earlier growth stage. The work should be extended from tracer dye to actual product, and efficacy assessed.

Further work undertaken in HGCA project RD-2008-3562

A subsequent project *Application factors that influence the distribution of pesticide within a cereal crop canopy* is currently following up on some of the findings of this work, focussing on application volume and nozzle design, but looking at a single, later growth stage when the crop density presents more of a challenge for penetration. The project also considers the wider aspects of distribution over the whole plant, rather than focusing on solely the lower stem.

Summary of relevant results to date

Wind tunnel studies showed that increasing wind speed reduced the quantity of spray able to penetrate into the bottom of the canopy with a fine spray. However, the spray from a drift-reducing nozzle (in this case, the Billericay Bubble Jet) was unaffected by wind speed. At the lowest wind speed, the air induction nozzle resulted in lower levels of spray on the lower part of the canopy than the flat fan, similar levels at the medium wind speed and significantly greater levels at the highest wind speed. This suggests that the environmental conditions can have as big an effect on penetration into a canopy as application parameters.

A field experiment was also undertaken, similar to the one in this eyespot project in 2008. Five replicate plots were sprayed with one of eight different application treatments. The aim was to investigate the effect of volume, spray angles and nozzle design on distribution of spray deposit. Results for deposit on the lower stem showed that there was no statistically significant difference in deposits with volume. Again, the 80° flat fan nozzle and a small droplet air induction nozzle (this time the Billericay Bubblejet which has no backwards angling) gave significant increases in deposit on the lower stem. In addition, it was shown that the deposit on the whole plant was significantly lower with the highest volume and the largest droplets.

Key messages for growers

A greater quantity of spray will reach the lower part of the stem at GS 31-32 compared with GS 37. It is not possible to achieve a higher level of lower stem deposits at the later growth stage with conventional spray nozzles. Increasing volumes above 100 l/ha is not necessary to increase penetration into a dense crop. Two seasons of experiments suggest that

- 80° degree spray angles
- A small droplet air induction nozzle (such as the Guardian Air or the Billericay Bubblejet) gives the highest levels of lower stem deposits in a cereal crop.

Improvements in lower stem deposits can be made by appropriate use of nozzles, particularly at the later growth stage, should the later timing be necessary.

3.5. Possible impacts on eyespot occurrence as a consequence of climate change

3.5.1. Aim

The aim of this section of work was to provide a brief overview of the impact that a changing climate might have on the severity and distribution of eyespot in the UK.

3.5.2. Materials and methods

The previous HGCA Report 347 (Burnett and Hughes, 2004) detailed the impact of spring rainfall on eyespot severity. Using this criterion, the UK Climate Projections (http://ukclimateprojections.defra.gov.uk/) weather projections 2009 database was used to plot projected rainfall for March, April and May using the medium emissions scenario and the time frames 2010 - 2039 up to 2070 – 2099, at a range of probabilities. The previous project had also suggested that temperature in the winter months was a risk factor so cumulative temperature in December, January and February was also plotted.

An alternative method was also used, whereby locations were identified where the disease is currently common. The first was the Penrith area in north-west England and the second was the Perth area of Scotland. UK climate data for March, April and May were matched to Penrith and Perth to identify areas with similar climates (rainfall, rainfall pattern and temperature). These were then used to map the distribution of similar climates using a Climex Model which produced maps of climate matches – colour coded to indicate the degree of climate match on a scale of 0.5 -1 (0.5 = 50% match, 1= 100% match).

3.5.3. Results

The UK Climate Projections data on spring rainfall suggests that there will be a very small reduction in spring rainfall in the period up to 2059, with increase of up to 10% in precipitation thereafter, although under the more extreme probabilities. The more likely scenario is closer to a 3% increase at the 50% probability up to 2089, shown in Figure 22.
Temperature is predicted to increase at a fairly constant rate under all the probabilities plotted (Figure 23). Increases of up to 4°C are predicted by 2099.

Temperature is predicted to increase at a fairly constant rate under all the probabilities plotted in Figure 23. Increases of up to 4°C are predicted by 2099, with changes of less than 2°C up to the 2030-2059 time period.

Climex mapping shows that the distribution of conducive weather is likely to reduce in the short term 2020 model (Figure 24), and reduce further by 2050 (Figure 25), leaving areas in the East of England and East Anglia at a reduced risk of eyespot.

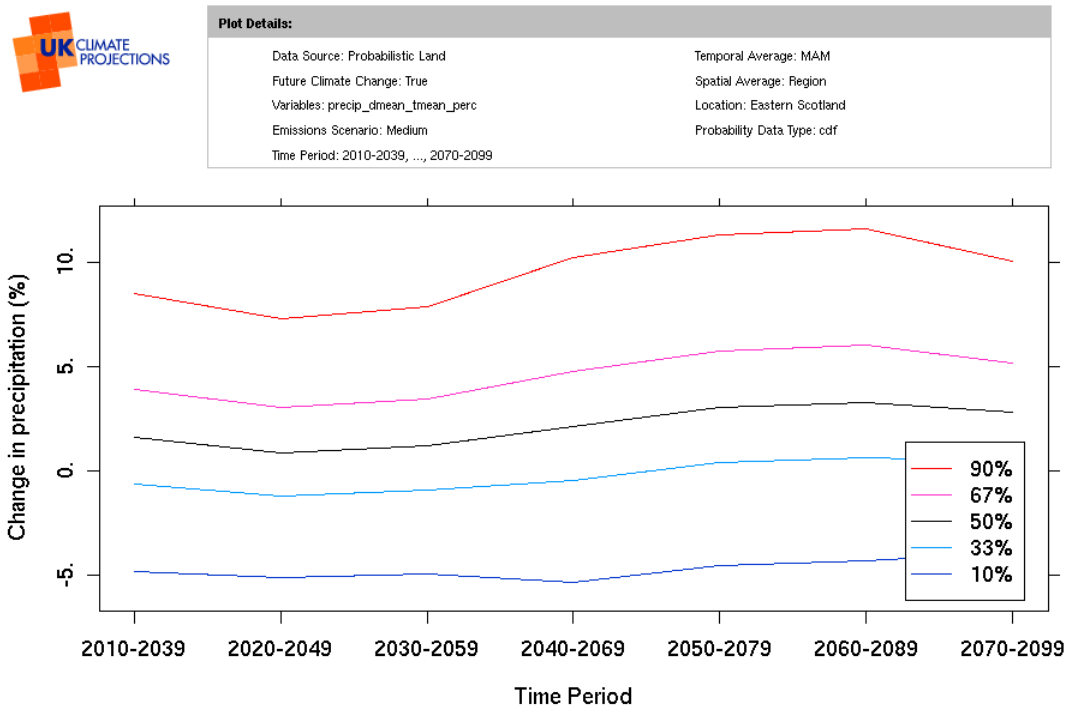


Figure 22. Predicted changes in rainfall for March, April and May.

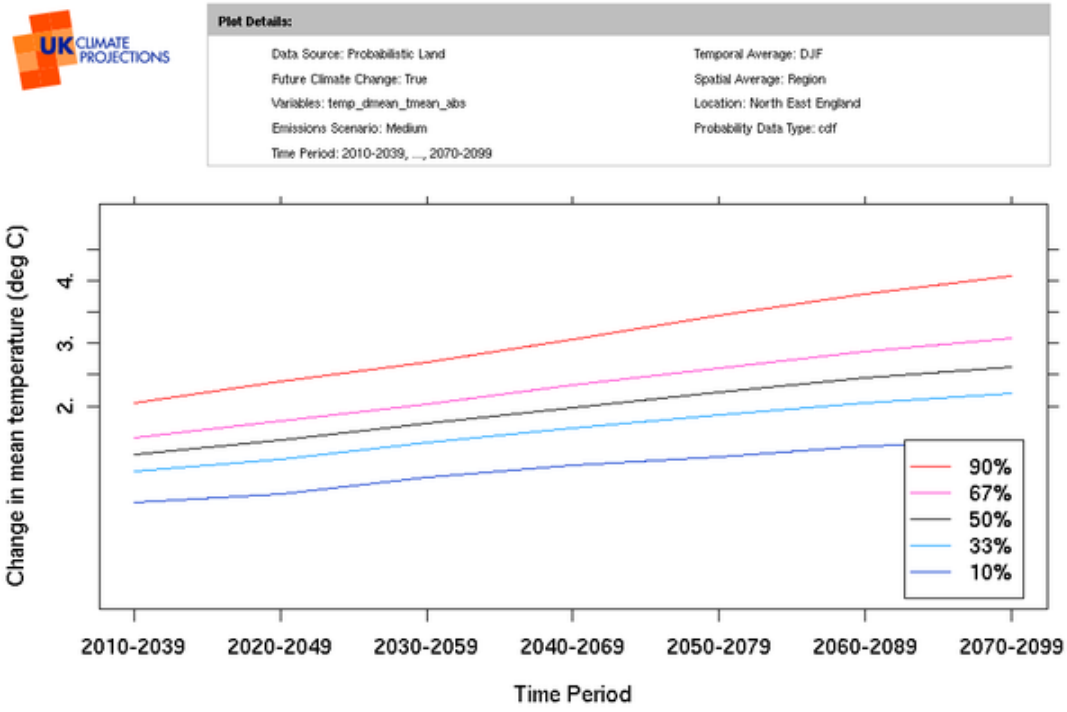
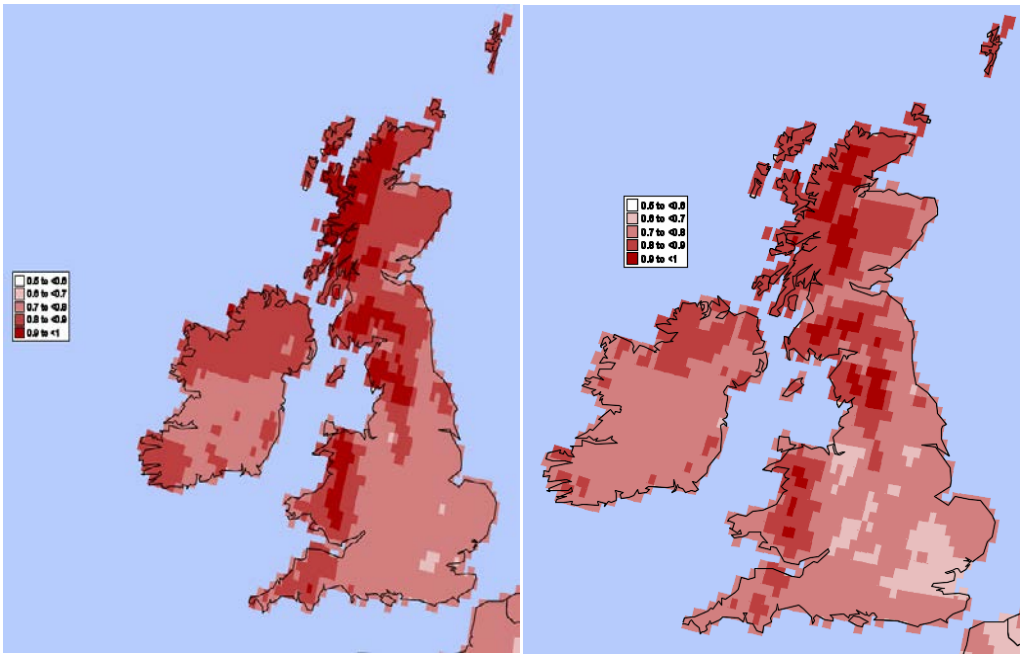
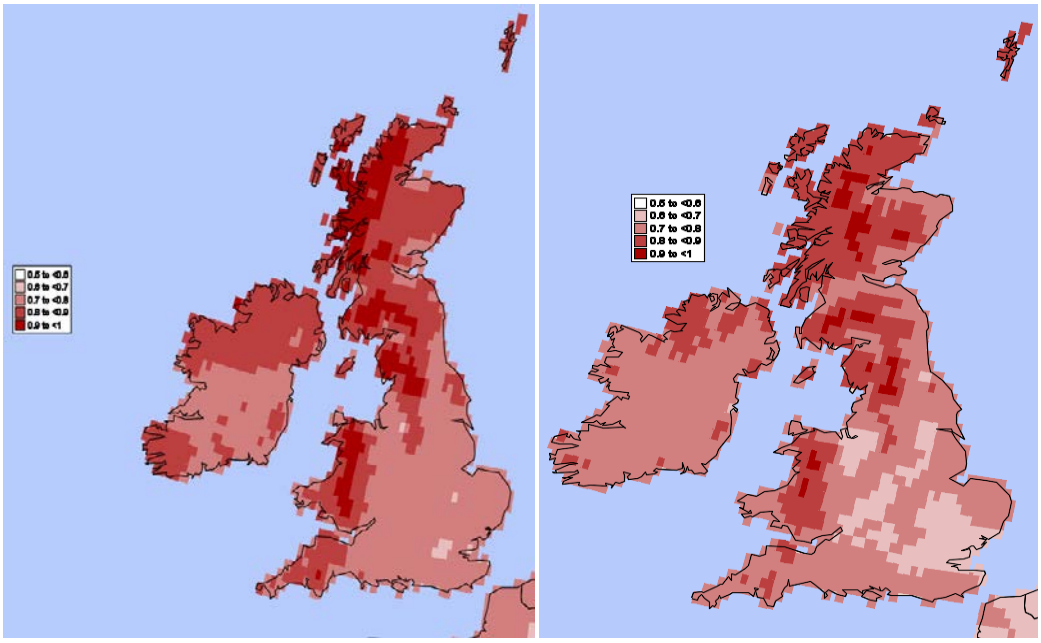


Figure 23. Predicted changes in temperature for December, January and February.



0.5 - <0.6 0.6 - <0.7 0.7 - <0.8 0.8 - <0.9 0.9 - <1

Figure 24. Current (left) and 2020 (right) March, April and May climate matching (0.5 = 50% match, 1= 100% match)



0.5 - <0.6 0.6 - <0.7 0.7 - <0.8 0.8 - <0.9 0.9 - <1

Figure 25. Current (left) and 2050 (right) March, April and May climate matching (0.5 = 50% match, 1= 100% match)

3.5.4. Discussion

The two methods of predicting the climatological risk of eyespot increasing or decreasing would suggest that in the short term there is a diminishing risk up to the 2050 time frame; and the longer term the UK Climate Predictions data base would suggest that thereafter there will be an

increasing risk, both as a consequence of rainfall and of increased winter temperatures. Eyespot resistance should therefore be retained in wheat breeding programmes as a useful trait for long term sustainable production.

3.6. Eyespot disease risk assessment

3.6.1. Aim

HGCA Report No. 347 (Burnett and Hughes, 2004) described the development of a risk assessment method to identify wheat crops at risk from eyespot. The analysis presented here builds on that previous work. Thus the objectives here are to update that work:

- by incorporating experience gained from deployment of the methodology described in HGCA Report No. 347, and
- by incorporating new developments in epidemiological thinking since HGCA Report No. 347 was published.

Since the underlying risk assessment methodology described in HGCA Report No. 347 is retained, we begin with a brief review of the principles and procedures on which that work was based.

3.6.2. Review of risk assessment method from HGCA Report No. 347

Overview of risk assessment methodology

Briefly, the risk assessment method presented in HGCA Report No. 347 was based on analysis of data from a total of 341 wheat crops untreated for eyespot. These crops were retrospectively classified definitively as either 'needed treatment' or 'did not need treatment' on the basis of the level of eyespot incidence at the end of the season, which was based on levels of eyespot causing economic yield loss. In the shorthand epidemiological terminology, crops that needed treatment are referred to as *cases*, those that did not need treatment are referred to as *controls*. The determination of cases and controls is the *gold standard* classification. The gold standard is definitive, but of course comes too late to be of practical use in disease management decision-making. What is required is a predictor of the need for treatment that can be deployed by providing decision guidelines at an appropriate stage in crop development. Although the predictor cannot be definitive, it can be developed on the basis that crops will be assessed for risk (in this case, in relation to the need for treatment for eyespot). For this purpose, it is necessary:

- to identify the most important eyespot disease risk factors related to the host-crop, the environment and the pathogen;
- to quantify the risk associated with individual risk factors and with the set of risk factors to which an individual crop has been exposed; and
- to allow for the fact that not all decision-makers will have the same response to a particular specified level of risk.

Statistical methodology

The statistical methodology required for the development of risk-based decision guidelines must allow classification of crops into 'predicted cases' and 'predicted controls', in such a way that most of the predicted cases really are cases that needed treatment, and most of the predicted controls really are controls that did not need treatment. This requires that during model development we have access to data on both the predicted status (from the risk assessment) and the true status of crops (from the data collected on disease outcome).[1] Two statistical methods have been widely used in analyses where the basic problem is the classification of subjects on the basis of proxy data (the risk factors) that are related to the actual variable of interest (the requirement for treatment or otherwise): logistic regression analysis and discriminant function analysis (see, for example, Hughes and Madden (2003) for a comparison). In HGCA Report No. 347, logistic regression was used to identify the following six risk factors: *soil type*, *previous crop*, *tillage method*, *sowing date*, *eyespot at GS31-32* and *March/April/May rainfall*. A risk points score was calculated for each level of each factor, such that the maximum risk score was 50 points. Risk points were tabulated. Then two different thresholds for treatment were identified: a lower threshold points score (20 points) for application by relatively risk-sensitive users, and a higher threshold points score (29 points) for application by relatively risk-tolerant users.

3.6.3. Updating the risk assessment method from HGCA Report No. 347

General principles

Generally, we can think of disease risk as an accumulation of risks. This accumulation may take place over an extended period of time during which the subject of a risk assessment is monitored, or a shorter period of time during which a sequence of diagnostics is administered to the subject. Or, indeed, a combination of the two may be used to assess overall risk for an individual subject. An example (from a clinical perspective) of the sequential diagnostic approach can be seen in Van den Ende et al. (2007), who present a figure showing the evolution of probability following consecutive diagnostic steps. The approach of Van den Ende et al. (2007) allows decision-makers to apply Bayesian logic without formal calculations. That analysis has influenced the way we have chosen to update the risk assessment method presented in HGCA Report No. 347.

[1] In application, of course, decision-makers will only have access to the predicted status of the crop, and will base their decision on this prediction.

For a crop, there are some disease-related risk factors, such as those associated with geographic location, site topography and soil physical properties, over which a decision-maker can exert little or no control. Then, the level of risk associated with factors such as previous cropping, tillage method, variety choice and sowing date is already decided at the start of the growing season. Subsequently, only risk factors relating to the environmental conditions during the growing season and the level of disease observed in the growing crop remain for the decision-maker to take into account. The underlying principle on which we have based the update of the risk assessment method presented in HGCA Report No. 347 is to assign as much of the accumulation of disease risk as possible to factors that can be assessed prior to the crucial eyespot disease assessment at GS31-32.

While it is possible to identify factors relating to the host, the pathogen and the environment (i.e., the classic 'disease triangle') that are important contributors to crop disease risk, it is also the case that individual decision-makers may respond differently to a specified risk accumulation. Thus we must allow some flexibility for individual decision-makers to calibrate accumulated risk according to their personal circumstances.

Data

In addition to the original data set comprising 341 wheat crops untreated for eyespot (but treated to control foliar disease), data from a further 324 untreated wheat crops were available for analysis.[2] The two data sets were not combined. As for the original data set, the new data set was incomplete in the sense that not all risk factors were recorded for each crop. No single crop had all risk factors recorded, and in general, the sub-set of the overall data that comprised the 324 untreated crops was more unbalanced than the original data set analysed in HGCA Report No. 347. Some factor levels were missing entirely from the new data set; for example, none of the untreated crops were grown on a heavy soil (all either light or medium), while all had wheat as a previous crop. Only 15 of the 324 untreated crops were resistant (Pch-1) varieties, insufficient for a quantitative analysis of their contribution to risk reduction. No new data were available on the eyespot risk related to brash or limestone soils as reported in HGCA Report No. 347.

[2] Data from untreated crops are used because the objective is to develop guidelines for decision-makers who are considering the need or otherwise for treatment in crops, and who must therefore predict what would happen in the absence of treatment.

3.6.4. Results

Pre-disease risk accumulation

Because of the severely unbalanced nature of the new data set, most of the analysis of pre-disease risk accumulation was carried out using the original data set. Where cross-checking was possible, no incompatibilities were found in the analysis of the individual risk factors selected for inclusion in the risk algorithm described in HGCA Report No. 347.

As explained in HGCA Report No. 347, there may be internal correlations between risk factors that are not apparent when they are analysed separately. If two risk factors are correlated (i.e., they account for the same component of the overall risk), then there is no need to include both of them when making a prediction of disease, and one is eliminated. This was the case with the risk factors *region* and *March/April/May rainfall*. Previously *region* was excluded from the eyespot risk algorithm and *March/April/May rainfall* included. We have now included *region* and excluded *March/April/May rainfall*. The main reason for this change is that it allows us to classify all the risk accumulation except for the component associated with the crucial eyespot disease assessment at GS31-32 as 'pre-disease' risk. We also note that *region* (levels: East, North or West) is likely to be regarded as easier to determine than *March/April/May rainfall* (levels: less than or equal to 170mm or greater than170mm).

Table 21 shows the pre-disease risk factors together with the calculated odds ratio (a measure of relative risk, see HGCA Report No. 347) and the $\log_{10}$(odds ratio) (an additive measure of risk).[3] The revised risk points scale is derived by transforming the calculated $\log_{10}$(odds ratio) values to integer values on a scale from zero to twenty-five points.

In evidence-based medicine, a 5-point ordinal categorical scale is often used as a basis for classifying diagnostic results (see, for example, Swets, 1988). Using this approach we can write a 5-point ordinal categorical scale for pre-disease risk accumulation (Table 22), based on the accumulated risk points for a particular crop, as shown in Table 21. Thus, for example, a winter wheat crop in Scotland (*region*/North), following a wheat crop (*previous crop*/wheat), sown in late September (*sowing date*/early), on medium soil (*soil type*/medium) after minimum till (*tillage*/minimum till) accumulates 1+8+2+1+0 = 12 pre-disease risk points (Table 21) and so on this basis is classified in the medium pre-disease risk category (Table 22).

[3] Base 10 logarithms are used here for consistency with Van den Ende et al. (2005).

Table 21. Pre-disease risk factors*

Factor	Level	Odds ratio (OR)	Log_{10}(OR)	Risk points
Region	East	1	0	0
	North	1.149	0.0603	1
	West	1.788	0.2524	5
Soil type	Light	1	0	0
	Medium	1.071	0.0298	1
	Heavy	1.559	0.1928	4
Previous crop	Non-host	1	0	0
	Other cereal	2.245	0.3512	7
	Wheat	2.420	0.3838	8
Tillage	Minimum Till	1	0	0
	Plough	2.044	0.3105	6
*Sowing date***	Late	1	0	0
	Early	1.336	0.1258	2

* The table refers to pre-disease factors for which there is currently an evidence base for quantitative risk assessment. This does not mean that other potential sources of risk should be ignored, as discussed in the text and Table 22.

** early = before or including 6 Oct, late = after 6 Oct.

Table 22. Pre-disease risk categories (conditional risk)

Pre-disease risk points	Verbal description of pre-disease risk category*
1-4	Low risk (L)
5-9	Low-medium risk (LM)
10-14	Medium risk (M)
15-19	Medium-high risk (MH)
≥20	High risk (H)

* These descriptions are advisory rather than prescriptive. They are based on the accumulation of risk points relating to risk factors as shown in Table 13, but the risk category may also be adjusted by an individual decision-maker where information relating to additional factors is available. For example, growing a crop with a high target yield or use of a susceptible variety might be reasons to place a crop in a higher pre-disease risk category than indicated solely on the basis of the risk factors as shown in Table 13. In addition, the subjective sensitivity to or tolerance of risk for an individual decision-maker may result in adjustment of the risk category without requirement for further specification of a particular cause.

At this point in the two phase assessment process the grower or operator could decide to select a different field to use or plant a variety which incorporates eyespot resistance in the form of the *Pch1* gene. The risk categorization may require further adjustment. For example, there are at present insufficient data to calculate risk points relating to use of a susceptible variety compared with a resistant (Pch-1) variety. From the data in section 3.2, these varieties did sometimes respond to eyespot-active fungicide treatment but eyespot disease was reduced by 20%. It is therefore reasonable to assume from the trials that were carried out within the project that use of a resistant variety should place a crop one (for a risk-sensitive decision-maker) or even two (for a risk-tolerant decision-maker) categories down the pre-disease risk category scale. Indeed, the risk sensitivity or tolerance of the individual decision-maker is an important consideration. In some cases, it may not be difficult to elicit the basis for sensitivity to risk. A crop with a high target yield, for example, may be regarded as an investment worth protecting, and so be placed by a decision-maker in a higher pre-disease risk category than otherwise.

So, for the above example of a crop initially classified in the medium pre-disease risk category on the basis of a pre-disease risk point accumulation of 12 points, a target yield greater than 10 t/ha would reasonably lead to re-classification in the medium-high risk category. If the crop were a susceptible variety, a further re-classification to high risk would be appropriate. The process of assessing pre-disease risk described here is meant to be analogous to the specification of a Bayesian prior probability. While the risk points scale provides the best guidelines for a generic eyespot risk assessment on the basis of the evidence currently available, risk assessment for a particular crop must be flexible enough to accommodate for the expert opinion of the individual decision-maker in relation to risk (without necessarily eliciting a particular basis for a stated attitude to risk).

Finally, here, we note that the pre-disease risk accumulation is actually an assessment of *conditional risk*. That is to say, we know (referring again to the disease triangle) that even if a susceptible variety is being grown in conditions conducive for the spread of disease, that in the absence of the pathogen the disease will not develop. So while the pre-disease (conditional) risk accumulation categorises a predisposition to risk, this is not realised as an actual risk until the outcome of the eyespot disease assessment at GS31-32.

Disease risk assessment

The analysis here is based on the new data set of 324 untreated wheat crops, in which a total of 299 crops had recorded a % eyespot disease score at both GS31-32 (recorded as eyespot incidence) and GS70-80 (recorded as eyespot severity index – calculation shown in methods section 3.2) (Figure 26).

Treatment is carried out in order to prevent the GS70-80 (end-of-season) % disease score reaching a pre-specified level. Above this level of eyespot, a crop is assumed to have required treatment; below it, treatment was not required (of course, disease control decisions in practice must be based on predictions of whether or not the end-of-season % disease score will reach the pre-specified level). The analysis of interest then is based on classifying crops as either 'needed treatment' (cases) or 'did not need treatment' (controls) on the basis of the level of eyespot incidence at the end of the season, and a logistic regression of the binary (case/control) variable on the explanatory variable % eyespot disease score at GS31-32 (recorded as eyespot incidence).[4] The analysis was carried out using the epidemiological software EGRET®.[5] Five different thresholds were used to classify crops as either cases or controls on the basis of % eyespot disease score at GS70-80 (recorded as eyespot index): 45%, 30%, 20% 15% and 10% (Table 23).

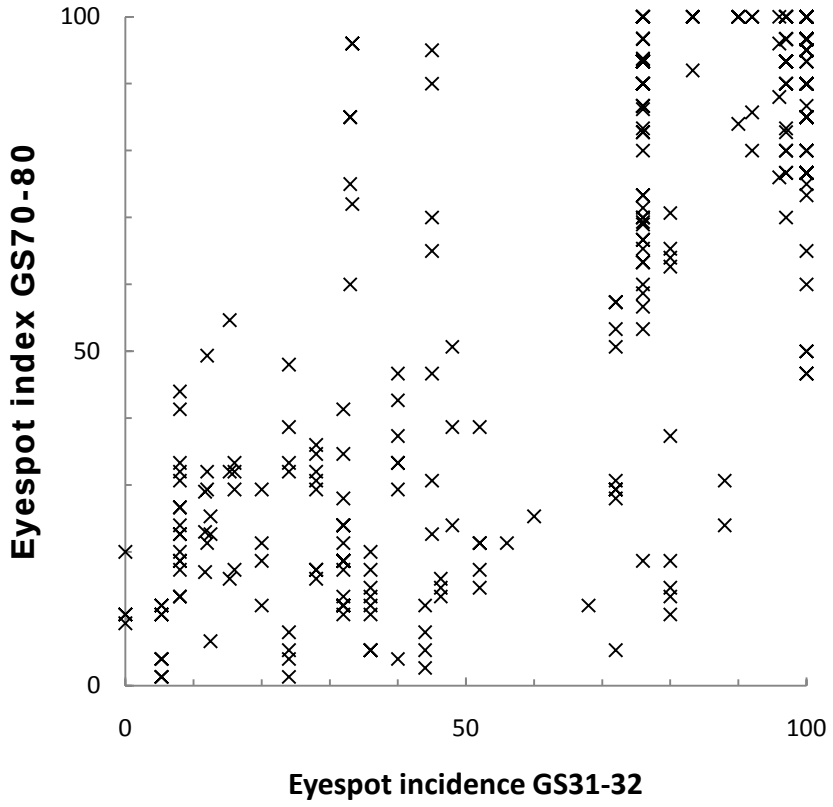


Figure 26. The relationship between eyespot index (GS70-80) and eyespot incidence (GS31-32) for 299 untreated wheat crops. The correlation coefficient = 0.798. (Figure 13, earlier shows all crops treated and untreated)

[4] Actually, the analysis is carried out with eyespot disease scores recorded on a 0 – 1 scale and the results are then converted to % scales for presentation purposes.

[5] CYTEL Software Corporation, Cambridge, MA 02139, USA.

To obtain a response curve (referred to as a *disease risk curve*) for eyespot risk varying with % eyespot disease score at GS31-32 (*eyespot incidence*), we calculate:

$$eyespot\ risk = \frac{\exp(a + b \times eyespot\ incidence)}{1 + \exp(a + b \times eyespot\ incidence)} \qquad \text{(Eq. 1)}$$

using the *a* and *b* coefficients for the appropriate case/control threshold (Table 23). Following this procedure for each case/control threshold in turn, we obtain Figure 27.[6]

From Figure 27 we note the following.

- The intercept of the disease risk curve on the vertical axis of Fig.27 is the pre-disease risk accumulation (i.e., attributable to risk factors preceding the GS31-32 disease assessment). The realization of this risk is conditional on the occurrence of disease.
- When the % eyespot disease score at GS31-32 (*eyespot incidence*) is low, eyespot risk depends to a large extent on the choice of case/control threshold.
- As the % eyespot disease score at GS31-32 (*eyespot incidence*) increases, the effect of choice of case/control threshold on eyespot risk decreases.
- At high levels of the % eyespot disease score at GS31-32 (*eyespot incidence*), the choice of case/control threshold has relatively little influence on eyespot risk.
- A decision-maker who was tolerant of eyespot risk would tend to use a higher case/control threshold, while a decision-maker who was sensitive to eyespot risk would tend to use a lower case/control threshold (the lower the threshold, the more crops are classified as cases).

Table 23. Logistic regression of case/control status

Threshold	Regression term	Estimate	Standard error	*P*-value
45%	*constant* (*a*)	−4.447	0.51	<0.001
	slope (*b*)	0.07992	0.007997	<0.001
30%	*constant* (*a*)	−2.198	0.3206	<0.001
	slope (*b*)	0.05419	0.005855	<0.001
20%	*constant* (*a*)	−0.9405	0.2791	<0.001
	slope (*b*)	0.04392	0.005803	<0.001
15%	*constant* (*a*)	−0.02867	0.2865	0.920
	slope (*b*)	0.0372	0.006296	<0.001
10%	*constant* (*a*)	1.108	0.3888	0.004
	slope (*b*)	0.03912	0.01029	<0.001

[6] Note, in passing, that this analysis avoids the need to assess the % eyespot disease score at GS31-32 against a threshold value, as was the case for the risk algorithm described in HGCA Report No. 347. The 7% threshold value adopted there proved rather difficult to implement in practice, hence the modification.

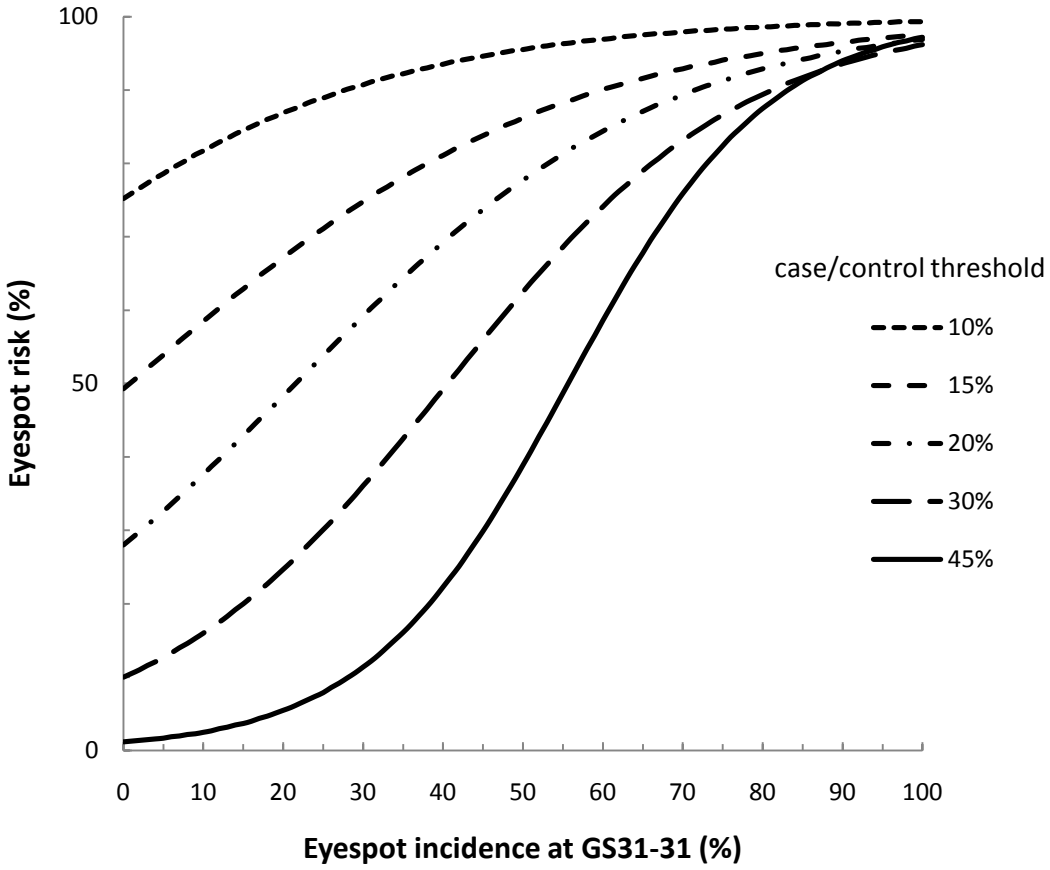


Figure 27. Disease risk curves for different case/control thresholds (from Eq. 1). Risk-sensitive decision-makers have relatively low thresholds (e.g., 10-15%); risk tolerant decision-makers have relatively high thresholds (e.g., 30-45%).

Each different case/control threshold has a different intercept. It works like this – the logistic regression equation models the dependence of risk on the GS31-32 disease assessment (given the particular case/control threshold), so both a slope (the dependence on the disease assessment) and an intercept (the dependence on other things) are calculated. If you are risk sensitive (low case/control threshold) you will already have accumulated a lot of risk (conditional risk) before you even see the disease. If you are risk tolerant (high case/control threshold) you do not accumulate much risk pre-disease, and indeed (see 45% line) need to see quite a lot of disease at GS31-2 before you get any substantial increase in eyespot % risk.

Disease risk curves and the pre-disease risk accumulation

As eyespot incidence (at the GS31-32 disease assessment) increases, the effect of pre-disease (conditional) risk accumulation on eyespot risk decreases, and at high levels of eyespot incidence, the pre-disease risk accumulation has relatively little influence on eyespot risk (Figure 27). This is in accord with the view that the process of assessing pre-disease risk accumulation is analogous to the specification of a Bayesian prior probability. If the evidence provided by the GS31-32 disease

assessment is of high eyespot incidence, this overwhelms a pre-disease risk accumulation that pointed to relatively low risk. From Figure 27, we see that the more sensitive a decision-maker is to eyespot risk, the more important is pre-disease risk accumulation as a component of the total risk. For a decision-maker tolerant of eyespot risk, the pre-disease risk accumulation is less important as a component of the total risk.

Decision guidelines based on disease risk accumulation

Table 24 provides a basis for decision guidelines that combine the pre-disease (conditional) risk accumulation with a GS31-32 eyespot incidence assessment. A decision-maker simply specifies the level of pre-disease risk accumulation (based on Tables 13 and 14, and including any other risk factors relevant to their personal situation), then obtains the corresponding eyespot disease risk category for the % eyespot incidence assessment at GS31-32. Eyespot disease risk can be interpreted as a categorical scale related to probability of need for treatment, taking into account important pre-disease (conditional) risk factors, the level of disease at the GS31-32 eyespot incidence assessment, and an individual decision-maker's attitude to risk.

Table 24. Eyespot disease risk categories*

Pre-disease risk points (conditional risk)	Eyespot disease assessment % incidence at GS 31-32				
	1-4	5-9	10-14	15-19	≥20
1-4	L	LM	M	MH	H
5-9	LM	M	M	MH	H
10-14	M	M	MH	MH	H
15-19	MH	MH	MH	H	H
≥20	H	H	H	H	H

*Verbal description of category: Low risk (L), Low-medium risk (LM), Medium risk (M), Medium-high risk (MH), High risk (H).

Eyespot disease economic threshold

Fitt et al. (1988) wrote, "For predicting yield losses, and hence the likely benefits of control measures, a simple relationship between some measure of disease incidence and/or severity and future yield loss is needed." However, subsequent eyespot research – including the present study – has not seen such a relationship described. The reasons for this are debateable, but are likely to include the fact that eyespot disease is only one factor among many that may contribute to the multi-factorial determination of wheat yield.

In fact, there are reasons why it may not be necessary – or even desirable – to describe a simple relationship between yield loss and disease (we refer to such a relationship as a *damage curve*). The main reasons for this are as follows.

- A damage curve based on a wide range of factors that affect both yield and disease may characterize a version of an overall average rate of yield loss per unit disease, yet not be applicable to the particular conditions in individual crops.
- Further, even a damage curve based on a wide range of factors is unlikely to be based on data for every level of every factor that might be met in actual field crops, so the overall average rate of yield loss per unit disease is itself questionable.
- Changes over time in cropping systems and in crop-pathogen interactions mean that, once described, a damage curve that characterizes an average rate of yield loss per unit disease from empirical data (experimental or observational) will soon become outdated. New agricultural technologies, the introduction of new varieties, and changes in the pathogen population are examples of dynamic factors that may affect a damage curve.

Here, instead of basing likely benefits of control measures on a simple overall average relationship between yield loss and disease, we adopt an alternative approach to the problem.

The economic threshold approach

Background

What we refer to as the economic threshold was developed as part of the integrated control concept (Stern et al., 1959). In its original form, this was primarily concerned with the management of arthropod pest populations. However, the conceptual basis for combining and integrating chemical and non-chemical methods – often referred to now generically as integrated pest management (IPM) – is equally applicable to crops at threat from disease. Indeed, this is the basis on which HGCA has commissioned the development of disease risk assessments, including those for eyespot.

At the centre is the idea that the *economic threshold* is the population density at which control measures should be used to prevent an increasing pest population from reaching the economic injury level (Stern et al., 1959). The *economic injury level* is the lowest population density that will cause an amount of crop injury that justifies the cost of artificial control measures (Stern et al., 1959). This scheme recognizes that control measures should be taken in response to the threat of pest injury, but that it is not a practical proposition to wait until a pest population reaches the economic injury level before taking action. In that case, the eventual loss of revenue resulting from crop yield and/or quality reduction would exceed the cost of control. Instead, decisions on whether or not to deploy artificial control measures are made in relation to the economic threshold. Thus pest control decisions are made based on predictions of their consequences. Where the harmful organism in question is a pathogen, the economic threshold and the economic injury level are

usually denominated in units based on the observation of disease symptoms, rather than population densities.

Threshold graph calculations

Generally, revenue from an untreated crop (R_U) is calculated from:

$$R_U = (Y \times V) - (V \times B) \times x \qquad \text{(Eq. 2)}$$

in which

Y is the potential yield of the crop (t/ha),

V is the value of that yield (£/t),

B is the yield loss per unit disease intensity

and

x is the disease intensity (in appropriate units).

Revenue from a treated crop (R_T) is calculated from:

$$R_T = (Y \times V - C) - (V \times B \times [1 - D]) \times x \qquad \text{(Eq. 3)}$$

in which

C is the cost of treatment (£/ha),

D is the proportional reduction in disease intensity from treatment ($0 < D \leq 1$),

and everything else is as above for an untreated crop.

Essentially, then, we have two simultaneous equations relating revenue to disease. When *Y*, *V*, *C*, *B*, and *D* are constant (i.e., do not vary with disease intensity *x*), the equations describe linear relationships between revenue and disease intensity for untreated crops (Eq. 2) and treated crops (Eq. 3). If we consider such linear relationships, the values of *Y* and *V* must be based on the same yield unit (here, t) so that $Y \times V$ (Eq. 2) and $Y \times V - C$ (Eq. 3) have the same revenue units (here, £/ha). These terms are the intercepts (revenue when $x = 0$) on the vertical axis of a graphical plot of revenue reduction against disease. We can see that where there is no disease ($x = 0$), R_T (= $Y \times V - C$) < R_U (= $Y \times V$). In words: in the absence of disease, the revenue from a treated crop is lower than the revenue from an untreated crop, all other things being equal (because the cost of an unnecessary treatment has been incurred).

Now, consider the rate of yield loss[7] in the presence of disease. In the case of eyespot, the measure of disease intensity (*x*) that is the basis for crop protection decision-making is % disease incidence at GS31-32, obtained by a field sampling. The value of *B* is then the rate of yield loss per % disease incidence at GS31-32, so that $B \times x$ has units of t/ha and $V \times B \times x$ (Eq. 2) has units of

[7] Yield loss (yield reduction) has the same units as yield (here, t/ha).

£/ha. D (Eq. 3) is a measure of efficacy, measured on a proportional scale.[8] As a proportion, D is dimensionless, so $V \times B \times (1-D) \times x$ (Eq. 3) also has units of £/ha. The term $(1-D)$ (rather than D) appears in Eq. 3 because it is the disease remaining after treatment that causes a problem, not the disease reduction. The terms $-(V \times B)$ (Eq. 2) and $-(V \times B \times [1-D])$ (Eq. 3) are the slopes of linear revenue reduction against disease relationships. We can see that for an untreated crop ($D = 0$), the (downward) slope $-(V \times B)$ (Eq. 2) is steeper than that for a treated crop ($0<D\leq1$) where the (downward) slope is $-(V \times B \times [1-D])$ (Eq. 3). In words: in the presence of disease, the rate of revenue loss from a treated crop is lower than the rate of revenue loss from an untreated crop, all other things being equal.

Thus we have two lines on a graphical plot of revenue reduction against disease. Where the lines cross, $R_T = R_U$ and the corresponding value of % disease incidence at GS31-32 is the economic threshold. Above the threshold, revenue from a treated crop exceeds revenue from an untreated crop (Figure 28).

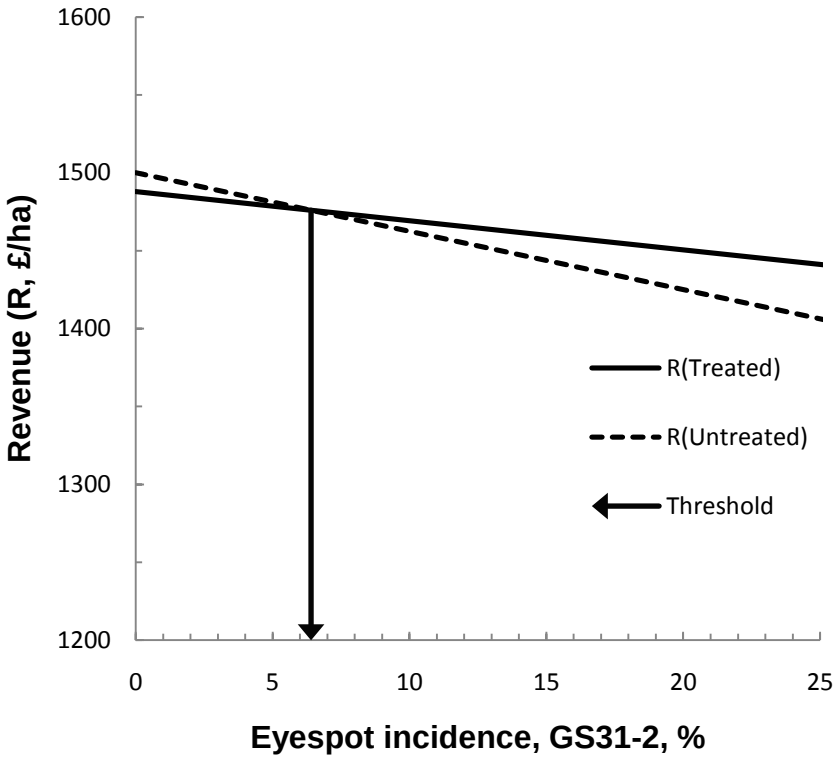


Figure 28. An example of a graphical plot of revenue reduction against disease (based on Eqs. 2 and 3), illustrating the economic threshold. The rate of revenue reduction is steeper in an untreated crop than in a treated crop. The economic threshold is the point where the revenue reduction resulting from the disease is equal to the revenue reduction incurred for treatment. In this example, calculations are based on Y = 10t/ha,

[8] The efficacy term D includes both the effectiveness of a product against eyespot and the effectiveness of its application in particular specified circumstances.

V = 150£/t, C = 12£/ha, D = 0.5, B = 0.025t/ha/% eyespot incidence at GS 31-32, and the economic threshold value is then 6.4% eyespot incidence at GS 31-32.

Parameter values

Values of:

Y the potential yield of the crop (t/ha),

V the value of that yield (£/t),

C the cost of treatment (£/ha),

D the proportional reduction in disease intensity from treatment ($0<D\leq1$),

and

B the rate of yield loss (t/ha/% eyespot incidence at GS 31-32),

are required for the calculation of the economic threshold graph (e.g., Figure 28).[9]

With just V and C we can calculate the equivalent cost of eyespot treatment in yield units (Table 25). Taking the values as used in Figure 28, the equivalent cost of eyespot treatment in yield units is 0.08 t/ha (Table 25). If we divide by the efficacy (D = 0.5, Fig. 28), we obtain the yield loss at the threshold (0.08/0.5 = 0.16 t/ha). Dividing again by the rate of yield loss (B = 0.025t/ha/% eyespot incidence at GS 31-32, Fig. 28), we obtain the threshold (0.16/0.025 = 6.4%).

Normally, appropriate values of Y, V, C and D can be specified by a decision-maker without resort to the apparatus of statistical analysis of agricultural field trials. If we consider B, the rate of yield loss, this would be where the relationship between yield loss and disease envisaged by Fitt et al. (1988) comes in. If we imagine a graphical plot of yield (vertical axis) against disease (horizontal axis), we expect this damage curve to be downward sloping. If the downward sloping relationship is linear, the slope provides an estimate of B (an average value over all the conditions under which yield and disease were measured). However, because field experimentation has not yet been successful in identifying such an estimate of B, an appropriate value must instead be elicited from the decision-maker. The advantage of this procedure is that the elicited value of B is based specifically on the experience and expertise of the decision-maker in relation to the particular conditions under which a specific crop is being grown. B must be expressed in units of t/ha/% eyespot incidence at GS 31-32. So, for example, the statement “a 20% increase in eyespot (at GS 31-32) causes a 0.5 t/ha yield loss” is equivalent to B = 0.025 (=0.5/20) t/ha/% eyespot incidence at GS 31-32 (see Figure 28). We assume a linear response.

[9] If we just deal with Eqs. 2 and 3 as simultaneous equations, we can set $R_T = R_U$ and solve for x. We find that the economic threshold value of x (% eyespot incidence at GS 31-32) is equal to $C/(V\times B\times D)$. Thus, perhaps rather counter-intuitively, the economic threshold value does not actually depend on the potential yield. However, a value of Y is required if we want to plot the graph.

Table 25. The body of the table shows the equivalent cost of eyespot treatment in yield units (t/ha) for different assumptions about the grain price (*V*) and the cost of treatment (*C*)*

Grain price (£/t)	Cost of eyespot treatment (£/ha)		
	6.00	12.00	14.00
100	0.060	0.120	0.140
110	0.055	0.109	0.127
120	0.050	0.100	0.117
130	0.046	0.092	0.108
140	0.043	0.086	0.100
150	0.040	0.080	0.093
160	0.038	0.075	0.088
170	0.035	0.071	0.082
180	0.033	0.067	0.078
190	0.032	0.063	0.074
200	0.030	0.060	0.070
210	0.029	0.057	0.067
220	0.027	0.055	0.064
230	0.026	0.052	0.061
240	0.025	0.050	0.058
250	0.024	0.048	0.056

* The “cost of treatment” is the extra cost of adding eyespot treatment to the cost of other fungicide treatments.

The revenue response graph

There is an alternative graphical presentation of the economic threshold. After first calculating R_T (Eq. 2) and R_U (Eq. 3) separately, we calculate the *revenue response* $R_T - R_U$ and plot this against x (% disease incidence at GS31-32). This is shown in Figure 29, based on the same data as for the example shown in Figure 28. The economic threshold is the point where the revenue response line cuts the horizontal axis. At eyespot incidences (% at GS31-32) above the threshold, the revenue from a treated crop exceeds the revenue from a treated crop (for the specified parameter values), so the revenue response to treatment is positive.

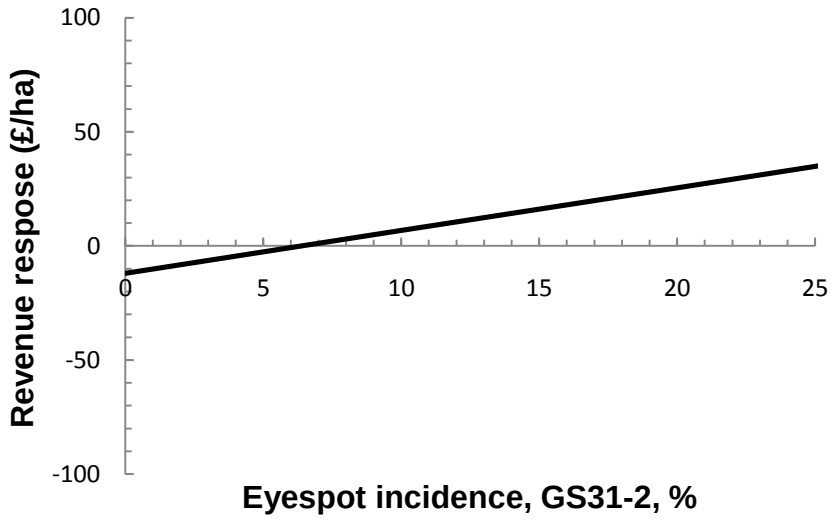


Figure 29. An example of a graphical plot of revenue response to treatment against disease (based on Eqs. 2 and 3), illustrating the economic threshold. This example is based on the same data as Fig. 28, where *Y* = 10t/ha, *V* = 150£/t, *C* = 12£/ha, *D* = 0.5, *B* = 0.025t/ha/% eyespot incidence at GS 31-32, and the economic threshold value is 6.4% eyespot incidence at GS 31-32.

Examples

From the relationship:

$$Economic\ threshold = \frac{C}{V \times B \times D}$$

we can see the following.

- The economic threshold will be lower if the value of the crop (*V*) is higher, all other things being equal. In other words, if our commodity is more valuable, it is worth protecting at a lower threat level (see Fig. 30.
- The economic threshold will be lower if the rate of yield loss (*B*) is higher, all other things being equal. In other words, if the threat is more severe, it is worth protecting a crop at a lower threat level.

The first example (see Figures 28 and 29) shown uses yield loss at 0.025 t/ha/% eyespot which was the level of loss in the inoculated experiments. The second example (see Figure 30) uses a yield loss figure of 0.005 t/ha which was the yield loss per percentage eyespot taken over the whole data set.

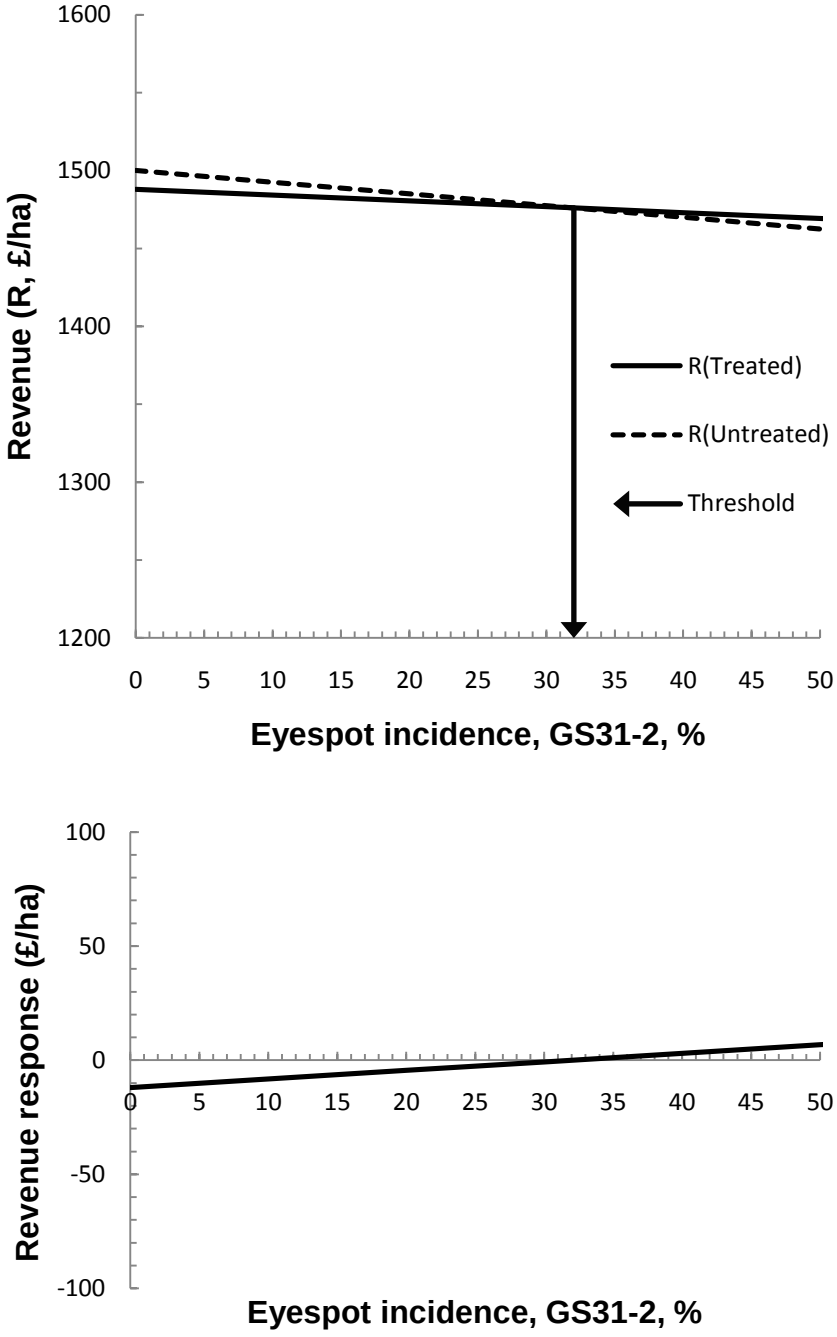


Figure 30. Upper panel: graphical plot of revenue reduction against disease (based on Eqs. 2 and 3). In this example, Y = 10t/ha, V = 200£/t, C = 12£/ha, D = 0.5, B = 0.005t/ha/% eyespot incidence at GS 31-32, and the economic threshold value is 32% eyespot incidence at GS 31-32. Lower panel: graphical plot of revenue response to treatment against disease (based on Eqs. 2 and 3), based on the same data as in the upper panel.

- The economic threshold will be lower if the rate of yield loss (*B*) is higher, all other things being equal. In other words, if the threat is more severe, it is worth protecting a crop at a lower threat level. Conversely, the economic threshold will be higher if the rate of yield loss (*B*) is lower, all other things being equal (see Fig. 30).
- The economic threshold will be lower if the cost of treatment (*C*) is lower, all other things being equal. Recall that in essence, the economic threshold characterizes a balance between the cost of treatment (£/ha) and the potential revenue (*Y*×*V*, £/ha). If the cost of treatment is lower but the potential revenue is unchanged, we can 'trade' less of our potential revenue to obtain the same amount of crop protection, so the economic threshold is lower.
- The economic threshold will be lower if the efficacy of treatment (*D*) is higher, all other things being equal. An increase in efficacy is equivalent to a decrease in the cost of treatment, because we can obtain the same amount of crop protection for a smaller financial outlay.

These examples are illustrative of the effects that directional changes in parameter values can have on the economic threshold. We do not suggest that calculations made on the basis of one parameter changing and "all other things being equal" will often be the case in routine crop protection decision-making. However, the principle of using threshold graphs to examine "what if?" scenarios in response to simultaneous changes in parameter values is a useful one.

Decision guidelines combining disease risk with economic data

Figures 28 and 29 provide a basis for decision guidelines that combine economic data with a GS31-32 eyespot incidence assessment. A decision-maker specifies values for *Y* [the potential yield of the crop (t/ha)], *V* [the value of that yield (£/t)], *C* [the cost of treatment (£/ha)], *D* [the proportional reduction in disease intensity from treatment ($0<D\leq1$)], and *B* [the rate of yield loss (t/ha/% eyespot incidence at GS 31-32)]. In the absence of experimental evidence that provides a value of *B* appropriate for all circumstances, the decision-maker selects an appropriate value for the particular circumstances. As a guideline for this selection, see Table 26. The table shows selected ranges of eyespot increase (X, %) and yield loss (Y, t/ha) in the margins, and in the body of the table, the corresponding value of *B* (=Y/X). Read the table as follows: "An X% Increase in eyespot (select an appropriate value from the top margin of the table) causes a yield loss of Y t/ha (select an appropriate value from the left-hand margin of the table)", then obtain the value of *B* (the rate of yield loss) from the body of the table, corresponding to the selected X and Y values. More severe rates of yield loss (appropriate for relatively risk-sensitive decision-makers) are situated towards the bottom left-hand corner of the table; less severe rates of yield loss (appropriate for relatively risk-tolerant decision-makers) are situated towards the top right-hand corner of the table. Then, either the economic threshold graph or the revenue response graph will show the economic

threshold, i.e., the level of eyespot at the GS31-31 disease assessment above which treatment is economically justified under the specified conditions. So, if the actual GS31-32 disease assessment is then above this calculated threshold, treatment is economically justified.

This approach allows for the economic threshold for treatment to be adapted to new information on yield loss which may arise from future research, and allow changes in eyespot species and varieties and their impacts on yield losses to be incorporated in a similar manner.

Table 26. Rate of yield loss to eyespot (highlighted values are those found in the current data set and used as worked examples).

		X% increase in eyespot					
		X					
	Y	**5**	**10**	**15**	**20**	**25**	**50**
causes	**0.05**	0.010	0.005	0.003	0.003	0.002	0.001
yield loss of	**0.10**	0.020	0.010	0.007	0.005	0.004	0.002
of **Y** t/ha	**0.15**	0.030	0.015	0.010	0.008	0.006	0.003
	0.20	0.040	0.020	0.013	0.010	0.008	0.004
	0.25	0.050	0.025	0.017	0.013	0.010	0.005
	0.30	0.060	0.030	0.020	0.015	0.012	0.006
	0.35	0.070	0.035	0.023	0.018	0.014	0.007
	0.40	0.080	0.040	0.027	0.020	0.016	0.008
	0.45	0.090	0.045	0.030	0.023	0.018	0.009
	0.50	0.100	0.050	0.033	0.025	0.020	0.010
	0.60	0.120	0.060	0.040	0.030	0.024	0.012
	0.70	0.140	0.070	0.047	0.035	0.028	0.014
	0.80	0.160	0.080	0.053	0.040	0.032	0.016
	0.90	0.180	0.090	0.060	0.045	0.036	0.018
	1.00	0.200	0.100	0.067	0.050	0.040	0.020

3.7. General discussion

Treatment for the stem base disease eyespot, caused by *Oculimacula* spp., represents an additional cost to the standard spray programme that would be applied to the winter wheat crop for control of the foliar diseases, which are the main target of the stem extension fungicide sprays. The primary aim of this project was to help growers predict which crops are at risk of eyespot and will give a cost effective yield response to specific eyespot treatment. It was therefore problematic that no overall yield loss association between eyespot levels and yield could be determined. This problem was solved by developing a risk assessment that would predict eyespot risk and, separately, a revenue calculator that allows for the cost of treatment, the grain price, the efficacy of treatment and the yield loss accrued by non-treatment to be entered by the user. This approach has several benefits over a risk prediction system that uses a prescribed yield loss approach in that it can be adapted to different situations and past experiences and it can be updated to incorporate new information on yield losses to eyespot as well as new information on the occurrence and incidence of the two causal species of eyespot. Varietal developments and changes in fungicide efficacy can also be incorporated. This work, and previous work has shown that the two causal species of eyespot fluctuate in prevalence and this dynamic situation can also be incorporated as the yield losses attributable to each species become better understood.

This tool can be used by agronomists and researchers who have their own, local experiences of eyespot and yield loss. In addition, by using worked examples based on the fungicide efficacy determined in this project and current grain and treatment costs combined with a high and low estimate of yield loss taken from experiments within the project data set or from the data set as a whole, growers and users without experience of eyespot yield loss can use the risk assessment and decide on a treatment threshold.

A previous model (Burnett and Hughes, 2004) enabled growers to determine the need for fungicide treatment in the spring but the introduction of varieties with the Pch1 gene conferring better resistance to eyespot means that growers needed to be able to judge the risk of eyespot in the autumn. The two phase approach to risk assessment that has been developed is novel in crop protection and allows growers to assess options prior to drilling and, based on the eyespot risk score, either select an alternative field for drilling or decide to drill a variety of wheat with eyespot resistance.

The second phase of the risk assessment allows growers to decide on the need for fungicide treatment in the spring by combining this pre-disease autumn score with information on the incidence of eyespot at stem extension. Here a methodology borrowed from medical literature was used to ascribe a risk status to crops based on low, low moderate, moderate, moderate high and high categories. This allows for more sensitivity in deciding on the need to treat than would a more

simple low, moderate or high description. A logical development of this methodology would be to develop a web based / on-line tool that would allow calculations on eyespot risk to be made by entering relevant parameters.

One development that can be incorporated in such a system would be improvements in treatment efficacy. Fungicides evaluated in the course of this project gave 50% eyespot control at best. Improved targeting of the stem base has the potential to improve this. Application technology trials were carried out in the first year of this project (and continued for a second year in a separate HGCA project RD-2008-3562) and identified several developments that could improve spray deposition on the stem base. Angled nozzles and small droplet air induction nozzles showed the greatest potential for improved targeting. It has commonly been assumed that increasing water volumes would improve crop penetration but this work would suggest that volumes over 100 l/ha are not required. This has benefits to speed and efficiencies of working at peak spray periods.

A further aim of the project was to better understand how the two eyespot species, *O. yallundae* and *O. acuformis*, cause yield loss. The inoculated trials described establish that the quantity of inoculum to which the crop is exposed influence final disease outcome and yield loss. This concurs with the risk assessment developed in this project that ascribes increased risk to previous susceptible crops in the rotation. There were differences in how yield losses accrued between the two species. Eyespot disease caused by *O. yallundae* was associated with a greater occurrence of lodging whilst *O. acuformis* caused increased numbers of whiteheads. The inoculated studies confirm evidence from previous studies that *O. yallundae* creates more rapid and visible symptoms compared to *O. acuformis*. Early epidemiological differences between species typically become less apparent late in the season (Goulds and Fitt, 1988; Ray *et al*., 2006), and the current work concludes that both species cause similar disease levels by the end of the season. The inoculated work confirms a significant seasonal effect, suggesting that final disease symptoms are the result of an interaction between inoculum level and environmental conditions. Spring rainfall was associated with increased disease development in the inoculated trials and this concurs with the risk prediction model developed from the wider data set. Overall yield loss was similar for both species and was related to early inoculum quantity irrespective of season.

In order to develop the risk prediction system, data on yield, disease and agronomy was collected from field trials located throughout the UK between 2004 and 2010, and combined with data from a previous eyespot project running from 2000 to 2003 to give a data set of over 700 untreated scenarios.

PCR analysis of stem base samples from the 50 field sites evaluated in the development of the risk model, showed that *O. yallundae* was the dominant strain – with 10 times the quantity of DNA

recovered compared to *O. acuformis*. Most sites had mixed infections. This is a reversal of the situation in the previous HGCA project 347, when *O. acuformis* was the dominant strain in trials throughout the UK. Core treatments were also included in the data set. Epoxiconazole applied at GS31-32 was included, as it is commonly applied in practice at this time for control of foliar diseases, but not for control of eyespot. The expectation in designing the experiments was that this active ingredient would reduce any confounding effect that foliar disease development might have and enable yield losses to eyespot to be better defined by comparing this treatment to treatments where boscalid was applied in mixture with epoxiconazole or prothioconazole was applied. Yield was highly variable between trials and no consistent significant relationship between eyespot levels could be established. This is perhaps not surprising as eyespot is likely to be a relatively small factor amongst the other factors that drive wheat yield. Using the yield response to treatment figure for each site we hypothesised at the start of the project that the influence of these other factors would be removed and the response to eyespot control elicited. This was not the case and it seems likely that the unexpectedly high levels of control seen from epoxiconazole treatment may have partially negated the benefits of the application of fungicides with specific eyespot activity. The shift towards *O. yallundae* and away from *O. acuformis* would be one explanation why epoxiconazole was more effective as the azole group of chemistry has more activity on this species. This shift may be due to a decline in the use of prochloraz, which selects strongly for *O. yallundae*.

Despite not being able to attribute a consistent correlation between yield loss and eyespot severity, there were significant yield benefits to fungicide treatment in the data set. Yield response to fungicide treatment at GS31-32 was significant for boscalid + epoxiconazole, cyprodinil, epoxiconazole and prothioconazole, and significant levels of eyespot control were also noted. The relative efficacy of cyprodinil is interesting as it is seldom applied now in commercial practice as it represents a more expensive addition to the spray programme. In early commercial trials boscalid and prothioconazole were more effective than cyprodinil but this data would suggest that control between products is now comparable.

An evaluation of predicted climatic changes and expected eyespot distribution do not suggest any large shift in current risk. Climate change predictions would suggest that because of an increasing likelihood of dry springs, eyespot severity and distribution might be expected to decline in the UK in the 2020 and 2050 time frames, but weather predictions beyond this time frame suggest a slightly increased risk so it is important that resistance to eyespot remains a target in wheat breeding programmes. Trials within this project demonstrated that varieties that incorporate the Pch1 resistance mechanism can reduce final eyespot severity by up to 30%. Combining eyespot resistance with fungicide treatment often improved control and a combined approach to eyespot control using both approaches might be more sustainable in the longer term than an over reliance

on one or the other, where pathogen adaptations can quickly erode fungicide efficacy or overcome varietal resistance mechanisms.

3.7.1. Future work

The lack of any significant correlation between eyespot levels and yield in the data set is an important finding of this work and suggests that responses to eyespot occur on a far more local scale than the factors that were evaluated in the project could predict. Factors such as region, weather, soil type, sow date and previous crop which were useful in predicting final eyespot severity did not assist in predicting the likelihood of a yield response.

It is likely that surrounding stems can compensate for infected plants under certain circumstances, and timing of infection will impact on the formation of the various components of yield such as plant number, grain site number and grain filling. The mechanisms of yield loss caused by individual *Oculimacula* spp. also require further investigation and it is likely that intrinsic differences between species on their effect on plant physiology are greater than previously considered.

3.8. Acknowledgements

The authors would like to thanks the many farmers who hosted trial sites and the commercial sponsors who funded the trials, Bayer CropScience, Syngenta UK and BASF, as well as the British Wheat Breeders Association who provided details on varietal resistance and advised on experimentation.

3.9. References

Anon, 1987 Winter wheat - managing disease control. ADAS leaflet 843 (revised). MAFF, Alnwick.

Bateman GL, Edwards SG, Marshall J, Morgan LW, Nicholson P, Nuttall M, Parry DW, Scrancher M, Turner AS, 2000. Effects of cultivar and fungicides on stem-base pathogens, determined by quantitative PCR, and on diseases and yield of wheat. *Annals of Applied Biology* 137, 213-221.

Burnett F J and Oxley, S J P, 1996. The importance and control of common eyespot in wheat Proceedings Crop Protection in Northern Britain, 1996, 1, 121 - 126.

Burnett F J, Oxley, S J P and Harling, R, 1997. The use of PCR diagnostics to monitor development of eyespot in winter wheat. HGCA Project Report Number 150.

Burnett F J, Oxley, S J P and Laing, A P, 2000. The use of PCR diagnostics in determining eyespot control strategies in wheat. Proceedings of the 2000 British Crop Protection Conference - Pests and Diseases, 1, 107-112

Burnett F J, 1999. The use of fungicide sequences to maximise the control of eyespot in cereals and minimise the risk of sharp eyespot. Project Report No. 200. Home Grown Cereals Authority, London.

Burnett FJ and Hughes G, 2004. The development of a risk assessment method to identify wheat crops at risk from eyespot. Project Report No. 347. Home Grown Cereals Authority, London.

Bierman SM, Fitt BDL, Van Den Bosch F, Bateman GL, Jenkyn JF and Welham, SJ, 2002. Changes in populations of the eyespot fungi *Tapesia yallundae* and *T. acuformis* under different fungicide regimes in successive crops of winter wheat, 1984–2000. Plant Pathology 51, 191-201.

Clarkson J D, 1981. Relationship between eyespot severity and yield loss in winter wheat. Plant Pathology 30, 125-131.

Cook R J, 1993. Eyespot - agronomic influences in the United Kingdom. In Exploring the depths of eyespot. Ed. G D Palmer, Shering AG, Berlin, pp 83 - 89.

Crook MJ and Ennos AR, 1995. The effect of nitrogen and growth regulators on stem and root characteristics associated with lodging in tow cultivars of winter wheat. *Journal of Experimental Botany* 46, 931-8.

Crous PW, Groenewald JZE, Gams W, 2003. Eyespot of cereals revisited: ITS phylogeny reveals new species relationships. *European Journal of Plant Pathology* 109, 841-850.

Daniels A, Lucas JA, Peberdy JF, 1991. Morphology and ultrastructure of W and R pathotypes of *Pseudocercosporella herpotrichoides* on wheat seedlings*. Mycological Research* 95, 385-397.

Daniels A, 1993 (a) Early infection processes of *Pseudocercosporella herpotrichoides* pathotypes. In Exploring the depths of eyespot. Ed. G D Palmer, Shering AG, Berlin. pp 29-37.

de Boer RF, Steed GR, Kollmorgen JF, Macauley BJ, 1993. Effects of rotation, stubble retention and cultivation on take-all and eyespot of wheat in northeastern Victoria, Australia. *Soil and Tillage Research* 25, 263-280.

Fitt BDL, Goulds A, Polley RW, 1988. Eyespot (*Pseudocercosporella herpotrichoides*) epidemiology in relation to prediction of disease severity and yield loss - a review. *Plant Pathology* 37, 311-328.

Fitt BDL, Nijman DJ, 1983. Quantitative studies on dispersal of *Pseudocercosporella herpotrichoides* spores from infected wheat straw by simulated rain. *Netherlands Journal of Plant Pathology* 89, 198-202.

Fitt BDL, White RP, 1988. Stages in the progress of eyespot epidemics in winter wheat crops. *Journal of Plant Diseases and Protection* 95, 35-45.

Glynne MD, 1944. Eyespot, *Cercosporella herpotrichoides* Fron, and lodging of wheat. *Annals of Applied Biology* 31, 377-378.

Glynne MD, Dion WM, Weil JW, 1945. The effect of eyespot (*Cercosporella herpotrichoides* Fron.) on wheat and the influence of nitrogen on the disease. *Annals of Applied Biology* 32, 297-303.

Glynne MD, Salt GA, 1958. Eyespot of wheat and barley. Report of Rothamsted Experimental Station 1958.

Goulds A, Fitt BDL, 1990. The development of eyespot on seedling leaf sheaths in winter wheat and winter barley crops inoculated with W-type and R-type isolates of *Pseudocercosporella herpotrichoides*. *Journal of Phytopathology* 130, 161-173.

Goulds A, Fitt BDL, 1991. Prediction of eyespot severity on winter wheat or winter barley inoculated with W-type or R-type isolates of *Pseudocercosporella herpotrichoides*. *Journal of Phytopathology* 132, 105-115.

Hocart MJ and McNaughton JE, 1994. Interspecific hybridisation between *Pseudocercosporella herpotrichoides* and *P. anguioides* achieved through protoplast fusion. Mycological Research, 98, 47.56.

Hughes G, McRoberts N and Burnett, FJ, 1999. Decision-making and diagnosis in disease management. Plant Pathology, 48, 147-153.

Hughes G and Madden LV, 2003. Evaluating predictive models with application in regulatory policy for invasive weeds. *Agricultural Systems* 76: 755–774.

Jalaluddin M and Jenkyn J F, 1996. Effects of wheat crop debris on the sporulation and survival of *Pseudocercosporella herpotrichoides*. Plant Pathology, 45:1052-1064.

Jenkyn JF, Gutteridge RJ, Bateman GL, Jalaluddin M, 2010. Effects of crop debris and cultivations on the development of eyespot of wheat caused by *Oculimacula* spp. *Annals of Applied Biology* 156,387-399.

Jørgensen J, 1964. Investigations on loss in yield due to attack by *Cercosporella herpotrichoides* Fron in field experiments with winter wheat. *Acta Agriculture Scandinavica* 14, 12-20.

Jones D R, 1994. Evaluation of fungicides for control of eyespot disease and yield loss relationships in winter wheat. Plant Pathology 43, 831 - 98.

King J E and Griffin M J, 1985. Surveys of benomyl resistance in *Pseudocercosporella herpotrichoides* on winter wheat and barley in England and Wales in 1983. Plant Pathology 34, 272 - 283.

Lucas JA, Dyer PS, Murray TD, 2000. Pathogenicity, host-specificity, and population biology of *Tapesia* spp., causal agents of eyespot disease of cereals. *Advances in Botanical Research* 33, 225-258.

Matusinsky P, Mikolasova R, Klem K, Spitzer T, 2009. Eyespot infection risks on wheat with respect to climatic conditions and soil management. *Journal of Plant Pathology* 91. 93-101.

Matysiak K, 2006. Influence of trinexapac-ethyl on growth and development of winter wheat. *Journal of Plant Protection Research* 46, 133-143.

Nicholson P, Rezanoor H N, Simpson DR and Joyce D, 1997. Differentiation and quantification of the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis* using a PCR assay. Plant Pathology, 46, 842-856.

Nicholson P and Turner AS, 2000. Cereal stem-base disease – a complex issue. In: Proceedings of the Crop Protection Conference, British Crop Protection Council, Farnham, UK. 99-106.

Nicholson P, Turner AS, Edwards SG, Bateman GL, Morgan LW, Parry DW, Marshall J, Nuttall M, 2002. Development of stem-base pathogens on different cultivars of winter wheat determined by quantitative PCR. *European Journal of Plant Pathology* 108, 163-177.

Migeon J L, Mathop M P and Leroy J P, 1995. Le cyprodinil: une nouvelle solution dans la lutte contre le pietin-verse des cereales [*Pseudocercosporella herpotrichoides* (fron) Deighton] trois annees d'experimentation en France. *Mededelingen van de Faculteit Landbouwwetenschappen Universiteit Gent*, 60 (2b), 393 - 399.

Polley RW and Clarkson JDS, 1978. Forecasting cereal disease epidemics. In: Plant disease epidemiology (Eds PR Scott and A Bainbridge), pp 141-150. Blackwell Scientific Publications, Oxford.

Rapilly F, Laborie Y, Eschenbrenner P, Chisnel E, Lacrose F, 1979. La prévision du piétin-verse sur blé d'hiver. *Perspectives Agricoles* 23, 30-40.

Ray RV, Crook MJ, Jenkinson P, Edwards SG, 2006. Effect of eyespot caused by *Oculimacula yallundae* and *O. acuformis*, assessed visually and by quantitative PCR, on stem strength associated with lodging resistance and yield of winter wheat. *Journal of Experimental Botany*. 57, 2249-2257

Ray RV, Jenkinson P, Edwards SG, 2004. Effects of fungicides on eyespot, caused predominantly by *Oculimacula acuformis*, and yield of early-drilled winter wheat. *Crop Protection*. 23, 1199-1207

Rowe R C, Powelson RL, 1973. Epidemiology of *Cercosporella* footrot of wheat: Disease spread. *Phytopathology* 63, 984-988.

Scott P R, Hollins T W and Muir P, 1975. Pathogenicity of *Cercosporella herpotrichoides* to wheat, barley, oats and rye. Transactions of the British Mycological Society 65, 529-538.

Scott PR, Hollins TW, 1974. Effects of eyespot on the yield of winter wheat. *Annals of Applied Biology* 78, 269-279.

Scott PR, Hollins TW, 1978. Prediction of yield loss due to eyespot in winter wheat. *Plant Pathology* 27, 125-131.

Stern V M, Smith R M, van den Bosch R and Hagen K. S. 1959. The integrated control concept. *Hilgardia* 29: 81–99.

Sutherland K G, Oxley S J P, 1993. Effect of GS 31 fungicide sprays on yield benefit and disease control in winter wheat. Proceedings Crop Protection in Northern Britain 1993, 115-120.

Swets J A 1988. Measuring the accuracy of diagnostic systems. *Science* 240: 1285−1293.

Turner AS, Nicholson P, Edwards SG, Bateman GL, Morgan LW, Todd LD, Parry DW, Marshall J, Nutall M, 2001. Evaluation of diagnostic and quantitative PCR for the identification and severity assessment of eyespot and sharp eyespot in winter wheat. *Plant Pathology* 50, 463-469.

Walsh K, Korimbocus J, Boonham N, Jennings P, Hims M, 2005. Using Real-time PCR to discriminate and quantify the Closely Related Wheat Pathogens *Oculimacula yallundae* and *Oculimacula acuformis*. *Journal of Phytopathology* 153, 715-721.

Wan AM, Bock CH, Fitt BDL, Harvey JL, Jenkyn JF, 2005. Development of *Oculimacula yallundae* and *O. acuformis* (eyespot) on leaf sheaths of winter wheat in the UK in relation to thermal time. *Plant Pathology* 54, 144-155.

West SJE, Booth GM, Beck JJ, Etienne L, 1998. A survey of *Tapesia yallundae* and *Tapesia acuformis* in UK winter wheat crops using a polymerase chain reaction diagnostic assay. In: *Proceedings of the Brighton Crop Protection Conference*. British Crop Protection Council, Farnham, UK, pp. 1029-1034.

Zadoks JC, Chang TT, Konzak C.F, 1974. A decimal code for the growth stages of cereals. *Weed Research* 14, 415-421.

Zagonel J, Venancio WS, Kunz RP, 2002. Effect of growth regulator on wheat crop under different nitrogen rates and plant densities. *Planta Daninha* 20,147-476.

Van den Ende J, Bisoffi Z, Van Puymbroek H, Van Gompel A, Derese, A, Lynen, L, Moreira J and Janssen P A J, 2007. Bridging the gap between clinical practice and diagnostic clinical epidemiology: pilot experiences with a didactic model based on a logarithmic scale. *Journal of Evaluation in Clinical Practice* 13: 374–380.

Van Der Plank JE, 1963. Plant diseases: epidemics and control. Academic Press, New York.

APPENDIX FIELD SITES USED IN DATA SET

Table 26. Sites used for fungicide trials, and recorded agronomic details

Year	Trial code	County	Region N/E/W	Tillage	Actual sow date	Variety	Soil type	Rotation
2004	WW04-068	Suffolk	East	Ploughed	15/09/2003	Consort	Sandy clay loam	1st WW
2004	LO15r	Shropshire	West	Ploughed	03/10/2003	Einstein	Sandy loam	1st WW
2004	861	Lothians	North	Min-till	10/09/2003	Riband	Clay loam	3rd WW
2005	WW05-019	Yorkshire	East	Mulch Tillage	10/09/2004	Robigus	Silt clay loam	1st WW
2005	WW05-019	Kent	East	Non-inversion	15/09/2004	Robigus	Loam	1st WW
2005	WW05-019	Suffolk	East	Ploughed	28/09/2004	Robigus	Sandy clay loam	1st WW
2005	WW05-093	Yorkshire	East	Mulch Tillage	08/09/2004	Robigus	Silt clay loam	1st WW
2005	WW05-093	Essex	East	Ploughed	01/10/2004	Einstein	Silt	2nd WW
2005	WW05-101	Norfolk	East	Ploughed	15/09/2004	Robigus	Sandy	1st WW
2005	BT05-247	Northumberland	North	Ploughed	29/09/2004	Robigus	Clay loam	1st WW
2005	MO17r	Shropshire	West	Ploughed	05/10/2004	Gladiator	Sandy loam	1st WW
2005	961	Lothians	North	Min-till	15/09/2004	Consort	Clay loam	2nd WW
2005	962	Lothians	North	Min-till	15/09/2004	Consort	Clay loam	2nd WW
2005	984	Perthshire	North	Min-till	28/09/2005	Einstein	Silt clay loam	1st WW
2006	WW06-104	Yorkshire	East	Mulch Tillage	08/09/2005	Solstice	Silt clay loam	1st WW
2006	WW06-019	Yorkshire	East	Mulch Tillage	08/09/2005	Robigus	Silt clay loam	1st WW
2006	WW06-019	Norfolk	East	Ploughed	12/09/2005	Robigus	Silt clay loam	1st WW
2006	BT06-247	Northumberland	North	Ploughed	28/09/2005	Robigus	Clay loam	1st WW
2006	NO16r	Shropshire	West	Ploughed	19/09/2005	Robigus	Sandy loam	1st WW
2006	NO25	Shropshire	West	Ploughed	19/09/2005	Robigus	Sandy loam	1st WW
2006	1051	Lothians	North	Min-till	12/09/2005	Consort	Clay loam	2nd WW
2006	1053	Lothians	North	Min-till	12/09/2005	Consort	Clay loam	2nd WW
2007	WW07-116	Yorkshire	East	Mulch Tillage	27/09/2006	Robigus	Silt clay loam	1st WW
2007	PO15	Shropshire	West	Ploughed	19/09/2006	Alchemy	Sandy loam	2nd WW
2007	PO14	Shropshire	West	Ploughed	02/10/2006	Robigus	Sandy loam	1st WW
2007	PO13	Shropshire	West	Ploughed	19/09/2006	Alchemy	Sandy loam	2nd WW
2007	1132	Lothians	North	Min-till	05/09/2006	Consort	Clay loam	1st WW
2007	1133	Perthshire	North	ploughed	05/09/2006	Einstein	Silt clay loam	1st WW
2007	1134	Lothians	North	Min-till	05/09/2006	Consort	Clay loam	2nd WW

Year	Trial code	County	Region N/E/W	Tillage	Actual sow date	Variety	Soil type	Rotation
2008	WW08-039	Yorkshire	East	Mulch tillage	12/09/2007	Robigus	Silt clay loam	1st WW
2008	WW08-039	Bedfordshire	East	Non inversion	19/09/2007	Robigus	Clay loam	1st WW
2008	WW08-039	Hampshire	West	Ploughed	18/09/2007	Oakley	ZCL over chalk	1st WW
2008	RO26	Shropshire	West	Ploughed	18/10/2007	Timber	Sandy loam	2nd WW
2008	1230	Perthshire	North	Ploughed	29/09/2007	Duxford	Silt clay loam	1st WW
2008	1231	Lothians	North	Ploughed	30/08/2007	Consort	Clay loam	2nd WW
2008	1232	Lothians	North	Ploughed	30/08/2007	Consort	Clay loam	2nd WW
2009	N09r	Leicestershire	West	Min-till	01/10/2008	Robigus	Clay loam	2nd WW
2009	WW09-051	Yorkshire	East	Mulch tillage	16/09/2008	Robigus	Silt clay loam	1st WW
2009	WW09-051	Essex	East	Non inversion	03/10/2008	Robigus	Clay loam	1st WW
2009	WW09-059	Yorkshire	East	Mulch Tillage	16/09/2008	Robigus	Silt clay loam	1st WW
2009	1308	Dundee	North	Ploughed	08/10/2008	Duxford	Silt clay loam	1st WW
2009	1309	Lothians	North	Min-till	15/09/2008	Consort	Clay loam	2nd WW
2009	1310	Lothians	North	Min-till	15/09/2008	Consort	Clay loam	2nd WW
2009	1311	Lothians	North	Min-till	15/09/2008	Consort	Clay loam	2nd WW
2010	O10p	Leicestershire	West	Ploughed	27/10/2009	Panorama	Clay loam	2nd WW
2010	WW10-051	Yorkshire	East	Mulch tillage	10/09/2009	Robigus	Silt clay loam	1st WW
2010	WW10-051	Bedfordshire	East	Non inversion	13/10/2009	Robigus	Clay loam	1st WW
2010	WW10-094	Yorkshire	East	Mulch Tillage	25/09/2009	Cordial	Silt clay loam	1st WW
2010	1437	Lothians	North	Min-till	20/09/2009	Consort	Clay loam	2nd WW
2010	1440	Lothians	North	Min-till	20/09/2009	Consort	Clay loam	2nd WW