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The development of a risk assessment method to identify wheat crops at risk from eyespot

by

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TABLE OF CONTENTS

1. ABSRACT

The aim of the project was to develop a risk algorithm allowing growers to accurately determine the need for eyespot treatment in their wheat crop. Over three seasons, commercial crops and detailed fungicide trials were evaluated to test the influence of agronomic factors on eyespot development and to judge the cost effectiveness of treatment. Many factors affected disease outcome. Soil type, sowing date, previous crop, presence of eyespot at stem extension, spring rainfall and tillage were identified as being significant influences, independent of other factors. For development of the risk algorithm, an additive measure of risk was adopted to form a single explanatory variable, incorporating elements of the individual risk factors identified as useful predictors of the probability of eyespot, scaled according to their contribution to overall risk. To facilitate calculation by users, these values were scaled as follows:

Factor	**Level**	**Risk points**
Sowing date	≤ 6 October	0
	> 6 October	5
Eyespot GS 31-32	≤ 7%	0
	> 7%	10
Rain (mm) in March / April / May	≤ 170 mm	0
	> 170 mm	5
Tillage	Minimum till	0
	Plough	10
Soil type	Light	0
	Medium	1
	Heavy	5
Previous crop	Non-host	0
	Other cereal	10
	Wheat	15

The maximum risk score a crop could be assigned was 50. Two treatment thresholds were set, a risk-sensitive threshold and a risk-tolerant threshold. The risk-sensitive threshold treatment was set at 20 points, predicting a final disease incidence of 30%. Using the risk-tolerant threshold, treatment would not be triggered until 29 risk points were accumulated which would predict a final disease incidence of 45%. This approach allows flexibility on the part of the user in determining what level of risk is acceptable to them.

2. SUMMARY

The aim of the project was to develop a risk algorithm allowing growers to accurately predict the need for eyespot treatment in their wheat crop. The relative influences of factors such as previous cropping, cultivation method, drilling date, varietal resistance, straw removal method, level of eyespot in the crop at stem extension, region, soil type and weather were investigated. Survey sites in commercial crops and detailed fungicide trials were evaluated to test the influence of these factors on eyespot development and to judge the cost effectiveness of treatment. Samples were collected to assess disease progress visually and by analysis of eyespot DNA over three seasons, starting in the autumn of 2000 and continuing until the 2003 harvest.

Analysis of the data set showed that many agronomic factors affected disease outcome. Using logistic regression the probability of eyespot was modelled as a linear combination of explanatory variables, from which the probability of a treatable level of eyespot developing was calculated. Soil type, sowing date, presence of eyespot at stem extension, spring rainfall and tillage were selected as being significant influences, independent of other factors. These were attributed risk weightings in the model. For development of the risk algorithm, an additive measure of risk was adopted to form a single explanatory variable (the Risk Score), incorporating elements of the individual risk factors identified as useful predictors of the probability of eyespot, scaled according to their contribution to overall risk.

A useful predictor is one that can discriminate between crops that will need treatment for eyespot and those that will not, on the basis of information obtained at a sufficiently early stage of the growing season to enable action to be taken. No predictor can be entirely perfect as not all the variability in crops which causes them to fall into a 'treat' or 'don't treat category' can be explained by a statistical model. By setting a threshold 'risk score', imperfect discrimination means that some crops that really need treatment will fall below this threshold and some that really do not need treatment will fall above this threshold. Since different users may respond differently to these two different types of error, users should ideally be able to modify the threshold risk score to suit their own attitude to risk. It follows that predictors, such as the one described here are best considered as guidelines to be used as part of the crop protection decision making process, rather than rules that are meant to be followed without wider consideration of the circumstances in which the decision is made.

Factor	Level	Risk points
Sowing date	≤ 6 October	0
	> 6 October	5
Eyespot GS 31-32	≤ 7%	0
	> 7%	10
Rain (mm) in March / April / May	≤ 170 mm	0
	> 170 mm	5
Tillage	Minimum till	0
	Plough	10
Soil type*	Light	0
	Medium	1
	Heavy	5
Previous crop	Non-host	0
	Other cereal	10
	Wheat	15

1. Risk-sensitive user - treatment triggered at a Risk Score of 20
2. Risk-tolerant user - treatment triggered at a Risk Score of 29

*There was an increased risk of eyespot with brash or limestone soils but as this effect was identified from a limited number of sites it could not be included in the model. While this effect needs further investigation, for practical purposes adding a further 5 risk points would reflect the increased risk observed.

The maximum number of accumulated risk points a crop could be assigned was 50. Two treatment thresholds were set – a risk-sensitive threshold and a risk-tolerant threshold. This allows flexibility on the part of the user in determining what level of risk is acceptable to them. The risk-sensitive threshold was set using an external reference for yield loss from eyespot such that a final incidence of 30% eyespot at the end of the season would be worthwhile to treat. Using the data generated in the project the disease loss from eyespot was less than would be predicted by the external reference and the risk-tolerant threshold was set at this level whereby a predicted final incidence of 45% would trigger treatment. These thresholds were used to model the data and assign the risk points.

Receiver Operating Characteristic (ROC) curves provide a methodology for validation of guidelines for diagnostic decision-making when a binary (yes/no) decision is called for. The ROC curves generated in this project provide initial validation of the model in that they show the characteristic plot of a useful predictor. They also provide an opportunity for flexible implementation of decision guidelines as the ROC curves show the balance of risks associated with each threshold Risk Score. Thus, a very risk-sensitive user could choose to operate at a lower threshold Risk Score than 20. Similarly, a very risk-tolerant user could choose to operate at a higher threshold Risk Score than 29.

As well as generating a risk algorithm to allow growers to predict the need for treatment, the work also provided useful insights on the status of the eyespot population and responses to fungicides in trials. The data generated in this project confirmed the poor capability of eyespot levels at stem extension in predicting the level of eyespot that would develop in the crop.

PCR pathotyping showed that the majority of crops in the period of the project had a mixed infection of *Oculimacula acuformis* (R type) and *O. yallundae* (W type). R and W types were present in almost equal proportions which represent an increase in W type over the previous few seasons. Cyprodinil was used as a standard in all trials, and reduced eyespot levels. Prochloraz was also used in some trials. Effective control was also seen following prochloraz treatment which may reflect the higher levels of W type eyespot seen as this fungicide is known to control W type more effectively than it controls the R type. The use of azoxystrobin in the trials gave a consistent increase in eyespot levels where it was applied at stem extension. In one season this was related to a decrease in sharp eyespot levels although there was no direct correlation between levels of the two diseases in the data set as a whole.

Spore trapping revealed the presence of airborne spores of *O. acuformis*. This confirmed that there is a risk from surrounding crops even in true first wheats and may be one reason why the influence of previous cropping was relatively small.

It remains to validate the model in a commercial context but the model developed should allow growers to determine the need for treatment in their crops.

TECHNICAL DETAIL

3. INTRODUCTION

A complex of diseases infects 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 know 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. In severe infections the stem becomes brittle and can lead to lodging. Stiffer strawed varieties and improved use of growth regulators mean that lodging is now much less common. In the early stages of infection symptoms are much less clear and may appear as just small, honey brown smudges on the outer leaf sheaths. Symptoms are often confounded by the presence of other stem base diseases like *Fusarium* spp. and sharp eyespot (*Rhizoctonia cerealis*).

Early field work on eyespot control was carried out on the W type (*O. yallundae*) which predominated at the time. R type had become the dominant species in the UK by the mid 1980s (King & Griffin, 1985; Nicholson and Turner, 2000). There are differences in the infection processes for the two eyespot species. The R type has a more random and slower initial phase of growth than the W type that grows faster after germination (Daniels, 1993 (a)). The R type will invade all cell parts after it has penetrated the host whereas the W type will only infect the cell wall. After the formation of infection plaques R type plaques are more compact and symmetrical in comparison to the W type, probably due to its slower growth. R type isolates develop more slowly on leaf sheaths and on stems than W type isolates (Goulds & Fitt, 1990). They therefore are less likely to show visual browning and lesions early in the season when compared to the W type.

Yield losses from eyespot

The yield loss associated with eyespot infection and its impact on crop lodging is unclear. Sutherland & 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. Scott & Hollins (1978) also showed a relationship between eyespot and yield, but reported that the correlation between yield loss and lodging was stronger. The yield loss model by Scott and Hollins (1978) is 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 & Oxley, 1996).

The work of Scott and Hollins (1978) related to a period when W type eyespot was prevalent. It has been hypothesised that because the R type tends to infect later it is not damaging to yield but the work of Burnett and Oxley (1996) related to a site that was predominantly R type. Work at Harper Adams from 2000-2003 (Ray, *pers. com*.) 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 GS 69.

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), often applied as a split treatment. Previous HGCA funded work has identified prochloraz and cyprodinil as the two most effective fungicides for control of common eyespot and resultant yield benefit.

Work carried out for HGCA Project Report 150 showed that both fungicides were more effective at controlling W type than the R type. The greater efficacy of the two fungicides against the W type in that study concurs with reports in the literature. Prochloraz has been reported to control the W type better than the R type (Bateman *et al*., 1986) and cyprodinil showed better control of the W type in work carried out in France (Migeon *et al*., 1995).

HGCA Project Report 150 (Burnett *et al*., 1997) reports that control following treatment with prochloraz or cyprodinil was temporary. Populations of both R and W type eyespot recovered following treatment, so that the key to effectively reducing the degree of visual symptoms and the damage to the plant at the end of the season, was timing the fungicide application to achieve the longest respite from the disease possible. The optimum timing differed for prochloraz and cyprodinil. Prochloraz had to be applied early in the season, during tillering, for maximum effect on eyespot levels. Cyprodinil as more effective if applied after the start of stem extension. Spraying outside the optimum window allowed the eyespot populations to recover following treatment even if initial reductions in eyespot were achieved. Prochloraz applied too late did not reduce the eyespot population by enough for the reduction to be maintained until the end of the season. In contrast cyprodinil applied too early achieved an initial reduction that was not be maintained until the end of the season. Both active ingredients reduced W type eyespot levels. Prochloraz did reduce R type eyespot levels following spaying but cyprodinil gave the more persistent reduction of the R type.

Several other fungicides also claim activity against eyespot. The use of flusilazole, once widely used on wheat for eyespot control, was superseded by prochloraz (Burnett *et al*., 1997). Krexoxim-methyl plus epoxiconazole (as in the product Landmark) has a label claim for a reduction in eyespot rather than control (UK Pesticide Guide 2004). Two new products are at or approaching market. Flexity TP (http://www.pesticides.gov.uk/PSD_Databases/products/bbcrop-ap.cfm) with the active ingredients metrafenone and fenpropimorph was approved for the 2004 season and will reduce eyespot levels if applied at stem extension. Prothioconazole will be launched in 2005 and will also have a label claim for eyespot control.

Sharp eyespot

Sharp eyespot is caused by the soil borne fungus *Rhizoctonia solani*. The fact that it is ubiquitous in soils and also has a very wide host range means that there is no form of rotational control. All cereal crops can be affected, but as with other stem base diseases spring crops tend not to be severely affected. Winter wheat is the most susceptible of the cereals and there is no form of varietal resistance. The disease tends to be favoured by cool, dry conditions and therefore some fields are more prone to the disease than others.

Sharp eyespot causes symptoms very similar to those of common eyespot. The disease infects through outer leaf sheaths and causes eye-like lesions that have a much more defined edge and paler centre than those of common eyespot. Early in the season the lesions may have a more shredded appearance on the leaf sheaths than common eyespot. Mature lesions on the stem with sharp eyespot often contain a purplish

mycelial growth that can be scraped off and later in the season flat sclerotia or resting bodies forms against the stem and between leaf sheaths. Lesions have a slightly oblique shape are often seen as multiple lesions extending far up the stem. As with the other stem base diseases, sharp eyespot reduces uptake through the stem and as a consequence can cause shrivelled grains, reduced yields and whiteheads as well as weakening the stem so that lodging is more likely. It is generally perceived to be less damaging than common eyespot in terms of yield losses.

Burnett (1999) found a negative correlation between sharp eyespot and eyespot incidence, and found evidence that the fungicide azoxystrobin (as in the product Amistar) reduced sharp eyespot levels in the field. This raises the possibility that fungicides applied for foliar disease control may alter the balance of pathogens present on the stem base. Asoxystrobin has a label claim for the root disease take-all if applied at stem extension and this could potentially make eyespot problems more severe by removing the competition effect exerted by sharp eyespot. Competition effects between plant pathogens have been documented by several researchers (McRoberts *et al.,* 2003).

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).

This threshold was developed when W type of eyespot predominated whereas the R type is now more common (King and Griffin, 1985; Nicholson and Turner, 2000). The fungicides most commonly used on

wheat over the last 20 years were members of the DMI group which act differentially on the two types, and are far more effective in controlling the W type. This may be one reason why the R type now predominates throughout the UK. The R type often infects later and then increases fast which may make it less suitable for meeting the threshold criteria. The wheat type tends to cause more cell browning as it infects the stem and therefore may have been easier to assess as a visual threshold. HGCA Project Report 150 found that in one season there was a significant correlation between W type levels at stem extension and the final levels at the end of the season, indicating how thresholds may have been more effective when the W type was the dominant type of eyespot in the UK. In view of the changes in fungicides, in wheat cultivars and in the eyespot pathogen population itself since the currently recommended threshold was devised it clearly needed to be revised.

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 diseases 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 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 Report Nos. 150 and 200) 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.

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.

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. 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 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). There is no understanding of the mechanism for this effect which may be due to increased populations of bacterial or fungal antagonists, or to the dispersal of spores more rapidly from stubble on the surface compared to stubble ploughed down.

The range of cereal varieties has changed markedly over the past 20 years which may explain, partially, why the 20% threshold is not longer held to be useful in identifying crops at risk for eyespot. Moreover UK cereal production relies on varieties with, at best, moderate eyespot resistance. Recent 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. The influence of cultivar resistance is not currently considered by growers when assessing the likelihood of a cost effective response to the application of an eyespot specific fungicide. There are however effective resistance genes that have been identified by breeders and although not included in current commercial lines this is a potential future means of managing eyespot. The most effective resistance gene is known as Pch1 or VPM-1 resistance. A second source of resistance is known as Pch2 or Cappelle-Desprez (Dipek *et al*., 1999), and a third as Pch3 (Yildirim *et al*., 1997).

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, and 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. PCR testing of volunteer barleys from oilseed rape crops have detected severe levels of eyespot. (H. Philips, *pers.com*.). 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.

Developing a risk algorithm

A useful predictor is one that discriminates between crops that need treatment and those that do not on the basis of information that can be obtained at a sufficiently early stage of the growing season to enable action to be taken if the risk is deemed sufficiently high. At the outset, two important points should be recognized. Firstly, discrimination between crops that need treatment and those that do not need treatment, on the basis of a predictor, can never be perfect. Only a proportion of the variability in crops causing them to fall into one or other of these two categories is 'explained' by a statistical model. Added to this, uncertainty is increased because data are collected by sampling (so the 'true' status of crops is unknown), because there is no guarantee that disease assessments are free of inspection errors, and because there is a period of time between when the prediction is made and the end of the season, during which events occur that may affect the outcome. If a threshold 'risk score' is set for the predictor, imperfect discrimination means that some crops that really need treatment will fall below this threshold, and some crops that really do not need treatment will fall above this threshold. Since different users may respond differently to these two different types of error, a useful asset in a predictor is a capability for users to modify the threshold risk score to suit their own attitude to risk.

It follows, therefore, that predictors such as the one described in this project are best considered as guidelines to be used as part of the crop protection decision-making process, rather than rules that are meant to be followed without wider consideration of the circumstances in which a decision is made.

Objectives

Despite the fact that the 20% threshold is known not to work, it is still commonly used because there is no other method available to growers and advisers to allow them to predict the risk of eyespot in a crop. Factors known to influence eyespot development include previous cropping, drilling date, variety, cultivation method, plant population and weather. No work has been done to allocate a risk weighting to each of these factors to use them in predicting if an eyespot fungicide application will be cost effective. The aim of the project was to provide cereal producers with a risk algorithm for identifying which crops will give a cost effective response to an eyespot spray and should therefore be treated, and conversely

which crops are at low risk where specific eyespot fungicides are less important. The aim was that agronomic factors included in the risk algorithm would be information that growers would have readily to hand. If the approach is successful there is the scope to add to the data included in the risk algorithm. Additional data such as stem wetness, crop humidity and further information on the influence of the other stem base pathogens could be included in the future, but are too costly to investigate within the scope of this project.

i) Overall Aim

The aim of the project was to make available to all UK growers a straight forward risk algorithm where the cost benefits of spraying will be predicted so that accurate and cost effective eyespot fungicide decisions could be made

ii) Specific Objectives:

The factors that influence the development of eyespot in the crop will be assessed and a risk weighting applied to each factor. The relative importance of these factors will be assessed for different geographical area and cropping situations. The data will be utilised to produce an accurate eyespot risk forecast that will be validated using data from independent field trials and survey data.

This objective has the sub objectives of ascertaining:

- the importance of sowing date
- the importance of geographical location
- which crops act as true break crops
- the influence of surrounding grass / cereal weeds
- the influence of previous cropping in the field
- the influence of rainfall and temperature
- the influence of varietal resistance
- the importance of measured eyespot levels at stem extension
- the influence of cultivation method (ploughed versus minimal cultivation)
- the effect of removing or ploughing in straw
- the effect of controlling sharp eyespot

4. MATERIALS AND METHODS

The field work was carried out in three parts and took place over three seasons starting in the autumn of 2000.

Part A

Survey sites. Collection of plant samples from sites identified as meeting specific criteria described in the aims above. In order to determine the risk of eyespot associated with various agronomic practices the project measured the resultant eyespot infection from each.

Part B

Field trials. Detailed replicated field trials looking at the influences of fungicides, sowing date and weather on eyespot development and yield loss.

Part C

Influence of airborne spores. Investigation of the influence of surrounding crops by using bait plants and spore traps to detect the airborne phase of the fungus. The testing of samples from this last part of the work was funded and carried out by Syngenta Crop Protection Ltd.

General methodology

Part A

Survey sites

In each season 14 commercial crops were selected as survey sites in England and a further14 survey sites in Scotland were identified. Each included at least one of each agronomic characteristics shown in Table 1.

Table 1. Crop types sampled

	Potential Crop Sites	**Influence tested**
1	Early sown winter wheat after wheat or max. one year break from cereals	Sowing date
2	Late sown winter wheat after wheat or max. one year break from cereals	Sowing date

3	Winter wheat - minimal cultivation	Cultivation
4	Winter wheat - ploughed	Cultivation
5	Winter wheat with eyespot resistance	Varietal resistance
6	Winter wheat without eyespot resistance	Varietal resistance
7	Winter wheat following grass	Previous crop
8	Winter wheat following winter oilseed rape	Previous crop
9	Winter wheat following set aside -stubble	Previous crop
10	Winter wheat following set aside - non cereal	Previous crop
11	Winter wheat following beans / peas	Previous crop
12	Winter wheat following potatoes	Previous crop

Where possible, early and late sown (1 and 2), ploughed and minimum tilled (3 and 4) were co-located on the same farm so that other differences such as geography were minimised. Sampling sites were chosen to take account of the common agricultural practices in each area and to cover a reasonable geographical spread. Minimum tillage included scratch tillage and was defined as any ploughing of less than 5 cm depth.

Sampling and assessment details.

Sampling and assessment details are shown in the named section of the materials and methods.

Collection of data from Central Science Laboratory

Data was transferred by CSL to the project data base from the annual DEFRA disease survey. This reported incidence of eyespot at the end of the season along with agronomic details as above although soil type, weather and disease incidence early in the season was not recorded in the DEFRA survey. The aim was to add this data to the database used to create the model.

Part B

Replicated field work

Ten paired field trials looking at the influence if rotation, tillage and sow date on the need for eyespot treatment were established in each season. The paired trials were established on first and second wheats at the same location. Plants were monitored visually and with DNA probes for infection. Untreated plots were sampled for PCR testing at three points in the season and both fungicide treated and untreated plots

were sampled at GS 71-85. Visual assessments were also made at four points in the season. Plants for both forms of assessment were taken by randomly sampling plants across the plots. All the stem base diseases were assessed. The design of the trials was as shown in Figures 1 and 2, with the trial design as randomised treatments within split blocks. The variety used was Consort.

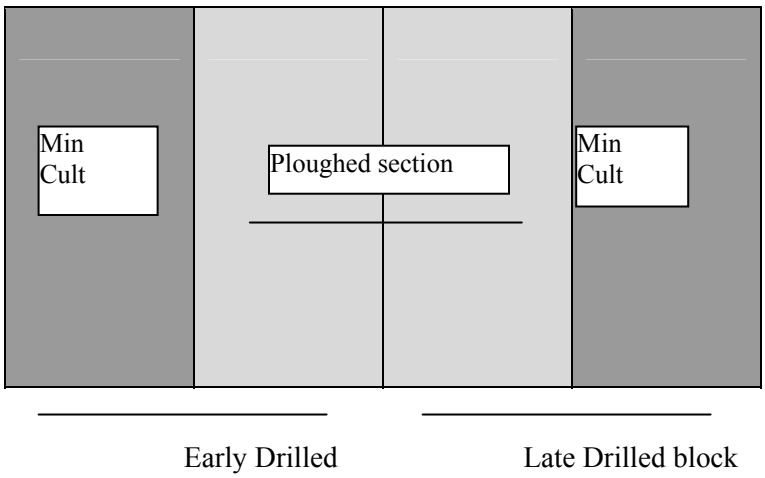
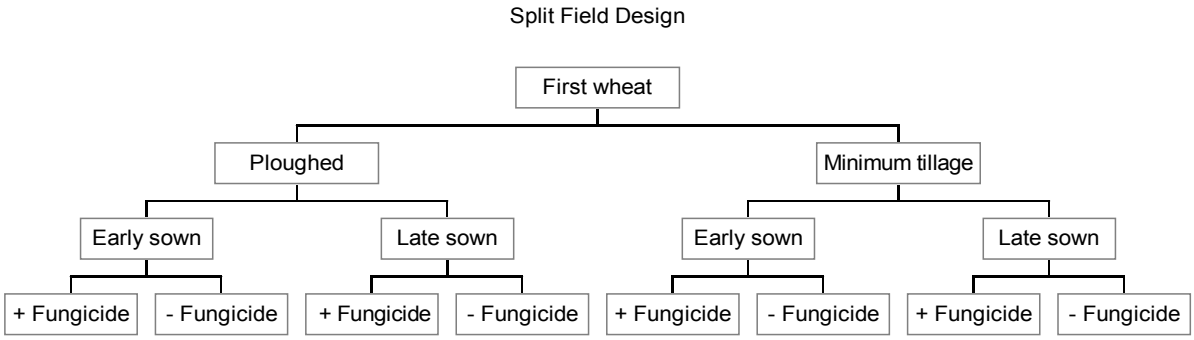


Figure 1. Tillage layout in trial fields

Within each of the 4 main sections above, there was a small plot, replicated trial. Plot size was a minimum of 2 m wide and 15 m long. There were four replicates of each treatment randomised within each block.



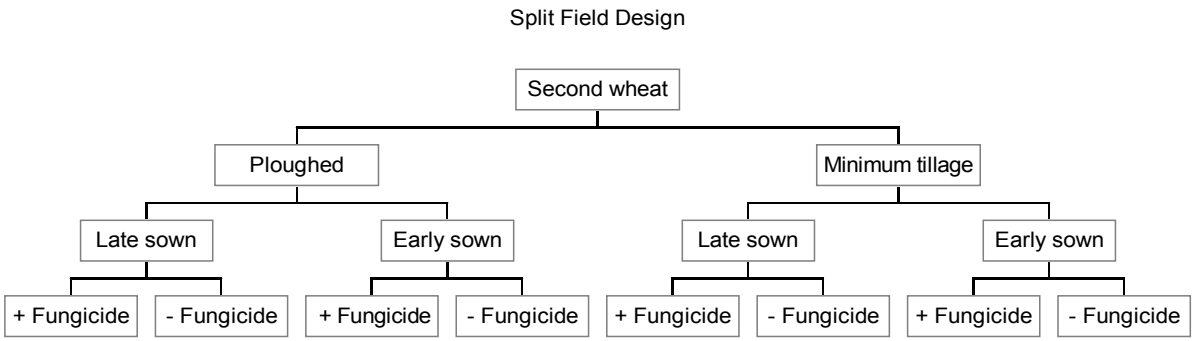


Figure 2. Design of the field trials.

Fungicide treatments

1. **+ Eyespot fungicide** i.e. 0.7 kg/ha Unix + Opus 1.0 l/ha at GS 31/32 (T1)
2. **No eyespot fungicide** i.e. Opus at 1.0 l/ha at GS 31/32 (T1)
3. In the final season (2002/2003) the + eyespot fungicide treatment was revised to 1.0 kg/ha Unix + Opus 1.0 l/ha at GS 31/32 (T1)
4. In the second season (2001/2002) an additional second eyespot fungicide was evaluated Poraz 0.6 l/ha + Opus 1.0 l/ha at GS 31/32 (T1)
5. In the second and third season (2001/2002 and 2002/2003) a sharp eyespot treatment was evaluated 0.6 l/ha azoxystrobin + Opus 1.0 l/ha (T1)

Minimum tillage was defined as the minimum required to allow adequate seed soil contact but still leaving plenty of stubble for cross contamination purposes. At the two paired Scottish sites this was direct drilling. At the eight paired English sites managed by Velcourt this was scratch tillage of no greater than 5 cm depth. Sowing dates for first wheats was aimed at the first week in September and second week in October i.e. a 6 week interval. Second wheats were drilled later, as a very early drilling date would have exacerbated take all problems. Date differences were the third week in September followed by third week in October.

Fungicides applied after T1.

GS 37-39 Twist 1.2 l/ha plus Opus 0.5 l/ha

GS 55 Folicur 0.5 l/ha

Flag leaf and heading sprays were selected to minimise the effect of foliar diseases and not to have activity against eyespot. Other inputs were in line with local practice.

Application details: Treatments were applied with a hand held CP3 Knapsack sprayer in 200 L of water per ha. Meteorological data and crop growth stage to be recorded at application timings.

Fungicide by sowing date by variety trials.

In each season, in addition to the field trials described above, a further trial was established to determine the influence of eyespot varietal resistance and sowing date on the need for eyespot treatment. There were four replicates of each treatment randomised within split blocks of different sowing date. Treatments are shown in Table 2.

Table 2. Treatments in variety by sowing date trials

Treat-ment code	Sowing date	Variety	T1 Fungicide
1	Early October	Consort	nil
2	Early October	Consort	0.8 kg/ha Unix + Opus 1.0 l/ha at GS 31/32
3	Early October	Consort	1.0 kg/ha Unix + Opus 1.0 l/ha at GS 31/32
4	Early October	Consort	1.0 l/ha Landmark
5	Early October	Consort	0.9 l/ha Stefes Poraz + Opus 1.0 l/ha
6	Early October	Eclipse	nil
7	Early October	Eclipse	0.8 kg/ha Unix + Opus 1.0 l/ha at GS 31/32
8	Early October	Eclipse	1.0 kg/ha Unix + Opus 1.0 l/ha at GS 31/32
9	Early October	Eclipse	1.0 l/ha Landmark
10	Early October	Eclipse	0.9 l/ha Stefes Poraz + Opus 1.0 l/ha
11	Early November	Consort	nil
12	Early November	Consort	0.8 kg/ha Unix + Opus 1.0 l/ha at GS 31/32
13	Early November	Consort	1.0 kg/ha Unix + Opus 1.0 l/ha at GS 31/32
14	Early November	Consort	1.0 l/ha Landmark
15	Early November	Consort	0.9 l/ha Stefes Poraz + Opus 1.0 l/ha
16	Early November	Eclipse	nil
17	Early November	Eclipse	0.8 kg/ha Unix + Opus 1.0 l/ha at GS 31/32
18	Early November	Eclipse	1.0 kg/ha Unix + Opus 1.0 l/ha at GS 31/32
19	Early November	Eclipse	1.0 l/ha Landmark
20	Early November	Eclipse	0.9 l/ha Stefes Poraz + Opus 1.0 l/ha

Fungicides applied after T1 (i.e. to flag and head).

All plots were over sprayed to minimise the effects of foliar diseases. At GS 37-39 Twist 1.2 l/ha plus Opus 0.5 l/ha was applied and on the ear at GS 55 Folicur 0.5 l/ha was applied.

Other inputs were in line with local practice.

Application details: Treatments to be applied with a hand held CP3 Knapsack sprayer in 200 L of water per ha. Meteorological data and crop growth stage to be recorded at application timings.

Fungicides used in trials

Full commercial doses for the products used were as follows:-

Active ingredient	*Product*	*Manufacturer*	*g a.i./ha*
krexoxim methyl +	Landmark	BASF	125
epoxiconazole			125
azoxystrobin	Amistar	Syngenta	250
prochloraz	Stefes Poraz	Stefes	405
cyprodinil	Unix	Syngenta	1000
epoxiconazole	Opus	BASF	125
tebuconazole	Folicur	Bayer	250
trifloxystrobin	Twist	Bayer	250

Treatments applied by CO_2 knapsack sprayer in 200 - 250 litres of water/ha at 200 -300 kPa

SAMPLING AND ASSESSMENT DETAILS FOR PARTS A AND B:

Stem base visual assessments were made as follows:-

GS 25-30 (pre T0)	untreated plots only
GS 31-32 (pre T1)	untreated plots only
GS 39-45	All plots
GS 70-85	All plots

At GS 25-30 and GS 31-32, 25 whole plants (including tillers) were sampled by digging whole 25 plants randomly throughout the plot, avoiding the outer two rows and the last 0.5m of each plot. Digging the plants with a small amount of root kept the stem base intact for inspection. At GS 39-45 and 70-85 single tillers were dug as above. At survey sites 50 plants or tillers, as above, were sampled at random in a 'W' pattern across the field.

At GS 25-30 and GS 31-32 the stem bases were assessed for eyespot which was recorded as penetrating or not penetrating the particular leaf sheaths. At these two timings Fusarium and sharp eyespot were scored as just present or absent. At GS 39-45 and GS 70-85 25 tillers eyespot was assessed and scored 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 such that lodging would readily occur. Sharp eyespot was recorded on the same scale and Fusarium was recorded as 0 = no symptoms, 1 = slight brown streaking on stem base, 2 = general browning on stem base and 3 = stem base rotted likely to cause lodging.

A percentage disease index was then calculated as follows:-

$$\frac{((\text{no. slightly infected stems})+(\text{no. moderately infected stems x 2})+(\text{no. severely infected stems x3}))\text{x4}}{3}$$

Polymerase chain reaction (PCR) methodology was used to test the amount of *O. acuformis* and *O. yallundae* that was present in each sample. PCR testing was carried out on the same sample as visual assessment was made on so plants were kept intact while assessing so that leaf layers were not lost. Plants were couriered immediately after visual assessment to CSL for PCR analysis.

Lodging did not occur in any of the trials and was therefore not assessed.

Yield from field trials was measured and corrected to 85% moisture content. Yield was not determined for Part A survey sites.

Quality measurements: Specific weight was determined from field trials. Specific weight was not determined for Part A survey sites.

Agronomic details. Inputs for all trial and survey sites were in line with local practice. Details were recorded of location, cultivar, sowing date, tillage, straw removal method, and fungicide inputs. The previous four years cropping was noted. Soil status and texture was provided. Met data was obtained from the nearest available Met Office station for the Scottish sites and, for the English sites, from the regional weather data available on line from the Met Office at :-
http://www.met-office.gov.uk/climate/uk/index.html. Temperature data was air temperature (°C).

Quantification of *Oculimacula spp.* in wheat stem base tissue by PCR

PCR diagnostics were used to study the progress of the eyespot epidemic, in conjunction with the visual assessments.

Sampling

Prior to DNA extraction, the samples were first processed to produce a representative stem-base sub-sample. A 3 cm stem-base section (measured upwards from the point at which the roots begin) was cut from the main tiller of each plant contained within the sample. All the resulting sections were combined to make one bulked stem-base sample, which was weighed and then frozen at -80°C in 50 ml Falcon tubes. A second sample of stem-base sections was also taken (from the 2nd tiller) and stored, to act as a back-up sample.

DNA Extraction

Frozen samples were kept frozen in liquid N before being ground briefly in a Waring blender. Then 1.25 volumes of 0.2 M Tris pH 8.0 was added and the sample ground further. The homogenate was decanted back into the 50 ml Falcon tube and debris centrifuged out (6,500g for 5 min). A 0.5 ml aliquot of clarified sap was taken (the remaining sap was frozen at -80°C, to act as a back-up sample) and an equal volume of buffer A (from the Promega Magnesil kit) added. This was vortexed for a few seconds to mix. DNA was then extracted using a Magnesil kit (Promega) following the manufacturer's instructions. The magnetic extractions were performed using a Kingfisher ML magnetic particle processor (Labsystems).

TaqMan testing

TaqMan® reactions were set up in duplicate in 96-well reaction plates using PCR core reagent kits (Applied-Biosystems), following the protocols supplied with the kit. For each reaction, 5 µl of DNA extract was added, giving a final volume of 25 µl. Plates were then cycled at generic system conditions (48°C/30 min, 95°C/10 min and 40 cycles of 60°C/1 min, 95°C/15 sec.) within the 7700 Sequence Detection System (Applied-Biosystems), using real time data collection. Each sample was tested using three individual assays: *Oculimacula yallundae*, *O. acuformis* and wheat PAL (the 'normaliser' assay). Included on each plate were three standard curves: one for each target, spanning four log dilutions. The standard curves were each constructed from a linearised plasmid containing the target amplicon, diluted in a background of DNA extracted using the Magnesil kit (Promega). Quantitation was achieved using 'standard curve' methodology, and results were presented as relative amounts of target for each sample

tested. Each plate contains standard controls: uninfected controls for each extraction and water controls (capped pre- and post-sample application).

Part C

Influence of airborne spores

In order to investigate the influence surrounding crops might have on the risk of eyespot spore trapping and bait plant work was carried out. Spore trapping was carried out in each year of the three year project. Work with bait plants was only carried out in the 2000/2001 season. Velcourt, Scottish Agonomy and SAC identified six sites in 2000/2001 and 2001/2002 to meet the criteria in Table 3.

Table 3. Spore sampling sites.

	Potential Crop Sites	**Influence tested**
1	Grass	Surrounding crop
2	Winter oilseed rape	Surrounding crop
3	Set aside - stubble	Surrounding crop
4	Winter barley after wheat or barley	Surrounding crop
5	Spring barley after winter barley or wheat	Surrounding crop
6	Winter wheat	Surrounding crop

Spore trapping

In 2000/2001 and 2001/2002 three Burkard cyclonic spore traps were run for 48 hrs at each of the sites identified at 4 times in the season, December, February, April and May. In the final season, 2002/2003 the spore traps were run continuously at a three sites and samples collected once per fortnight. One of the spore samplers was run in England and two in Scotland.

Spore samplers were run with the sampling vent at a minimum height of 1 meter. Once the spore sampler was in situ an Eppendorf vial was placed in the sampler. After the allotted sampling period (48 hrs or 2 weeks) this was removed and sealed and sent immediately to Syngenta, Whittlesford, Cambridge, CB2 4BR were it was analysed directly using a Fast DNA technique which is commercially confidential to Syngenta. This presents results on a scale of 0-4, with 4 representing high levels detected.

Bait Plants

Bait plants were placed at each site for two weeks at the same timings as were used for the cyclonic spore traps in 2000/2001; December, February, April and May. Twenty seedlings of the winter wheat variety Consort were grown in 10 cm plastic pots covered in a plastic humex propagator box and kept out of doors after sowing until they were placed on the sites identified. Five pots were placed at each site. When placed in the field, plants were raised up at approximately 30 cm to be out of range of soil splash. The humex box lid was removed but plants were left in the base tray. In summer the main hazard was drying out and in winter, waterlogging, so plants were checked regularly. Plants and the tray were covered with chicken wire to provide protection from grazing. After 10 days the bait plants were cut below soil level, using scissors. Any compost was washed off and samples patted dry on kitchen towel. Samples were wrapped in a paper towel and placed in an envelope and sent immediately to Syngenta, Whittlesford, Cambridge, CB2 4BR were they were analysed using PCR probes detecting R and W type DNA on a 0-4 scale. The full method is commercially confidential to Syngenta.

A summary of the sites used is shown in Table 4.

Table 4. Number of sites each year

Areas	**Part A** Number of sampling sites	**Part B** Number of replicated trials	**Part C** Number of spore trapping sites
SAC, Scotland	7	1	6
Velcourt	14	4	6
CSL	350		
Scottish Agronomy, Scotland	7	1	6
Total per year	**378**	**6**	**18**
Total whole project	**1134**	**18**	**54**

Part C samples are sent to Syngenta for PCR analysis. Samples from parts A and B were sent to CSL for PCR analysis. To compare the fully quantified results from CSL with the categorised results from Syngenta untreated samples from all trials sites used in 2003 were split and analysed by both methods.

Development of the risk algorithm

Data were available from a total of 341 untreated wheat samples (excluding the CSL data). The objective was to develop a calculator for prediction of the risk of eyespot disease in wheat. To this end, the untreated 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 (GS 70-80). The statistical method used to analyse these data was logistic regression. In logistic regression the logistic transformation of the probability of eyespot is modelled as a linear combination of the explanatory variables, from which the predicted response probabilities can be calculated (Collett, 2003). In this situation, the 'probability of eyespot' is a binary outcome, denoting the probability that the final level of disease in a particular crop will be such that treatment should be applied, or otherwise. For this analysis, eyespot was assessed on an incidence scale. Two different thresholds of final disease incidence were investigated as a basis for classifying crops retrospectively as either 'needed treatment' or 'did not need treatment', a lower value of 30% (such as might be adopted by a more risk-sensitive user) and a higher value of 45% (such as might be adopted by a more risk-tolerant user). The 'explanatory variables' are crop, pathogen and environment factors that affect this outcome. Thus, the process of developing a risk algorithm for eyespot involves investigation of the extent to which various factors - collectively referred to as risk factors - contribute to the ability to discriminate between crops that need treatment and those that do not need treatment.

Risk factors

At the outset, the following candidate risk factors were considered to be possible contributors to a risk algorithm for eyespot. Risk factors were recorded either on a discrete scale (denoted D) or as variables (denoted C). They are divided, for convenience, into crop-, pathogen-, and environment-based factors.

- Crop-based factors: Sowing date (C), Varietal rating (D).
- Pathogen-based factors: Eyespot incidence at GS 25-30 (C), Eyespot incidence at GS 31-32 (C).
- Environment-based factors: Region (D), Tillage (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), Total rainfall during March/April/May (C).

Summary of the analysis

The analysis was carried out using the statistical software EGRET for Windows Version 2.0 (Cytel Software Corporation, 1999). The data set was incomplete in the sense that not all risk factors were

recorded for each crop. In fact, no single crop had all risk factors recorded. For each step in the analysis, the largest possible number of crops was used for the particular calculation in question. However, it should be noted that this means that different steps in the analysis are based on different numbers of crops.

1. The ability of each risk factor by itself to predict the need or otherwise for treatment was tested using logistic regression. Crops with missing data for a risk factor were not used in the analysis pertaining to that particular risk factor.

2. For those risk factors that appeared to have some predictive capability:

- risk factors initially recorded as variables were re-coded on simplified discrete scales;
- some risk factors initially recorded as discrete were re-coded on simplified discrete scales;
- finally, all discrete scales were organised so that the higher number(s) on the scale corresponded to a higher probability of eyespot.

3. Subsequent to steps (1) and (2), a set of discrete risk factors that appeared to have some predictive capability was assembled and a factor level summary generated. In this summary, EGRET terminology refers to crops that really did require treatment as 'cases' and crops that really did not require treatment as 'controls'.

4. The set of risk factors from (3) was analysed by a backwards elimination procedure. This is a stepwise procedure that starts with a logistic regression model based on terms for all risk factors of interest, from which terms are removed if they do not meet a pre-specified statistical significance probability (set at P=0.15 for this analysis). Crops with missing data for any of the risk factors in the starting model were not used in the analysis.

5. For the reduced set of risk factors identified in (4), a simplified risk points scale was devised which reflected the additive risk attributable to each of the identified risk factors. A 'risk points sum' was then calculated for each crop and used as an explanatory variable for logistic regression analyses in which the binary outcome variable was either the need for treatment, or otherwise, based on whether the final disease incidence was above the 30% threshold (for more risk-sensitive users) or 45% (for more risk-tolerant users).

5. RESULTS

Model Development

Incidence of disease is a more accurate measure in model development than is a more subjective index. An index is conventionally used to assess eyespot but the correlation in the data between index and incidence was very strong (r^2 – 0.968, P<0.001), as shown in Figure 3.

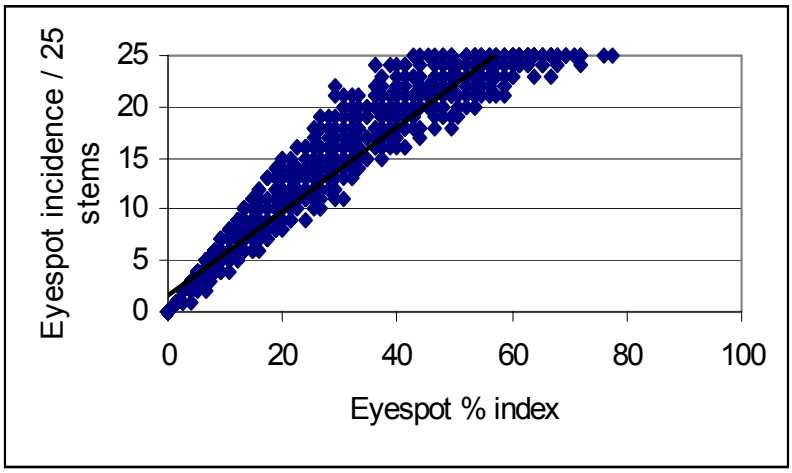


Figure 3: Relationship between index and incidence eyespot score

Logistic regression analysis

1. Individual testing of risk factors

This analysis was carried out twice using, in turn, the two different thresholds of final disease incidence that were investigated as a basis for classifying crops, 30% and 45%. In both cases, the following seven risk factors were found to have no useful predictive capability for probability of eyespot when tested by logistic regressions with individual risk factors as the single explanatory variable, and were eliminated from further consideration: Varietal rating, Eyespot incidence at GS 25-30, Soil P, Soil K, and Soil Mg, Total rainfall during September/October/November, Total rainfall during December/January/February.

2. Re-coding of risk factors still under consideration

Following individual testing of risk factors, twelve risk factors remained under consideration.

- Variables were re-coded on discrete scales as follows:

Sowing date (up to and including 6 October = 1, after 6 October = 2),

Eyespot incidence at GS 31-32 (≤ 7% = 1, > 7% = 2),

Soil pH (≤ 6.25 = 1, > 6.25 = 2),

Mean temperature during September/October/November (≤10.7° = 1, >10.7° = 2),

Mean temperature during December/January/February (≤3.3° = 1, >3.3° = 2),

Mean temperature during March/April/May (≤9.2° = 1, >9.2° = 2),

Total rainfall during March/April/May (≤170mm = 1, >170mm = 2).

- Discrete scales were coded (after re-coding if required) as follows:

Region (East = 1, North = 2, West = 3),

Tillage (Minimum till = 1, Ploughed = 2),

Straw (Incorporated = 1, Removed = 2),

Soil type (Light = 1, Medium = 2, Heavy = 3),

Previous crop (Non-host = 1, Other cereal = 2, Wheat = 3).

Explanation of selection of 7% incidence at GS 31/32 as presence or absence, as used in the model

The logistic regressions of probability of eyespot on Eyespot incidence at GS 31-32 (C), recorded as number diseased out of 25, gave the following results Tables 5 and 6.

Table 5. Final disease incidence threshold = 30%

	Estimate	Standard error	P-value
Constant term	-0.205	0.2061	0.32
Incidence	0.121	0.0265	< 0.001

Deviance (311 df) = 382; Likelihood Ratio Statistic (1 df) = 28.4; P-value < 0.001

Table 6. Final disease incidence threshold = 45%

	Estimate	Standard error	P-value
Constant term	-0.160	0.2015	0.42
Incidence	0.0896	0.0232	< 0.001

Deviance (311 df) = 418; Likelihood Ratio Statistic (1 df) = 16.1; P-value < 0.001

Thus, the corresponding response curves are:

$$\text{A: Probability of eyespot} = \frac{\exp(-0.205 + 0.121 \times \text{Incidence})}{1 + \exp(-0.205 + 0.121 \times \text{Incidence})}$$

and

$$\text{B: Probability of eyespot} = \frac{\exp(-0.160 + 0.0896 \times \text{Incidence})}{1 + \exp(-0.160 + 0.0896 \times \text{Incidence})}.$$

Of particular interest is the incidence at which probability of eyespot = 0.5, because this is a naturally appropriate disease incidence at which to divide the continuous incidence scale into two discrete classes. If we denote the Constant term α and the coefficient of Incidence β, this point occurs at $-\frac{\alpha}{\beta}$. Then:

A: Probability of eyespot = 0.5 when Incidence = –(–0.205/0.121) = 1.69, and
B: Probability of eyespot = 0.5 when Incidence = –(–0.160/0.0896) = 1.79.

Since 1.69/25 and 1.79/25 both represent about 7% incidence, this value was chosen to divide the continuous Eyespot incidence at GS 31-32 scale into two categories, corresponding to probability of eyespot ≤ 0.5 and probability of eyespot > 0.5.

3. Factor level summary

Table 7: Factor level summary

Factor Level	No. Controls	No. Cases	Total	% Controls	% Cases	Odds Ratio
Sowing Date						
1 ≤ 6 Oct	77	107	184	41.85	58.15	1
2 > 6 Oct	42	78	120	35.00	65.00	1.336
Total	119	185	304	39.14	60.86	
Eyespot GS 31-2						
1 –ve	22	23	45	48.99	51.11	1
2 +ve	88	180	268	32.84	67.16	1.957
Total	110	203	313	35.14	64.86	
Soil pH						
1 ≤ 6.25	45	45	90	50.00	50.00	1
2 >6.25	83	144	227	36.56	63.44	1.735
Total	128	189	317	40.38	59.62	
T°C S/O/N						
1 ≤10.7 °C	45	39	84	53.57	46.43	1
2 >10.7 °C	89	168	257	34.63	65.37	2.178
Total	134	207	341	39.30	60.70	
T°C D/J/F						
1 ≤3.3 °C	84	82	166	50.60	49.40	1
2 >3.3°C	50	125	175	28.57	71.43	2.561
Total	134	207	341	39.30	60.70	
T°C M/A/M						
1 ≤9.2 °C	31	45	76	40.79	59.21	1
2 >9.2 °C	103	162	265	38387	61.13	1.083
Total	134	207	341	39.30	60.70	
mm Rain M/A/M						
1 ≤170mm	31	45	76	40.79	59.21	1
2 >170mm	103	162	265	38387	61.13	1.385
Total	134	207	341	39.30	60.70	

Region						
1 East	70	87	157	44.59	55.41	1
2 North	28	40	68	41.18	58.82	1.149
3 West	36	80	116	31.03	68.97	1.788
Total	134	207	341	39.30	60.70	
Tillage						
1 Min till	72	75	147	48.98	51.02	1
2 Plough	62	132	194	31.96	68.04	2.044
Total	134	207	341	39.30	60.7	
Straw						
1 Incorporated	76	101	177	42.94	57.06	1
2 Removed	54	96	150	36.00	64.00	1.338
Total	130	197	327	39.76	60.24	
Soil type*						
1 Light	19	16	35	54.29	45.71	1
2 Medium	51	46	97	52.58	47.42	1.071
3 Heavy	32	42	74	43.24	56.56	1.559
Total	102	104	206	49.51	50.49	
Previous crop						
1 Non host	81	82	163	49.69	50.31	1
2 Other cereal	11	25	36	30.56	69.44	2.245
3 Wheat	40	98	138	28.99	71.01	2.420
Total	132	203	337	39.17	60.83	

*Brash or limestone soils appeared to be a factor in increased eyespot incidence but as this observation was made on relatively few crops it could not be included in the model.

The Odds Ratio can be interpreted as a measure of risk relative to the first factor listed.

4. Backwards elimination procedure

This analysis was carried out twice using, in turn, the two different thresholds of final disease incidence that were investigated as a basis for classifying crops, 30% and 45%. In both cases, the procedure eliminated the following risk factors from the analysis: Region, Straw removal, Soil pH, Mean temperature during September/October/November, Mean temperature during December/January/

February, and Mean temperature during March/April/May. The resulting analyses are shown in Tables 8 and 9.

Table 8. Backwards elimination using final disease incidence threshold = 30%

Model term	Estimate	Standard error	P-value	Odds Ratio
Constant term	-7.82	1.279	< 0.001	0.0004
Soil type = '2'	4.22	0.889	< 0.001	67.93
Soil type = '3'	3.44	0.769	< 0.001	31.03
Previous crop = '2'	3.92	0.813	< 0.001	50.61
Previous crop = '3'	2.05	0.487	< 0.001	7.76
mm Rain M/A/M = '2'	2.16	0.503	< 0.001	8.69
Eyespot GS 31-2 = '2'	1.37	0.541	0.011	3.92
Tillage = '2'	1.16	0.353	0.001	3.19
Sowing date = '2'	0.67	0.371	0.072	1.95

Table 9. Backwards elimination using final disease incidence threshold = 45%

Model term	Estimate	Standard error	P-value	Odds Ratio
Constant term	-9.46	1.538	< 0.001	0.0001
Soil type = '2'	5.02	1.244	< 0.001	150.65
Soil type = '3'	3.95	1.013	< 0.001	51.73
Previous crop = '2'	4.27	0.905	< 0.001	71.56
Previous crop = '3'	2.60	0.523	< 0.001	13.44
mm Rain M/A/M = '2'	3.29	0.611	< 0.001	26.91
Eyespot GS 31-2 = '2'	1.47	0.641	0.022	4.35
Tillage = '2'	1.16	0.346	0.001	3.20
Sowing date = '2'	0.64	0.366	0.080	1.90

The first level of each factor is set to zero and is the 'reference' level. Parameter estimates and standard errors refer to differences between the particular level and the reference level.

It is not the case that the risk factors with the highest Odds Ratios in (3) above are necessarily included in the model after invoking the backwards elimination procedure. This is because 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 'explain' the same bit of variability in probability of disease), then there is no need to include both of them when making a prediction of disease, and one is eliminated. Because this part of the analysis was carried out with a reduced data set, in which no missing data were allowed in any of the twelve risk factors used to start the analysis, some of the resulting parameter estimates are based on relatively small amounts of data. For this reason, the backwards elimination procedure is used in the selection of variables for inclusion in the risk algorithm, but the estimates used to generate risk points are calculated from the largest possible proportion of the data set, which is the factor level summary shown in (3) above. This also allows the same risk points scale to be applied whether working with a final disease incidence threshold of either 30% or of 45%.

5. Risk points

For development of the risk algorithm, an additive measure of risk (i.e., $\log_e$(Odds Ratio)) was adopted. To facilitate calculation by users, these values were scaled as follows in Table 10.

Table 10. Risk points allocated per factor.

Factor	Level	Risk points
Sowing Date	≤ 6 October	(reference)
	> 6 October	5
Eyespot GS 31-2	≤ 7%	(reference)
	> 7%	10
mm Rain M/A/M	≤ 170 mm	(reference)
	> 170 mm	5
Tillage	Minimum till	(reference)
	Plough	10
Soil type*	Light	(reference)
	Medium	1
	Heavy	5
Previous crop	Non-host	(reference)
	Other cereal	10
	Wheat	15

*For brash or limestone soils add a further 5 risk points (see Table 7)

A 'risk points sum' was then calculated for each crop. This represents a single explanatory variable, incorporating elements of the individual risk factors identified as useful predictors of the probability of

eyespot, scaled according to their contribution to overall risk. This variable, referred to as Risk Score, takes values between 1 and 50 (the maximum possible) for the crops in the data set under investigation. Logistic regression analysis of probability of eyespot on Risk Score was carried out using, in turn, each of the two different thresholds of final disease incidence were investigated as a basis for classifying crops, 30% and 45%. The resulting analyses were as shown in Tables 11 and 12.

Table 11. Logistic regression analysis of probability of eyespot on risk score for final disease incidence threshold = 30%

	Estimate	Standard error	P-value
Constant term	-1.643	0.399	< 0.001
Risk Score	0.0798	0.0138	< 0.001

Deviance (276 df) = 321.4; Likelihood Ratio Statistic (1 df) = 39.47; P-value < 0.001

Table 12. Logistic regression analysis of probability of eyespot on risk score for final disease incidence threshold = 45%

	Estimate	Standard error	P-value
Constant term	-2.730	0.439	< 0.001
Risk Score	0.0937	0.0142	< 0.001

Deviance (276 df) = 330.3; Likelihood Ratio Statistic (1 df) = 55.0; P-value < 0.001

The two logistic regression models can be plotted graphically to show the probability of eyespot as a response to Risk Score, as shown in Figure 4.

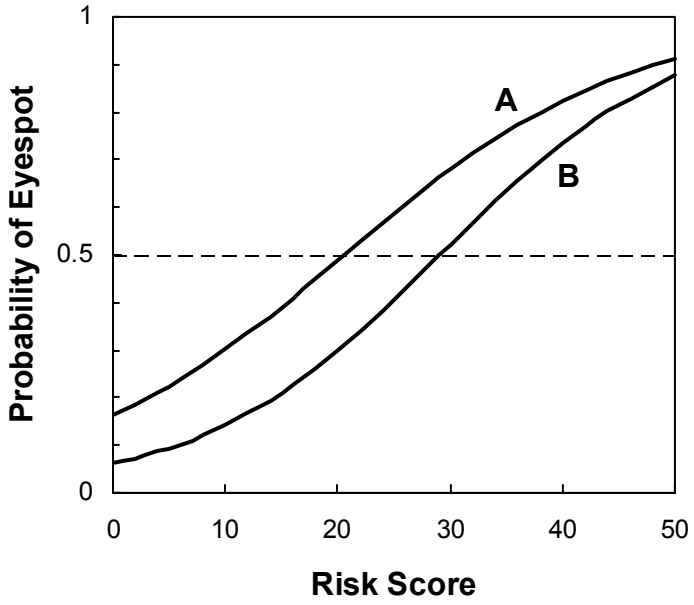


Figure 4. Probability of eyespot in response to risk score.

The above Figure shows the probability of eyespot varying with Risk Score for final disease incidence threshold = 30% (curve A) and final disease incidence threshold = 45% (curve B). Curve B shifted to the right because it corresponds to the final disease incidence threshold that might be adopted by a more risk-tolerant user. Thus, any given level of probability of eyespot (on the vertical axis) requires a larger value of Risk Score (on the horizontal axis) for a more risk-tolerant user than for a more risk-sensitive user.

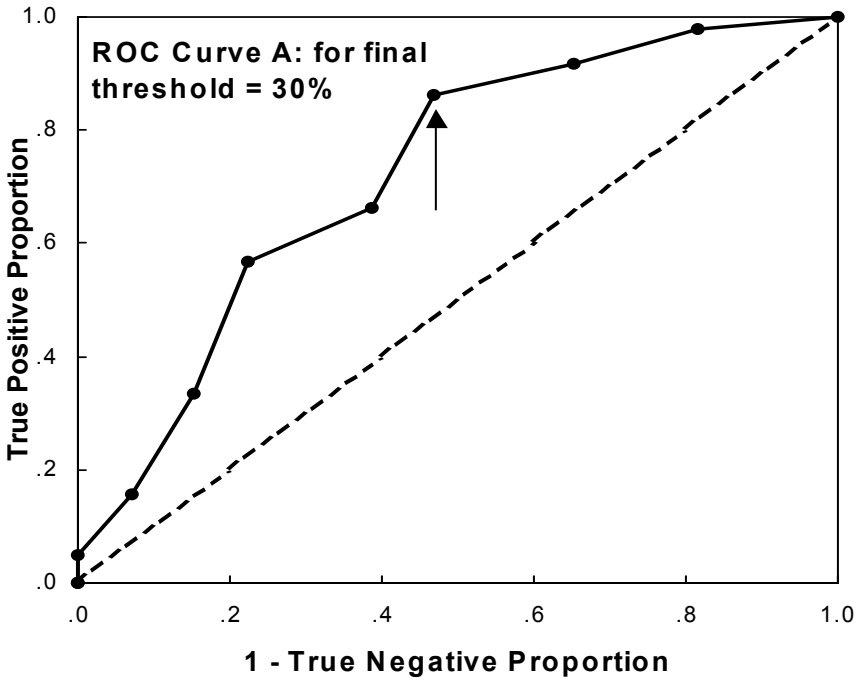
A natural decision guideline is to act if the probability of eyespot is > 0.5 (i.e. >50%) but not if the probability of eyespot is ≤ 0.5. This threshold probability is indicated in Figure 4 by a horizontal dashed line. On this basis, a more risk-sensitive user would adopt a threshold Risk Score of 20 and act if this was exceeded, while a more risk-tolerant user would adopt a threshold Risk Score of 29 and act if this was exceeded. Individual users can calculate the Risk Score for a particular crop by accumulating risk points as given in the Risk Points Table above, for the risk factors: Sowing date, Eyespot incidence at GS 31-32, Rainfall in March/April/May, Tillage method, Soil type, and Previous crop.

Receiver Operating Characteristic curves

Receiver Operating Characteristic (ROC) curves provide a methodology for validation of guidelines for diagnostic decision-making when a binary (yes/no) decision is called for. Swets *et al.* (2000) provide a good general introduction to the methodology, and applications in disease management decision-making

have been discussed by Yuen *et al.* (1996), Twengström *et al*. (1998) and Hughes *et al*. (1999). Briefly, the idea is as follows. At any given threshold Risk Score, we can identify four classes of crop. Those crops above the threshold that really did require treatment, and those at or below the threshold that really did not require treatment, have been correctly classified by the decision guideline. However, there will also be some crops above the threshold that really did not require treatment, and some at or below the threshold that really did require treatment. Such crops have been incorrectly classified by the decision guideline. We refer to crops that really did require treatment as 'cases' and crops that really did not require treatment as 'controls'. The proportion of correctly identified cases is referred to as the 'true positive proportion' (TPP, called 'sensitivity' clinical studies). The proportion of correctly identified controls is referred to as the 'true negative proportion' (TNP, called 'specificity' clinical studies). An ROC curve is a graphical plot of TPP against 1-TNP. The values of TPP and TNP required for this plot are generated by allowing the threshold Risk Score to vary over the whole range of scores (in this case, from 1 to 50). The ROC curve starts at (1,1) (i.e., in the top right-hand corner of the plot, corresponding to a the lowest threshold Risk Score) and ends at (0,0) (i.e., in the bottom left-hand corner of the plot, corresponding to a the highest threshold Risk Score). For a useful predictor, the plot is curved towards the top left-hand corner of the plot. This point (TPP=1, TNP=1) characterizes a perfect predictor. A predictor with no discriminatory capability has an ROC curve that is a straight line between (0,0) and (1,1) on the graphical plot. The area under the ROC curve is a statistic used both for the validation and comparison of predictors.

The ROC curves for both versions of the predictor, final disease incidence threshold = 30% (A: area under curve = 0.7213, standard error = 0.0303) and final disease incidence threshold = 45% (B: area under curve = 0.7399, standard error = 0.0296), are shown in Figure 5, overleaf. Both show the characteristic curve, towards the top left-hand corner of the plot, of a useful predictor. Starting at the top right-hand corner, points along the lines are marked at 0, 10, 15, 20, 25, 30, 35, 40, 45, and 50 points on the Risk Score scale. The 'no discrimination' line is shown as a dashed straight line between the bottom left-hand corner and the top right-hand corner.



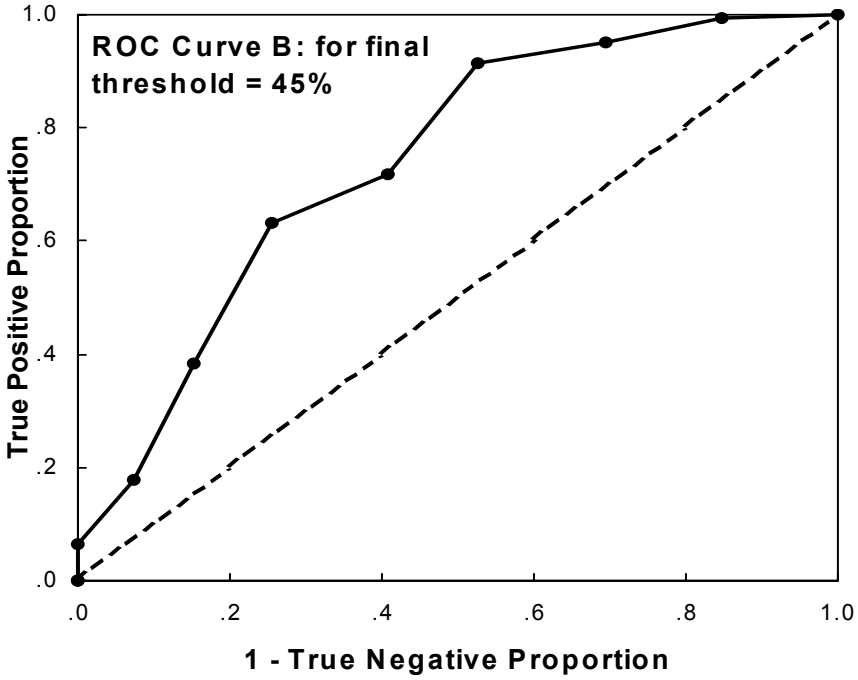


Figure 5. ROC Curves for final thresholds of 30% and 45% eyespot incidence

In the top right-hand corner of the plot, where the threshold Risk Score is zero, TPP = 1 and TNP = 0. This is the position of someone who always treats, whatever the risk. Thus, all cases are correctly identified (and treated), at the expense of incorrectly identifying all controls as cases, and unnecessarily treating them. In the bottom left-hand corner, where the threshold Risk Score is fifty, TPP = 0 and TNP = 1. This is the position of someone who never treats, whatever the risk. Thus, all controls are correctly identified (and not treated), at the expense of incorrectly identifying all cases as controls, and erroneously not treating them. For both these extreme positions, decision guidelines representing a balance of risks are irrelevant. For others, the ROC curves provide an opportunity for flexible implementation of decision guidelines. The balance of risks associated with each threshold Risk Score along the ROC curve is transparent. For example, on ROC curve A, the point indicated with an arrow corresponds to a threshold Risk Score of 20. At this point, TPP = 0.86 and TNP = 0.53. A very risk-sensitive user of ROC curve A could choose to operate at a lower threshold Risk Score than 20 (i.e., closer to the top right-hand corner of the plot). The effect of a reduction in the threshold Risk Score to 15 can be read from the ROC curve: TPP is increased to 0.92 and TNP decreased to 0.35. Thus more crops that really need treatment are correctly identified, at the expense of more crops that really do not require treatment being incorrectly identified. Conversely, note that more risk-tolerant users could choose to operate at a higher threshold Risk Score (i.e., closer to the bottom left-hand corner of the plot). The effect of an increase in the threshold Risk Score, in terms of increase in TNP and decrease in TPP, can again be read from the ROC curve.

Explanation of thresholds used in model

Two thresholds for treatment were used in the model.

1. Risk-sensitive threshold of 30% eyespot incidence at GS 70-85
2. Risk-tolerant threshold of 45% eyespot incidence at GS 70-85

The risk-sensitive threshold was determined using an externally referenced yield losses to eyespot equation (Scott and Hollins, 1978) which was used until 2002 to calculate eyespot losses in Defra survey data.

% yield loss = $0.1\ \chi_1 + 0.36\chi_2$ where χ_1 = % incidence of moderate eyespot and χ_2 = % incidence of severe eyespot. This, when meaned, gives a yield loss formula of % yield loss = 0.153χ, where χ is the incidence of disease. At a grain price of £100 per tonne, on an average 10 tonne per hectare crop then a predicted 220 kg gain from treatment is worthwhile with the assumption that the cost of an eyespot

specific fungicide at full commercial dose rate is around £22 / hectare and would reduce disease by 50% (HGCA project report 150). Using the formula a disease incidence of 30% would cause losses of 459 kg of which half would be recovered by a treatment.

The risk-tolerant threshold was set using the data generated in the project that shows a lower level of yield loss as a result of eyespot. The average realised loss, calculated from the difference between all Unix treatments and untreated was 5.145 kg per % point eyespot incidence based on 78 crops. At £100 / t it is worth treating to save 220 kg which at this rate of loss would result from a 45% incidence of eyespot at the end of the season (GS 70-85).

Field trial results

Eyespot population status

Eyespot levels varied by site and by year as shown in Table 13. Levels were highest in the 2002 season of the project and lower in the other two seasons.

Table 13. Mean eyespot % incidence at GS 70 where no eyespot fungicide applied.

Year	Eyespot % incidence at GS 70
2001	12.9
2002	62.4
2003	31.9
SED	19.14
P	<0.001

The population was predominantly mixed as shown in Tables 14 and 15. Just over 1% of samples had no detectable eyespot at GS 31-32. Over 84% of samples had a mixed population present and the number of samples where R was the dominant species only just exceeded those where W type was the dominant species at GS 31-32. By the end of the season the populations were even more evenly matched.

Table 14. The mean amount of pathogen DNA (ng/unit) in each year

Year	GS 25-30		GS 31-32		GS 39		GS 70-85	
	R type	W type	R type	W type	R type	W type	R type	W type
2001	0.57	0.57	0.18	0.52	0.52	2.89	4.2	8.83
2002	0.14	0.18	4.01	1.84	2.36	3.92	40.5	30.13
2003	0.00	0.00	0.01	0.00	0.33	3.21	6.8*	26.54
SD	0.712	0.677	21.92	5.114	2.070	9.991	19.51	36.20
P	0.006	0.005	0.488	0.135	<0.001	0.904	<0.001	0.004

*156.7 with Bankhead, Perthshire site included.

Table 15. Percentage of samples infected with R, W and mixed eyespot populations

Population	GS 31-32	GS 70-75
No eyespot	1.06	2.39
W only	11.0	7.66
R only	3.55	10.8
Mixed R dominant	45.7	39.5
Mixed W dominant	38.7	39.7

Figure 6 shows the development of the two eyespot species in untreated crop and trial plots meaned over the three seasons of the project. Both were present at approximately equal levels at stem extension.

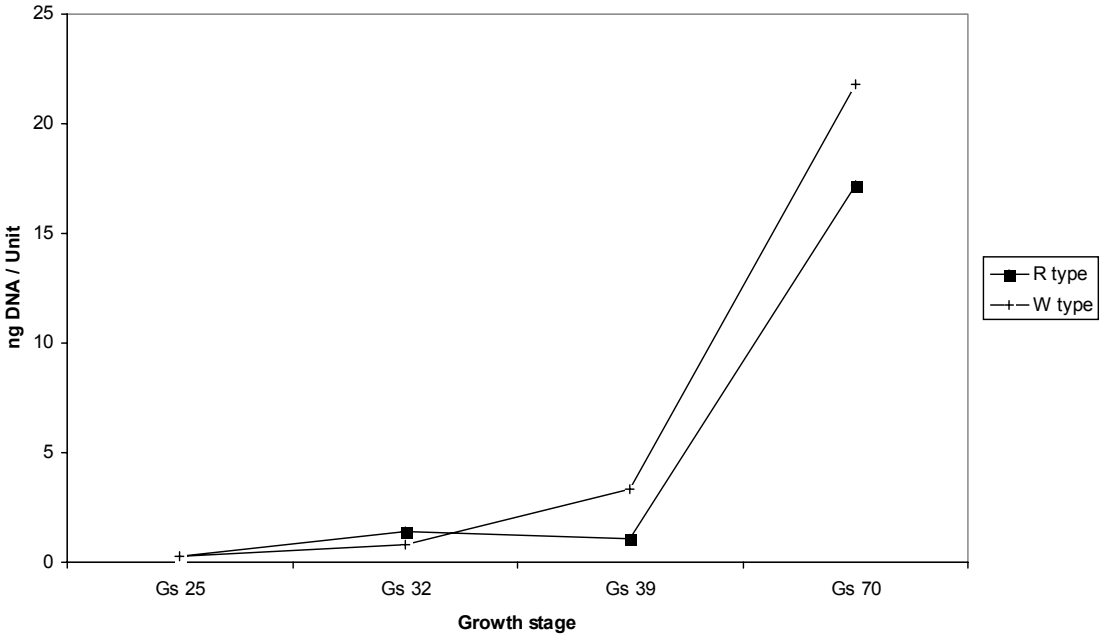


Figure 6. W and R type development through the season.

Relationship between early and late disease levels.

There was a significant correlation between eyespot levels at GS 31-32 and those assessed at the end of the season (Table 16). The regression equations was:-

Eyespot % incidence at GS 70 = 44.5 + 0.307 (eyespot % incidence at GS 31-32).

However, as seen in Figure 7 a large proportion of samples did not show high levels of eyespot early in the season but went on to develop high levels of infection.

Table 16. Correlation co-efficients between early and late disease data

Treatment	Eyespot GS 25-30	Eyespot GS 32	R type GS 32	W type GS 32
Eyespot GS 70-85	0.085	0.203	0.107	0.193
P	0.427	0.002	0.121	0.005
R type GS 70-85	-	-	-0.103	-
P			0.381	
W type GS 70-85				0.106
P				0.423

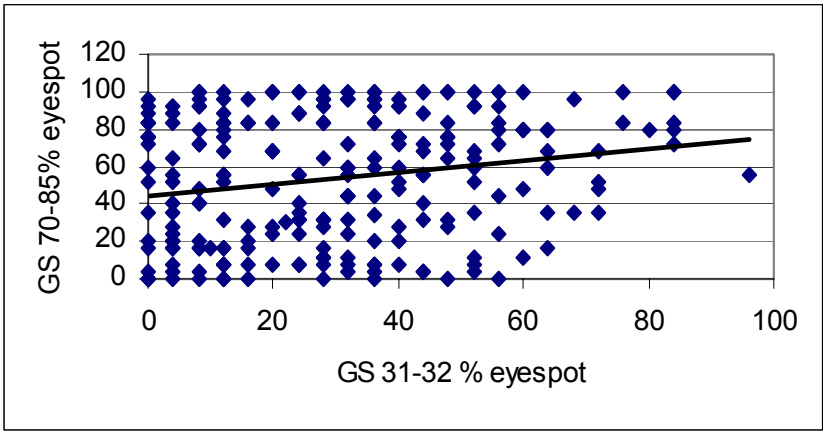


Figure 7. Scatter graph of eyespot incidence at GS 31-32 and eyespot incidence at GS 70-85.

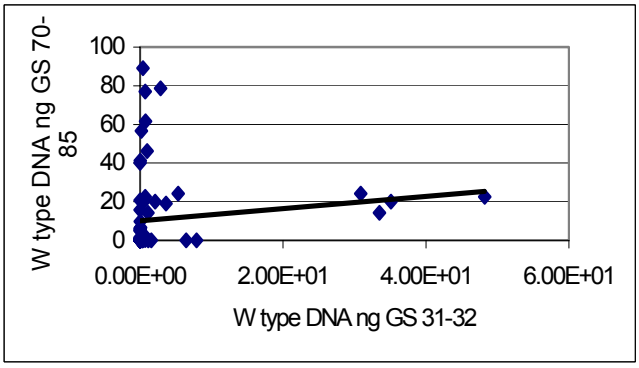


Figure 8. Scatter diagram of W type eyespot levels at stem extension and at the end of the season.

Figures 8 and 9 show the relationship between early R and W levels and late season levels. There was no significant correlation between the amount of DNA recovered at stem extension and the amount of disease that later developed for either R or W type, however the relationship appeared worst for R type (Figure 9).

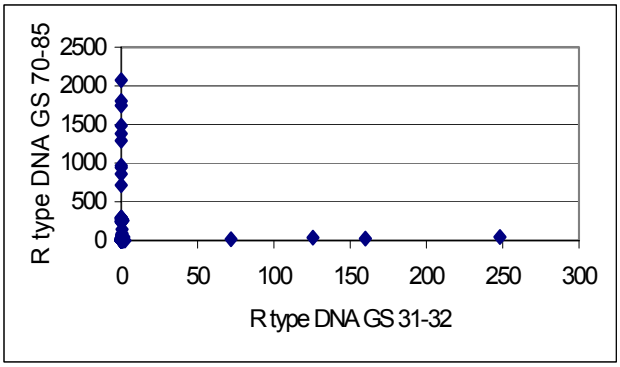


Figure 9. Scatter diagram of R type eyespot levels at stem extension and late season infection.

Fungicide trial results

Figures 10,11 and 12 show the mean eyespot levels at each trial site over the three years of the project. Levels in 2001 were very low, not exceeding a final incidence of 25%. Levels in 2002 were higher (24-96%). Levels in 2003 fell slightly (2-64%).

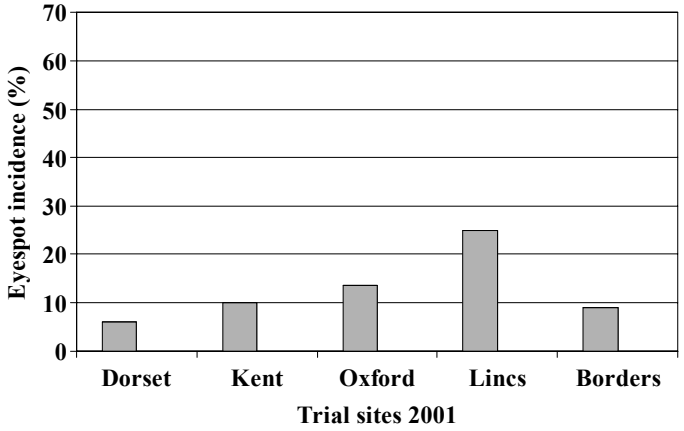


Figure 10. Eyespot levels at GS 70-85 at trial sites in 2001.

Figure 11. Eyespot levels at trial sites at GS 70-85 in 2002

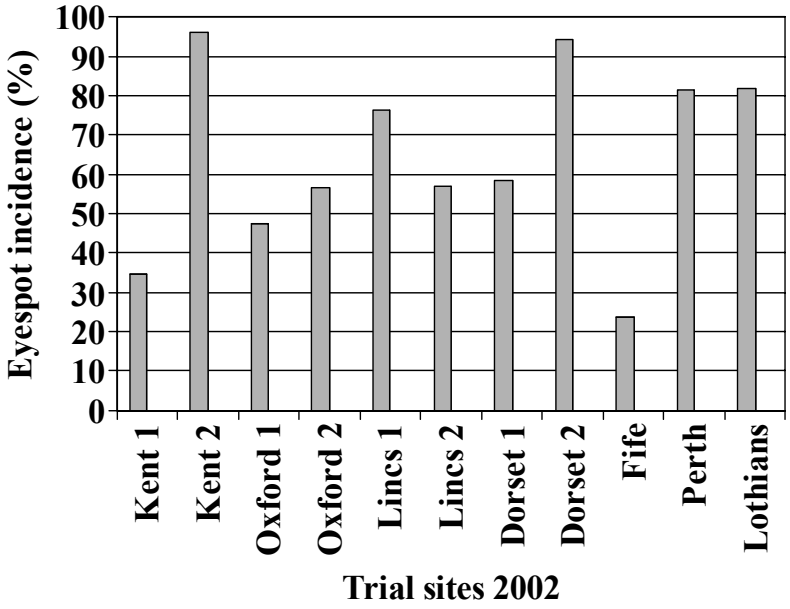
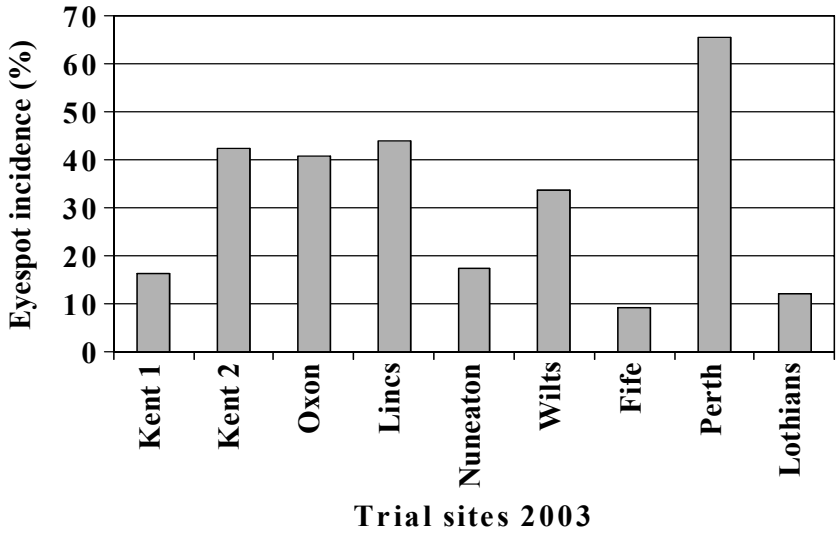


Figure 12. Eyespot levels at trial sites at GS 70 in 2003



The effect of azoxystrobin on eyespot levels

In 2002 and 2003 field trials were carried out by Velcourt to look at the effect on stem base diseases of using Amistar (a.i. azoxystrobin) at GS 31/32. In 2002 (Figure 13) eyespot levels were increased by a small but not statistically significant amount by the azoxystrobin treatment (Table 17). In 2002 there was an associated decrease in sharp eyespot levels. In 2003 there was no decrease in sharp eyespot levels and no increase in eyespot levels as assessed at the end of the season (Table 18). There was however a small increase in eyespot levels immediately after treatment at GS 39 (Figure 14), following the azoxystrobin treatment in this year although eyespot levels were still increased.

Table 14. Stem base disease levels in 2002.

Treat-ment	Eyespot %	R type ng DNA GS 39	W type ng DNA GS 39	Sharp eyespot %	Fusarium %	Yield t/ha	Specific weight kg/hl
UT	65.1	0.09	0.09	9.88	33.8	8.74	70.1
Unix 0.7	63.0	0.07	0.06	11.0	32.4	8.72	69.9
Amistar	69.1	0.09	0.25	5.84	35.5	8.82	70.3
Poraz	58.3	0.01	0.01	8.82	34.1	8.75	70.2
SED	10.44	0.049	0.119	9.675	19.05	1.903	3.041
P	0.031	0.581	0.517	0.019	0.833	0.992	0.900

Table 18. Stem base disease levels in 2003.

Treat-ment	Eyespot %	R type ng DNA GS 39	W type ng DNA GS 39	Sharp eyespot %	Fusarium %	Yield t/ha	Specific weight kg/hl
UT	31.4	0.11	4.29	21.1	36.4	7.84	71.5
Unix 1.0	23.6	0.10	8.66	22.0	36.0	7.82	71.4
Amistar	28.7	0.11	7.62	22.8	35.8	7.78	71.4
SED	13.98	0.537	13.21	10.39	12.77	1.442	1.838
P	0.030	0.994	0.289	0.738	0.973	0.979	0.955

Poraz reduced eyespot levels in 2002. Unix reduced eyespot levels in 2003 but not in 2002. Although not statistically significant in either season the trend for increased eyespot following azoxystrobin treatment seemed appeared to be associated with an increase in W type eyespot (Figure 15). There was a significant correlation between eyespot levels and sharp eyespot levels in the data set as a whole (GS 70-85 r^2= -0.152, P = 0.013 and at GS 39, r^2= -0.140, P = 0.022).

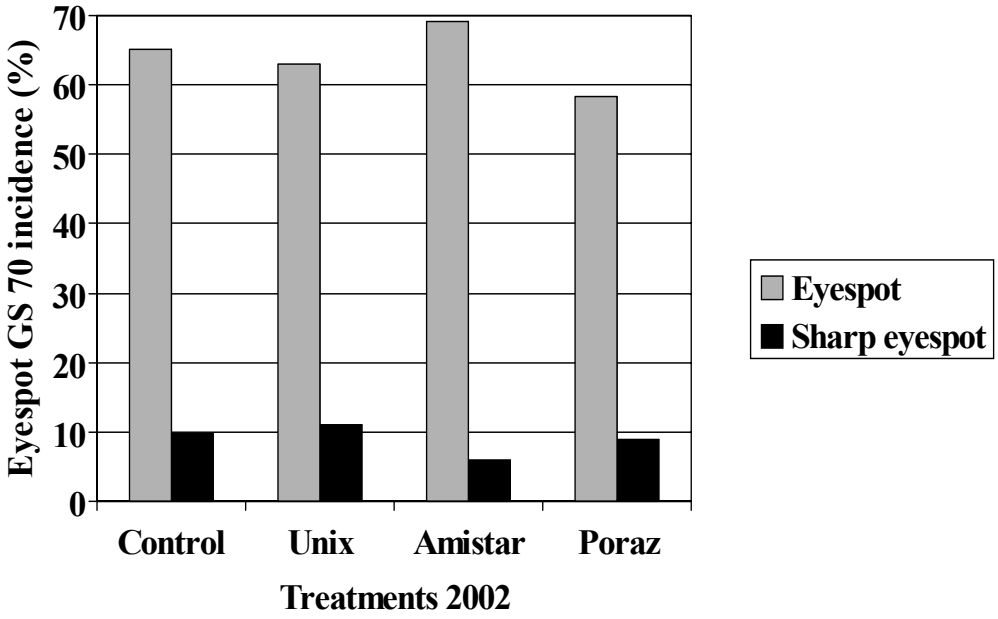


Figure 13. Stem base disease levels at GS 70 in 2002 trials

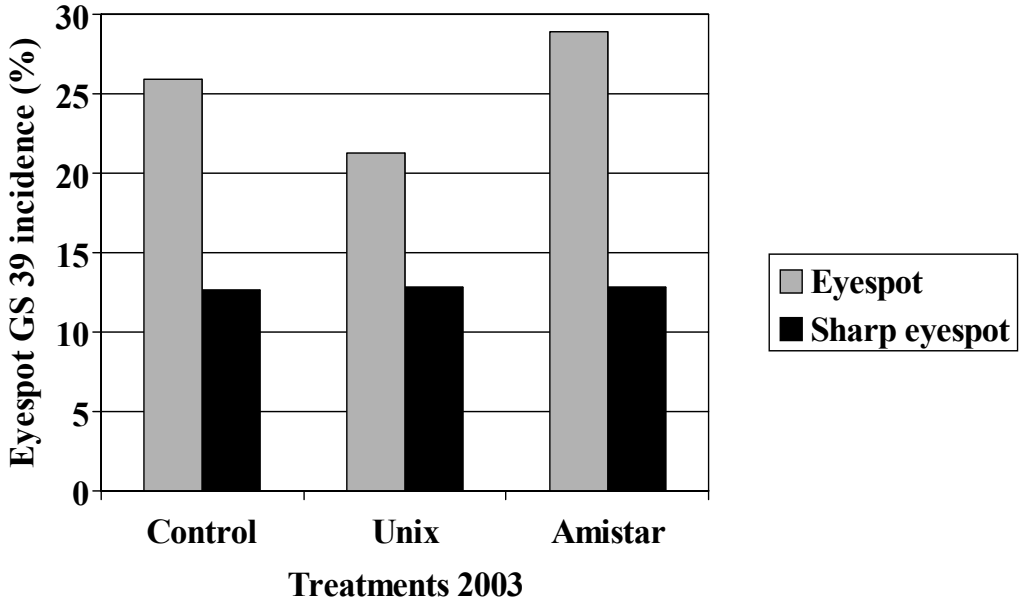


Figure 14. Stem base disease levels at GS 39 in 2003

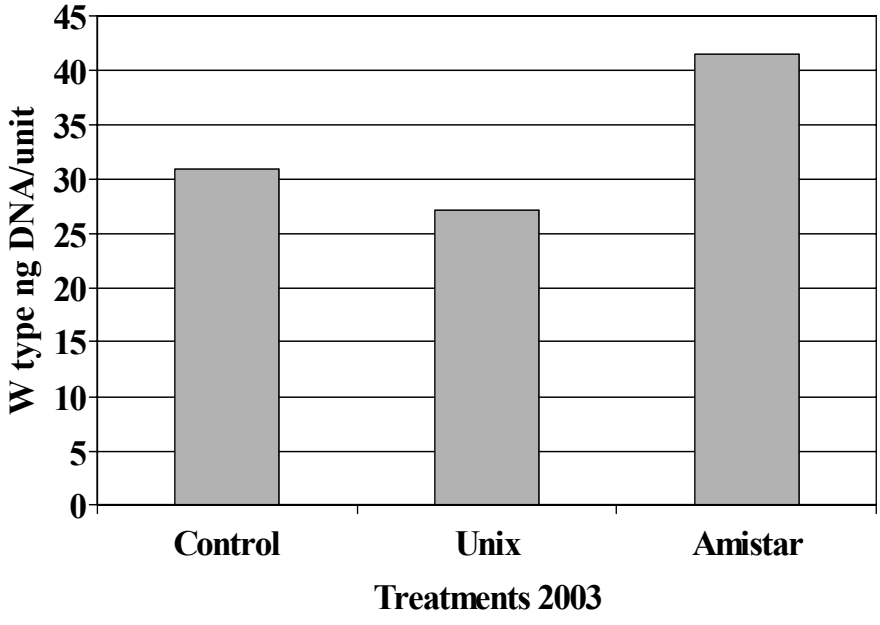


Figure 15. W type DNA levels at GS 39 in 2003.

Detailed Fungicide trials

Three fungicide trials were carried out by SAC, one in each season to determine the effects of sowing date and variety on the response to treatment. The results from the fungicide trial in 200/2001 are shown in Table 19. Eyespot levels were reduced in the variety Eclipse (Figure 16). Unix was the most effective treatment although Landmark also had an effect in the variety Eclipse but not in Consort.

Table 19. Influence of early and late sowing on eyespot and yield in 2001 SAC trial

Treatment	Eyespot % incidence	R type ng DNA	W type ng DNA	Yield
Early sown	7.50	0.57	0.05	8.8
Late sown	3.50	0.36	0.08	8.53
SED	4.307	1.102	2.655	0.822
P	0.004	0.547	0.344	0.333

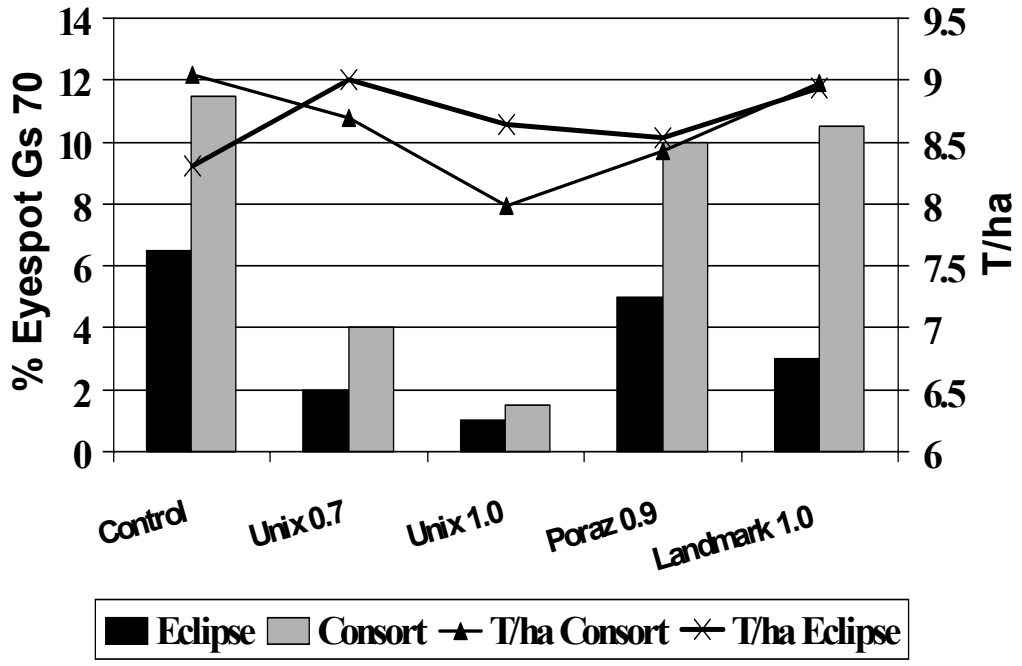


Figure 16. 2001 results for Eclipse and Consort following fungicide treatment

Eyespot levels were higher in the trial carried out in the 2002 season (Table 20)

Table 20. Influence of early and late sowing on eyespot and yield in 2002 SAC trial

Treatment	Eyespot % incidence	R type ng DNA	W type ng DNA	Yield
Early sown	70.5	0.78	0.06	7.60
Late sown	56.11	0.14	0.06	6.69
SED	15.52	0.599	0.277	0.080
P	0.004	0.001	0.993	<0.001

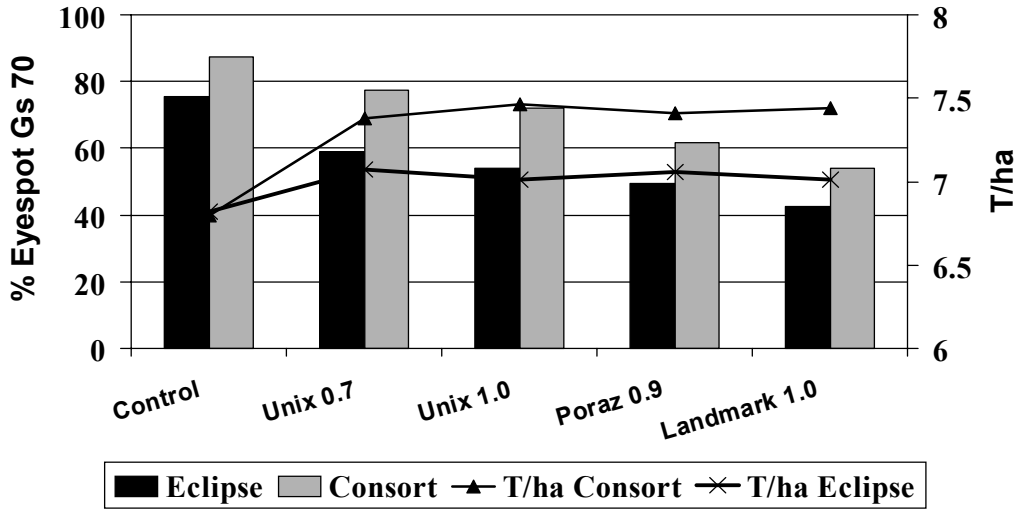


Figure 17. 2002 results for Consort and Eclipse following fungicide treatment.

Figure 17 shows that in 2002 eyespot levels were lower in Eclipse than in Consort. Landmark, Unix and Poraz all reduced eyespot levels and increased yield.

In 2003 there were also high levels of eyespot in the SAC trial – predominantly R type as seen in the previous two seasons. There was a significant reduction in eyespot with sowing date (Table 21).

Table 21. Influence of early and late sowing on eyespot and yield in 2003 SAC trial

Treatment	Eyespot % incidence	R type ng DNA	W type ng DNA	Yield
Early sown	59.0	1.07	>0.01	7.52
Late sown	37.4	0.02	>0.01	5.42
SED	19.14	1.022	0.000	0.877
P	0.001	0.002	0.199	>0.001

The most effective treatment was full rate Unix. Levels of eyespot were reduced in the variety Eclipse by more than in the previous two seasons (Figure 18).

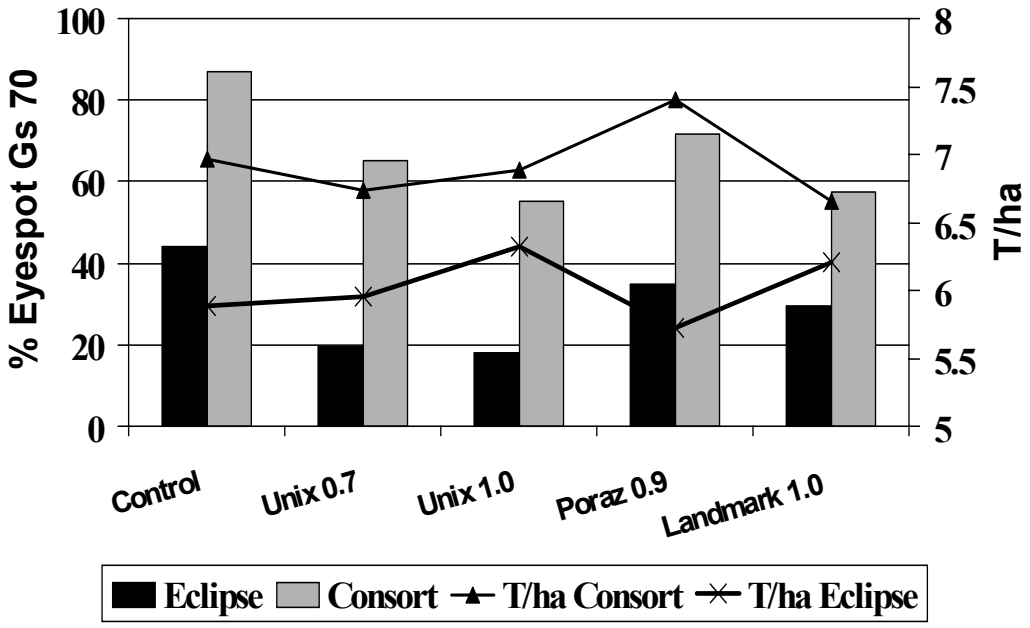


Figure 18. Eyespot levels in Consort and Eclipse following fungicide treatment 2003

CSL DEFRA Survey data

The data collected by CSL was not used to form the model. This was because the data was very unbalanced with only very low incidences of eyespot recorded. There were very few examples of crops that exceed the treatment threshold set in the model – i.e. only 212 out of the 1113 crops surveyed exceeded an incidence of 30% at the end of the season and only 85 crops exceeded the 45% threshold (i.e. less than 8%).

The mean levels of eyespot recorded in the survey were low in each season of the project as shown in Table 22. The factors used in the model were broadly in agreement with the trends shown in the survey data, in terms of resulting in low levels of disease. Earlier sown crops had higher levels of disease, minimum tilled crops had less disease than ploughed crops and straw removal had a small, but non significant, effect in increasing disease when compared to crops where the previous straw had been ploughed in.

Previous cropping had an influence and there was supporting evidence for the need for a two year break from cereals as compared to just a one year break. The influence of previous cropping was not large however and levels of disease in first wheats were often similar to those in second wheats. The CSL data concurred with the model data in showing lower levels of eyespot in crops from the north of England when compared to the South and West. There was no influence from seed treatment observed in the data.

Table 22. CSL / DEFRA data summary

Factor	Treatment	% eyespot incidence at end of season
Year	2001	15.5
	2002	18.2
	2003	15.3
		$P = 0.038$
Sowing date	September	18.3
	October	14.5
		$P < 0.001$

Area	North England	13.0
	South East England	16.7
	West England / Wales	16.3
		$P = 0.433$
Tillage	Ploughed	17.0
	Minimum tilled	13.8
	Shallow ploughed	14.9
		$P = 0.056$
Straw removal	Removed	18.5
	Ploughed in	16.8
		$P = 0.350$
Seed treatment	Single purpose	18.5
	Multi purpose	16.8
		$P = 0.802$
Previous Crop	Wheat	18.5
	Set-aside	19.3
	Oilseed rape	15.4
	Potatoes	18.6
	Grass	6.97
	Other cereal	18.0
	Other crop	14.2
	Pulses / Legumes	15.7
		$P < 0.005$

Previous crop	Previous crop a cereal	18.5
	One years break	16.9
	Two years break	14.2
	Three years break	15.0
		$P < 0.054$

The influence of airborne spores

The PCR testing by Syngenta on the spore trap samples did detect R type eyespot in the 2000/2001 season and in the 2002/2003 season. No spores were detected in the 2001/2002 season. Eyespot was not detected in the bait plants used in 2000/2001 and this methodology was abandoned after this first season. No W type eyespot was detected. Detection rates were low in season one when spore traps were run for 48 hrs at each sampling. In season three spore traps were run for 14 days per sampling and detection rates were higher. No airborne eyespot was detected in the autumn in any season. It was first detected in March 2001 and February 2003. Airborne R type eyespot was detected in all geographic regions tested i.e. Fife, Lothians, Hertfordshire (Table 23).

Table 23. Incidence positive eyespot detection after PCR testing on spore trap samples

Month of sampling	Season					
	2000/2001		2001/2002		2002/2003	
	R type	W type	R type	W type	R type	W type
November	0	0	0	0	0	0
December	0	0	0	0	0	0
January	0	0	0	0	0	0
February	0	0	0	0	2/7	0
March	1/5	0	0	0	6/6	0
April	0	0	0	0	4/4	0
May	4/5	0	0	0	1/4	0
June	0	0	0	0	1/3	0
July	0	0	0	0	4/4	0

Airborne R type eyespot was detected in spore samples taken from traps sited in wheat, barley, wheat, grass, set-aside and oilseed rape (Table 24). It was also detected in traps removed from immediate arable cultivations as used in the final 2002/2003 season.

Table 24. Incidence of detection by crop for spore samples taken in 2000/2001 season

Crop spore sampler sited	R type	W type
Grass	7/11	0/11
Oilseed rape	1/8	0/8
Wheat	1/12	0/12
Barley	0/10	0/10
Set-aside	1/11	1/11

Comparison of CSL and Syngenta PCR testing

In parallel testing of stem base samples by CSL and Syngenta, there was a very poor correlation between the results for R type eyespot from each method. There was also no significant correlation between the results for W type eyespot (Table 25).

Table 25. Correlation between results obtained from Syngenta and CSL PCR testing.

CSL v Syngenta results	R type	W type
Correlation coefficient	-0.037	0.146
P value	0.752	0.207

The two methodologies did not rank the eyespot level or type in a similar fashion when analysed by site. The Syngenta methodology over scored R type at several sites in comparison to the CSL results, but relatively underscored it for site 6. For W type both methodologies showed high levels of W type at site 2 and low levels at sites 1, 3 and 4 but did not agree for sites 5 and 6 (Figure 19).

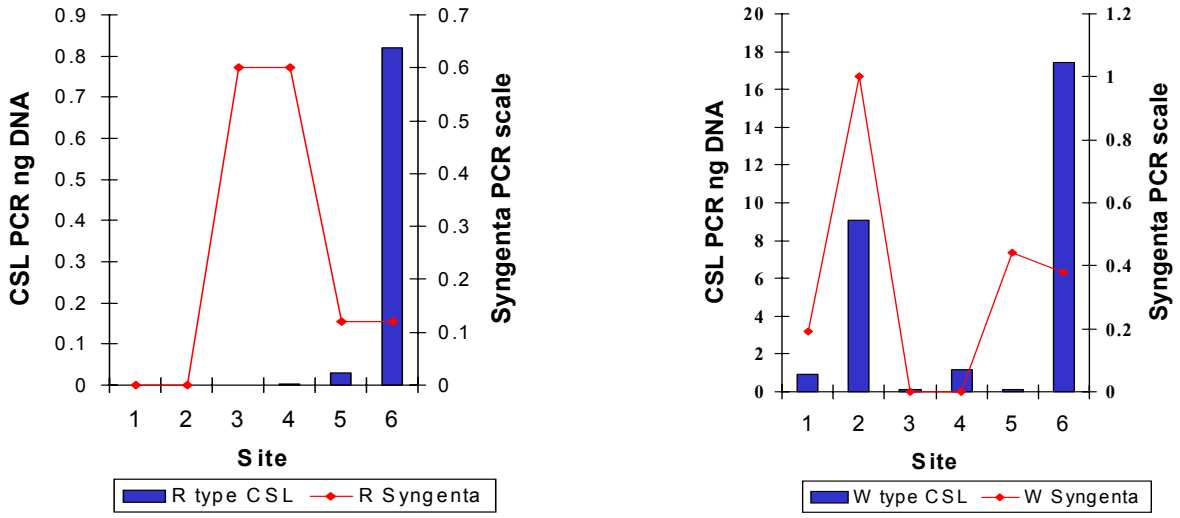


Figure 19. Comparison of Syngenta and CSL results by site.

There was no relationship between the W type DNA levels measured using the CSL and Syngenta methodology and some of the samples scoring highest on one scale scored lowest on the alternative scale. The R type methodology also correlated very poorly. There was a large spread of DNA quantities using the CSL methodology. Many of these scored as zero using the Syngenta methodology, but those that scored 1 or 2 on the Syngenta scale did not relate to those samples where most DNA had been recovered using the CSL method (Figures 20 and 21).

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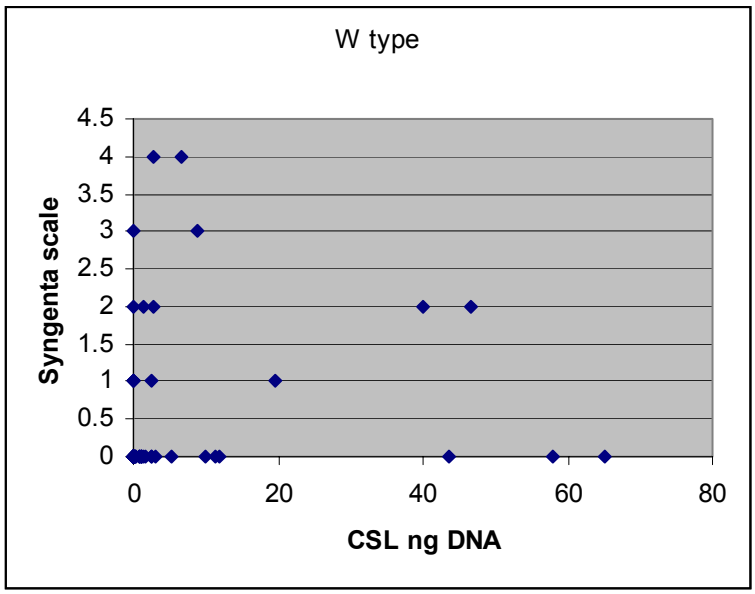


Figure 20. Relationship between W type test methods.

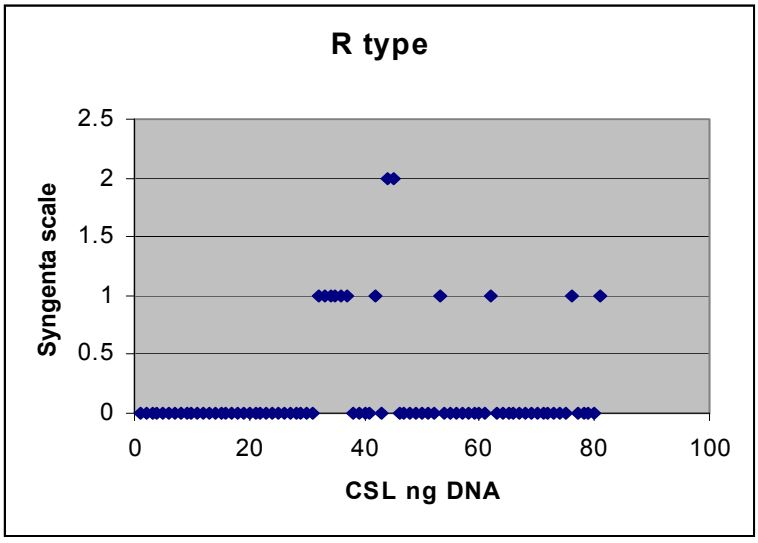


Figure 21. Relationship between R type test methods

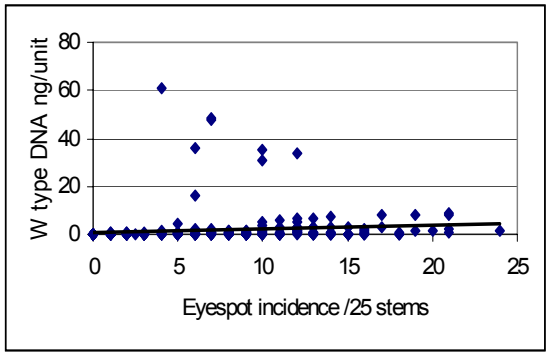
Comparison of PCR analysis with visual incidence of eyespot

The PCR analyses from GS 32 were not used in the model as a predictor of final eyespot levels. There was a significant correlation between W type DNA levels at GS 32 and visual incidence and amounts of W type DNA at the end of the season. There was however no significant correlation using R type DNA at GS 32 as a predictor of levels at the end of the season and the correlation for R type plus W type taken together was poorer than that obtained using visual incidence (Table 26). Visual eyespot incidence at GS 32 was retained by the model. There was a significant (5% level) correlation between the two methods of assessment at the end of the season assessment but no significant correlation at GS 31-32 (Table 26), although the correlation between the W type values and the visual incidence was significant at the 10% level.

Table 26. Correlation between DNA amounts and visual eyespot incidences.

	Eyespot incidence GS 31-32	Eyespot incidence GS 70-85
R type DNA GS 31-32	0.023	-0.107
P	0.714	0.121
W type DNA GS 31-32	0.113	0.193
P	0.071	0.005
R+W type DNA GS 31-32	0.043	0.125
P	0.497	0.071
R type DNA GS 70-85	-	0.494
P		<0.001
W type DNA GS 70-85	-	0.029
P		0.009
R+W type DNA GS 70-85	-	0.518
P		<0.001

Figures 22 and 23 show the relationship between visual assessment results and DNA quantification for samples assessed at stem extension and at the end of the season.



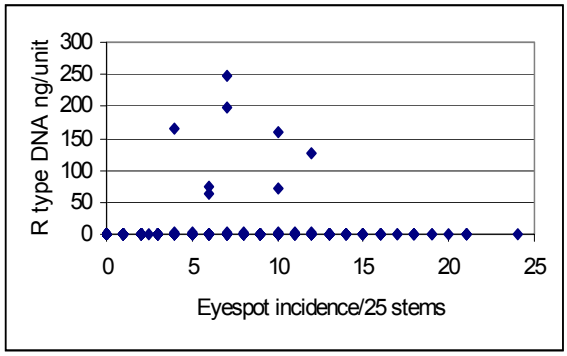
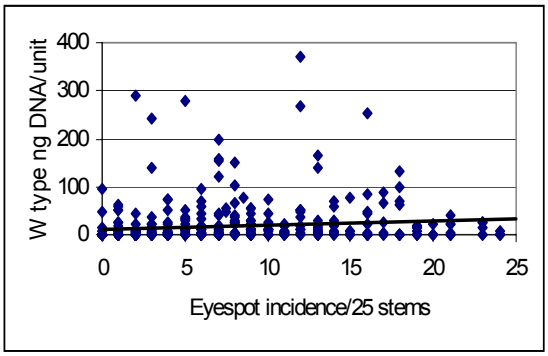


Figure 22. Correlation between eyespot visual % incidence at GS 31-32 and DNA amounts



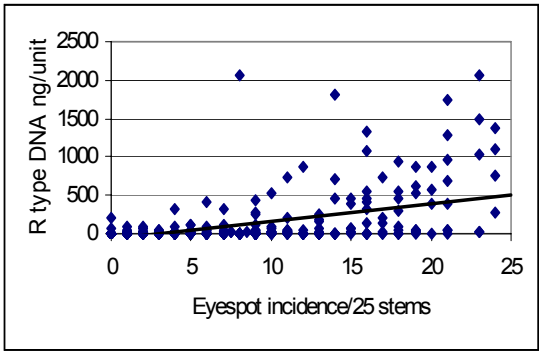


Figure 23. Correlation between eyespot visual incidence at GS 70-85 and DNA amounts

6. DISCUSSION

The aim of the project was to develop a risk algorithm to allow growers to accurately predict the need for eyespot treatment in their wheat crop. The relative influences of factors such as previous cropping, cultivation method, drilling date, varietal resistance, straw removal method, level of eyespot in the crop at stem extension, region, soil type and weather were investigated. Survey sites in commercial crops and detailed fungicide trials were evaluated to test the influence of these factors on eyespot development and to judge the cost efficacy of treatment. Samples were collected to assess disease progress visually and by PCR analysis over three seasons, starting in the autumn of 2000 and continuing until the 2003 harvest.

Analysis of the data set showed that many agronomic factors affected disease outcome. Using logistic regression the logistic probability of eyespot was modelled as a linear combination of explanatory variables, from which the probability of a treatable level of eyespot developing was calculated. Soil type, sowing date, presence of eyespot at stem extension, spring rainfall and tillage were selected as being significant influences, independent of other factors. These were attributed risk weightings in the model. Some factors such as the location of the sample appeared to have a large influence, but this variation could be fully explained in the model when soil type and weather were considered. Other factors such as autumn weather were unstable in the model, and were rejected as not being statistically significant. For development of the risk algorithm, an additive measure of risk was adopted to form a single explanatory variable (the Risk Score), incorporating elements of the individual risk factors identified as useful predictors of the probability of eyespot, scaled according to their contribution to overall risk (Table 27).

Table 27. Risk point allocated per factor

Factor	**Level**	**Risk points**
Sowing date	≤ 6 October	0
	> 6 October	5
Eyespot GS 31-32	≤ 7%	0
	> 7%	10
Rain (mm) in March / April / May	≤ 170 mm	0
	> 170 mm	5
Tillage	Minimum till	0
	Plough	10

Soil type*	Light	0
	Medium	1
	Heavy	5
Previous crop	Non-host	0
	Other cereal	10
	Wheat	15

1. Risk-sensitive user - treatment triggered at a Risk Score of 20
2. Risk-tolerant user - treatment triggered at a Risk Score of 29

*Brash and limestone increased the risk of eyespot but because of the limited number of samples on which this observation was made could not be included in the model. It is reasonable to propose that a further 5 risk points be added for either of these scenarios which would reflect an increased risk without giving the observation undue weight. Further work to confirm, or otherwise, this observed effect is required.

The maximum number of accumulated risk points a crop could be assigned was 50. Two treatment thresholds were set – a risk-sensitive threshold and a risk-tolerant threshold. This allows flexibility on the part of the user in determining what level of risk is acceptable to them. The risk-sensitive threshold was set using an external reference for yield loss from eyespot (Scott and Hollins, 1978) such that a final incidence of 30% eyespot at the end of the season would be cost effective to treat. Using the data generated in the project the disease loss from eyespot was less than would be predicted by the external reference and the risk-tolerant threshold was set at this level whereby a predicted final incidence of 45% would trigger treatment. These thresholds were used to model the data and assign the risk points. Receiver Operating Characteristic (ROC) curves provide a methodology for validation of guidelines for diagnostic decision-making when a binary (yes/no) decision is called for. The ROC curves generated in this project provide initial validation of the model in that they show the characteristic plot of a useful predictor. They also provide an opportunity for flexible implementation of decision guidelines. The balance of risks associated with each threshold Risk Score along the ROC curve is transparent. Thus, a very risk-sensitive user could choose to operate at a lower threshold Risk Score than 20. Similarly, a very risk-tolerant user could choose to operate at a higher threshold Risk Score than 29.

A useful predictor is one that can discriminate between crops that will need treatment for eyespot and those that will not, on the basis of information obtained at a sufficiently early stage of the growing season

to enable action to be taken. No predictor can be entirely perfect as not all the variability in crops which causes them to fall into a 'treat' or 'don't treat category' can be explained by a statistical model. By setting a threshold 'risk score' imperfect discrimination means that some crops that really need treatment will fall below this threshold and some that really do not need treatment will fall above this threshold. Since different users may respond differently to these two different types of error, a useful asset in a predictor is a capability for users to modify the threshold risk score to suit their own attitude to risk. It follows that predictors, such as the one described here are best considered as guidelines to be used as part of the crop protection decision making process, rather than rules that are meant to be followed without wider consideration of the circumstances in which the decision is made.

Several assumptions had to be made to attribute a yield loss to a given level of eyespot at the end of the season. An external reference was used to set the risk-sensitive threshold (Scott and Hollins, 1978), but this is widely thought to over estimate the risk from eyespot. The data generated in the project showed a yield response to Unix treatment in response to a reduction in eyespot levels. This level was actual yield recovered by treatment and therefore removes many suppositions that need to be made to apply the Scott and Hollins yield loss formula.

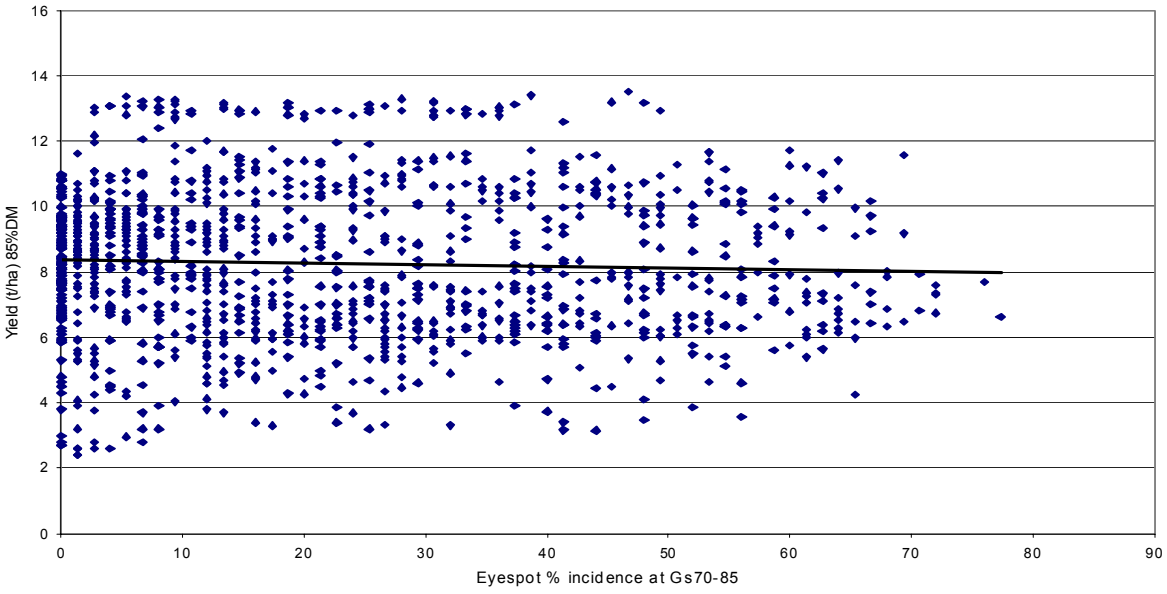


Figure 24. Scatter diagram of Eyespot % incidence and yield

In order to develop a functional model a formula for yield loss in response to a given level of eyespot had to be adopted. It was evident from the data set, however, that there is no clear association between eyespot levels and yield (Figure 24). Some sites still seem able to respond better to eyespot fungicides than others and this is a factor that would benefit from further study.

Variety and varietal resistance ratings (Anon, 2003) were rejected from the model as having no significant influence on eyespot levels. This reflects the fact that no current commercial varieties have very strong eyespot resistance – those included in the survey of commercial crops for this project had resistance ratings ranged from 3-6. It may also reflect the low number of trials on which the eyespot resistance ratings are made before inclusion in the CEL Recommended List. The more detailed fungicide work with the variety Eclipse that does have eyespot resistance showed the potential reduction that robust eyespot resistance could make. The inclusion of robust eyespot resistance, like the Eclipse resistance, in new varieties would negate the need for treatment in many cases and would allow varietal resistance to be added as a factor in the model.

As well as generating a risk algorithm to allow growers to predict the need for treatment, the work also provided useful insights into the status of eyespot population and responses to fungicide in trials. The data generated in this project confirmed the poor capability of eyespot levels at stem extension in predicting the level of eyespot that would develop in the crop. PCR quantification of DNA at stem extension was a poorer predictor of final eyespot incidence than was a visual assessment of incidence at GS 31-32, although there was a correlation between W type eyespot levels at stem extension and final levels at the end of the season. This provides further evidence that a threshold may be more effective in W type infections but in mixed or predominantly R type infections is not a useful predictor of eyespot risk. The project showed that the vast majority of cases in the UK are of mixed infections and the model was developed on this basis.

There was a very poor relationship between the two PCR methodologies evaluated. The full quantification of amounts of DNA carried out at the Central Science Laboratory, did not correlate with the 0-4 categories generated at Syngenta when samples were split and parallel tested. Some of this lack of agreement may be due to the inevitable variation that exists between plants in relatively small (25 plant) samples. Accurate sampling for eyespot, and information on the distribution of eyespot in a field would be a useful area for future study.

PCR pathotyping showed that the majority of crops in the period of the project had a mixed infection of *Oculimacula acuformis* (R type) and *O. yallundae* (W type). R and W type were present in almost equal proportions that showed an increase in W type over the previous few seasons. This may be as a result of the introduction of strobilurin fungicides to control foliar diseases of wheat. Triazole fungicides are more active against W type eyespot and their use over many years will have selected for R type eyespot. Triazole rates fell after the introduction of strobilurin fungicides, but, with the advent of *Septoria tritici* resistant to strobilurins, triazole rate have risen again (Fraaije *et al*., 2003). This means that it is very likely that R type eyespot will become more common and W type levels will fall.

Cyprodinil was used as a standard in all trials, and resulted in reduced eyespot levels. Prochloraz was also used in some trials. Effective control was seen following prochloraz treatment which may reflect the higher levels of W type eyespot seen in the course of the project as this fungicide is known to control W type more effectively than it controls the R type. In the 2002 season it was more effective than Unix which may also relate to the timing at which it was applied. It was applied early in the stem extension spray window – just prior to GS 31 whereas Unix is known to work best later in this window. The use of azoxystrobin in the trials gave a small increase in eyespot levels where it was applied at stem extension. In one season this was related to a decrease in sharp eyespot levels and there was a negative correlation between levels of the two diseases in the data set as a whole. Azoxystrobin has a label claim for the reduction of the root disease take-all when applied at stem extension. As a consequence of its efficacy against sharp eyespot the results of this project show that there is a danger of increasing eyespot levels where it is used at stem extension. A competition effect between the two pathogens would appear to allow eyespot to colonise where sharp eyespot has been reduced. Sharp eyespot is less damaging to yield and may play a role in keeping eyespot levels in check.

Spore trapping revealed the presence of airborne spores of *O. acuformis*. These spores were successfully trapped at a height of 20 m, showing that they were fully airborne as opposed to splash borne. This confirmed that there is a risk from surrounding crops even in true first wheats and may be one reason why the influence of previous cropping was not larger. There is a theoretical possibility that the spores trapped were airborne spores of the asexual stage that are normally splashed up from trash onto the crop in close proximity but it is probable that they were ascospores, released from the sexual stage of *O. acuformis*. No studies were carried out as part of this project to verify if the spores that were trapped were ascospores of the sexual stage of *O. acuformis*. Ascospores of the R type have not been specifically reported in the literature for *O. acuformis* in the UK but there is a weight of evidence to support the fact that a sexual stage does exist for the fungus. Reports supporting a sexual stage in *O. acuformis* are still more limited

than for *O. yallundae* but there is evidence of a sexual stage in both pathogens. W type apothecia (the sexual fruiting bodies) were first reported in 1987. R type apothecia have also been reported under field conditions (Douhan *et al*., 2002). Douhan also cites evidence supporting a sexual stage from the genetic variation within the R type population in the United States that would have arisen through sexual reproduction. Hocart and McNaughton (1994) also reported the genetic variation of R type eyespot to be typical of a sexually reproducing organism. For practical purposes to growers what is important is that the project clearly confirmed that eyespot can be airborne and therefore the risk to first wheats is greater than would be the case if the disease was entirely trash borne.

The risk algorithm developed provides clear but flexible guidance to growers on the probability that their crop will give a cost-effective response to an eyespot specific fungicide. The model could be recalculated for any given level of eyespot as the prices of grain and fungicide fluctuate, however, the two thresholds reported allow for individuals to reflect their own level of concern about eyespot. This, in combination with the ROC curves, allows discretion on the part of the user in setting their own threshold.

The factors included in the model give an accumulated risk score that was an acceptable predictor of final eyespot levels in the crop in initial validation using ROC curves. All factors selected as part of this single explanatory variable are available to the grower. The sowing date, tillage, soil type and previous crop are readily to hand. The presence of eyespot at the 7% level set in the model is easily assessed and represents between one and two infected plants out of the 25 plants that are commonly collected to assess the presence of eyespot. The rain fall in March, April and May is also a factor and in practical terms should be taken as the weather around and just after the stem extension spray window. Regional averages are available on line at http://www.met-office.gov.uk/climate/uk/averages/19712000/index.html. This Met Office site shows averages from 1971 – 2000. The long-range forecast should give an indication of wet or dry weather after spraying. In the longer term the risk algorithm has the potential to be developed into an interactive internet based system that producers could readily use to make decisions on the need for eyespot spray control. By linking with meteorological data the site could be kept live. Probabilistic weather modelling provides a method to determine if a season is likely to be wetter or drier than average and may be a useful area for future study in further developing the model.

It remains to validate the model in a commercial context but the model developed should allow growers to determine the need for treatment in their crops.

7. CONCLUSIONS

The aim of the project was to develop a straightforward risk algorithm to allow growers to predict which winter wheat crops will give a cost-effective response to eyespot treatment. The risk algorithm developed uses soil type, sowing date, tillage and previous cropping along with the presence of eyespot at stem extension and spring rainfall to predict the level of eyespot that will develop by the end of the season. Other factors that were rejected from the model as not being significant in isolation were area, autumn, winter and summer rainfall and temperature, and spring temperature. Straw disposal method, the level of eyespot at GS 32, the presence or level of eyespot at GS 25 were also rejected from the model. The risk weightings assigned dispel some commonly help misconceptions about eyespot. The influence of previous cropping was found to be relatively small. This means that it was almost as common in the data set generated to find damaging levels of eyespot in a first wheat crop as it was to find it in a second wheat. The level of eyespot at stem extension was not an accurate predictor of final eyespot levels, although the presence or absence of eyespot at stem extension was a risk factor identified in the model.

The level of eyespot which will give a cost effective response to treatment will obviously fluctuate with grain price and agrochemical efficacy and price. The predictive model described can be worked for any risk factor threshold but for the this report two levels are reported, one set at a risk-sensitive level and one at a more risk-tolerant level. This allows the grower to decide whether eyespot is a major concern or whether they will tolerate a higher level of risk. The more risk-tolerant approach would be more appropriate for example when grain prices are lower. If lodging is a risk then a more risk-sensitive approach would be justified.

The project also found that there was a risk from surrounding crops. Spore trapping detected wind borne R type eyespot. The presence of an air borne stage increases the risk of disease in first wheat crops and may explain why the influence of previous cropping was not larger. Most spores were detected in the spring and early summer that may lead to late infections in crops.

PCR analysis of samples showed that most sites within the UK have mixed R and W type infections with R and W type present in the database in almost equal proportions. This swing back to W type after R type being the dominant species since the mid eighties may reflect a decreased use of triazoles following the introduction of strobilurins for foliar disease control in wheat. With the onset of Septoria resistance to strobilurins, triazole rates have increased and this increase in W type may be very transient.

The use of Amistar (a.i. azoxystrobin) lead to a small increase in eyespot levels in the trials. This was related in one year to a decrease in sharp eyespot, There was a significant negative correlation between sharp eyespot and eyespot levels over the whole data set however. It is possible that because of its wide spectrum of activity azoxystrobin was also controlling other minor pathogens or saprophytes at the stem base and thereby allowing increased levels of eyespot to colonise.

The risk algorithm developed provides a useful guide to growers on the probability of yield damaging levels of eyespot developing in their crop. The algorithm has been presented in a flexible manner that allows some choice on the part of the user as to what level of risk, and certainty of pay back is acceptable to them. This approach presents growers with clear guidelines for treatment without being overly prescriptive where they have additional concerns or issues.

Acknowledgements

The field work in this project was carried out by colleagues within SAC and by staff with Velcourt, Central Science Laboratory and Scottish Agronomy Ltd. The support of Syngenta in testing samples from the spore trapping component of the work and from filed trials is also gratefully acknowledged, in particular that of Geraldine Booth who carried out the analysis and reported the results.

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9. APPENDICES

Site details

CODES USED IN TRIAL AND SURVEY TABLES

treatment code
1=No eyespot treatment
2= 0.7 Unix
3= 1.0 unix
4=Amistar
5=Poraz
6= Landmark

area
1=NORTH
2= DRY EAST
3=WET WEST

STRAW REMOVAL METHOD
1=REM
2=INCORP

TILLAGE
1=PLOUGH
2=MIN TILL

Rotation
1= WINTER WHEAT
2=WINTER BARLEY
3=SPRING BARLEY
4= SPRING WHEAT
5 = SET ASIDE
6= SET ASIDE NON CEREAL
7=WINTER OILSEED RAPE
8=SPRING OILSEED RAPE
9=POTATOES
10=WPEAS
11=WBEANS
12=GRASS
13=SPEAS
14=SBEANS
15=OTHER MONOCOT
16=OTHER DICOT
17=OATS

Location
1=PERTH
2=FIFE
3=LOTHIANS
4=BORDERS
5=ANGUS
6=LINCS
7=YORK
8=KENT
9=CAMBRIDGESHIRE
10=SUFFOLK
11=OXFORDSHIRE
12=WILTSHIRE
13=DEVON
14=DORSET
15= LANCS
16=ABERDEEN
17=HERTS
18=NORFOLK

SOIL Category
1=LIGHT
2=MEDIUM
3=HEAVY
4=LIME/CHALK
5=BRASH

VARIETY
1=CONSORT
2=ECLIPSE
3=DEBEN
4=HEREWARD
5=Xi19
6=ACCESS
7=CHARGER
8=TANKER
9=CLAIRE
10=SAVANNAH
11=MALACCA
12=RIBAND
13=EQUINOX
14=MADRIGAL
16=EQUINOX
17=WESTON

Table 29: Site details for commercial winter wheat crops surveyed

No.	Year	Site code	County	Region	Tillage	Straw removal	sow date	Variety	varietal rating	Soil type
1	2003	Vel-03A	9	2	1	2	11-Oct-02	4	5	1
2	2003	Vel-03B	9	2	2	2	17-Oct-02	1	7	1
3	2003	Vel-03C	9	2	2	2	06-Sep-02	1	7	1
4	2003	Vel-03D	9	2	1	2	12-Sep-02	1	7	1
5	2003	Vel-03E	9	2	2	2	13-Sep-02	1	7	1
6	2003	Vel-03F	9	2	2	2	27-Sep-02	5	5	1
7	2003	Vel-03G	9	2	1	2	14-Sep-02	6	4	1
8	2003	Vel-03H	9	2	1	2	11-Oct-02	5	5	1
9	2003	Vel-03J	9	2	2	2	05-Sep-02	1	7	1
10	2003	Vel-03L	10	2	1	1	18-Oct-02	7	4	2
11	2003	A1	5	1	1	2	23-Sep-02	1	7	2
12	2003	A2	2	1	1	2	18-Oct-02	1	7	2
13	2003	A3	2	1	2	2	03-Oct-02	1	7	2
14	2003	A4	2	1	1	2	03-Oct-02	1	7	2
15	2003	A5	2	1	1	2	03-Oct-02	10	5	2
16	2003	A6	2	1	1	2	03-Oct-02	11	4	2
17	2003	A7	2	1	1		08-Nov-02	10	5	2
18	2003	A8	1	1	1		25-Sep-02	1	7	2
19	2002	VELTS - 1a	14	3	2	1	25-Sep-01	9	7	8
20	2002	VELTS - 2a	14	3	2	1	30-Oct-01	1	7	8
21	2002	VELTS - 3a	14	3	2	2	13-Sep-01	9	7	2
22	2002	VELTS - 4a	14	3	1	2	20-Nov-01	3	6	2
23	2002	VELTS - 1b	6	2	2	2	28-Sep-01	1	7	1
24	2002	VELTS - 2b	6	2	1	2	05-Oct-01	11	4	1
25	2002	VELTS - 3b	6	2	2	2	08-Sep-01	13	5	1
26	2002	VELTS - 4b	6	2	1	2	07-Oct-01	1	7	1
27	2002	VELTS - 5	17	2	2	2	17-Sep-01	9	7	1
28	2002	VELTS - 6	17	2	1	2	23-Sep-01	4	5	1
29	2002	VELTS - 8	17	2	2	2	07-Sep-01	1	7	1
30	2002	VELTS -11	17	2	2	2	07-Sep-02	13	6	1
31	2002	VELTS - 9	18	2	1	1	17-Sep-03	9	7	
32	2002	VELTS - 10	6	2	2	2	14-Sep-01	9	7	12
33	2002	VELTS - 12	6	2	2	2	05-Oct-02	1	7	12
34	2002	Cunmont	5	1	1	2	15 sep-01oct 01	10	5	2
35	2002	Cunmont	5	1	1	1	14 oct 01nov 01	10	5	2
36	2002	Kilrie 2nd	2	1	2	1	01-13oct 01	10	5	6
37	2002	Kilrie 2nd	2	1	1	1	01-13oct 01	10	5	6
38	2002	Balmonth 2nd	2	1	1	1	01-13oct 01	12	6	6
39	2002	Balmonth 2nd	2	1	1	1	01-13oct 01	3	6	6
40	2002	Balmonth 1st	2	1	1		14 oct 01nov 01	3	6	6

No.	Year	Site code	County	Region	Tillage	Straw removal	sow date	Variety	varietal rating	Soil type
41	2002	Kilrie, 1st	2	1	1	2	01-13oct 01	10	5	6
42	2002	SAC Field 1	3	1	1	1		1	7	
43	2002	SAC Field 2	3	1	1	1		1	7	
44	2002	SAC Grain Store	3	1	1	1		1	7	
45	2002	West Clifton	3	1	1	1		1	7	
46	2002	Mull Park	3	1	1	1		1	7	
47	2001	DUNECHT	16	1	1	2	05-Oct-00	10	5	9
48	2001	DUNECHT	16	1	1	2	05-Oct-00	1	7	9
49	2001	HILTON OF FEARN	5	1	1	2	25-Sep-00	10	5	9
50	2001	HILTON OF FEARN	5	1	2	2	25-Sep-00	10	5	9
51	2001	HILTON OF FEARN	5	1	2	2	25-Sep-00	10	5	9
52	2001	BONERBO	2	1	1	1	12-Sep-00	1	7	4
53	2001	BONERBO	2	1	1	1	25-Oct-00	1	7	4
54	2001	BALMONTH	2	1	1		04-Nov-00	10	5	4
55	2001	BALMONTH	2	1	1	1	01-Nov-00	10	5	4
56	2001	MILTON,	2	1	1	2	25-Sep-00	11	4	4
57	2001	MILTON	2	1	1	2	25-Sep-00	1	7	4
58	2001	Queenston Bank 1	3	1	1			12	6	2
59	2001	Queenston Bank 2	3	1	1			12	6	2
60	2001	Queenston Bank 3	3	1	1			12	6	2
61	2001	Markle Mains 1	3	1	1			12	6	6
62	2001	Markle Mains 2	3	1	1			12	6	6
63	2001	Velts 1a	14	3	1	2	04-Oct-00	1	8	8
64	2001	Velts 1b	6	2	1	2	05.10.00	1	8	1
65	2001	Velts 2a	14	3	2	1	26-Oct-00	9	7	8
66	2001	Velts 2b	6	2			14-Sep-00	9	7	
67	2001	Velts 3a	14	3	2	1	08-Sep-00	9	7	8
68	2001	Velts 3b	6	2	2	2	01-Nov-00	10	5	1
69	2001	Velts 4a	14	3	1	2	10-Sep-00	14	7	8
70	2001	Velts 4b	6	2	1	2	04-Sep-00	11	4	10
71	2001	Velts 5	9	2				9	7	
72	2001	Velts 6	9	2				4	5	
73	2001	Velts 7	9	2						
74	2001	Velts 8	9	2				16	6	
75	2001	Velts 9	10	2	1	1	04-Oct-00	16	6	3
76	2001	Velts 10(out Marsh 4)	6	2	2	2	02-Oct-00	1	7	12
77	2001	Velts 11	9	2						
78	2001	Velts 12 (Lapwater 32)	6	2	2	2	20-Nov-00	17		12

No.	soil	soil pH	soil P	soil K	soil Mg	Fungicide	Rotation - 1	Rotation -2	Rotation -3	Rotation -4
1	3	8.1	0	3	n/a	1	1	11	1	1
2	3	7.9	2	2	n/a	1	11	1	7	1
3	3	8.2	2	2	n/a	1	11	1	1	7
4	3	7.9	1	1	n/a	1	7	2	1	10
5	3	7.5	3	2	n/a	1	7	2	1	10
6	3	7.6	2	2	n/a	1	10	1	7	1
7	3	7.3	2	2	n/a	1	7	2	1	11
8	3	7	1	1	n/a	1	1	1	1	1
9	3	8.2	1	2	n/a	1	10	2	1	
10	1	8.3	3	2	n/a	1	12		12	1
11	1	6.4	2	3	5	1	7	2	3	1
12	1	6.4	2	3	5	1	1	7	2	3
13	1	6.4	2	3	5	2	1	7	2	3
14	1	6.4	2	3	5	2	1	7	2	3
15	1	6.4	2	3	5	2	1	7	2	3
16	1	6.4	2	3	5	2	1	7	2	3
17	1	6	1	1	4	2	12	12	2	1
18	1	6.6	2	2	4	2	9	3	1	7
19	4	7.2	2	2	2	1	1	10	3	1
20	4	7.9	2	3	1	1	1	10	1	7
21	1	6.6	4	1	1	1	10	1	9	1
22	1	6.2	4	1.6	2	1	16	1	9	1
23	3	7.8	2	1	2	1	1	7	2	1
24	3	7.8	2	3	2	1	1	7	2	1
25	3	7.6	3	3	3	1	7	2	1	1
26	3	7.4	2	2	2	1	1	7	2	1
27	3	8.4	1	1	1	1	10	1	7	2
28	3	8.0	2	2	1	1	1	10	1	1
29	3	7.8	2	3	1	1	7	2	1	10
30	3	8.4	1	1	1	1	10	1	7	2
31		7.8	3	2	2	1	7	1	16	1
32	3	8.3	2	2	2	1	5	9	1	10
33	3	8.2	2	2	2	1	9	1	16	1
34	1	6.2	2.0	3.0	4.0	1	7	2	3	1
35	1	6.2	2.0	3.0	4.0	1	7	2	3	1
36	2	6.1	1.0	3.0	3.0	2	1	7	2	1
37	2	6.1	1.0	3.0	3.0	2	1	7	2	1
38	2	6.0	1.0	2.0	3.0	2	1	7	2	1
39	2	6.0	1.0	2.0	3.0	2	1	7	2	1
40	2	6.1	2.0	3.0	3.0	2	12	12	2	2

No.	soil	soil pH	soil P	soil K	soil Mg	Fungicide	Rotation - 1	Rotation -2	Rotation -3	Rotation -4
41	2	6.4	2.0	2.0	2.0	2	7	2	1	7
42						2				
43						2				
44						2				
45						2				
46						2				
47	2	5.9	1	2	2	2	2	1	8	3
48	2	5.9	1	2	2	2	2	1	8	3
49	2	6.3	2	3	4	2	17	17	1	8
50	2	6.3	2	3	4	2	17	17	1	8
51	2	6.3	2	3	4	2	17	17	1	8
52	3	6.5	1	2	3	2	1	7	3	1
53	3	6.5	1	2	3	2	7	3	3	1
54	3	6.4	2	2	5	2	12	2	3	1
55	3	6.2	2	2	4	2	12	12	2	1
56	3	6.6	2	2	4	2	5	1	1	1
57	3	6.6	2	2	4	2	5	1	1	1
58	1	5.7	3	2	2	2	7			
59	1	5.8	3	2	2	2	7			
60	1	5.5	3	2	2	2	1			
61	2	7.2	3	3	4	2	7			
62	2	7.3	2	2	4	2	1			
63	4	8.1	1	2	2.3	1	1	10	1	7
64	3	7.8	3	3	2	1	1	7	1	1
65	4	7.5	2	2	1	1	14	1	10	1
66										
67	4	7.9	3	2	1	1	10	1	7	2
68	3	7.7	2	3	2	1	1	7	1	1
69	4	8.1	1	1	1	1	10	1	7	2
70	5	7.1	3	2	1	1	1	2	1	7
71										
72										
73										
74							7			
75	2	7.7	1	1	2	1	5	2	1	16
76	3	8.3	2	3	3	1	5	16	1	1
77							10			
78	3	8.2	2	3	2	1	9	1		1

No.	Q1 mean temp	Q1 total rainfall	Q2 mean temp	Q2 total rainfall	Q3mean temp	Q3 total rainfall	Q4 mean temp	Q4 total rainfall
1	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
2	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
3	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
4	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
5	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
6	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
7	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
8	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
9	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
10	11.1	226.6	4.6	193.9	9.8	97.1	17.9	126.8
11	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
12	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
13	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
14	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
15	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
16	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
17	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
18	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
19	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
20	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
21	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
22	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
23	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
24	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
25	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
26	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
27	15.0	221.6	8.6	126.2	14.2	126.3	21.4	158.5
28	15.0	221.6	8.6	126.2	14.2	126.3	21.4	158.5
29	15.0	221.6	8.6	126.2	14.2	126.3	21.4	158.5
30	15.0	221.6	8.6	126.2	14.2	126.3	21.4	158.5
31	15.0	221.6	8.6	126.2	14.2	126.3	21.4	158.5
32	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
33	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
34	10.5	256	3.8	152.5	8.5	199.4	14.4	321.9
35	10.5	256	3.8	152.5	8.5	199.4	14.4	321.9
36	10.6	164	4.1	211.8	8.3	119.3	14.3	296
37	10.6	164	4.1	211.8	8.3	119.3	14.3	296
38	10.6	164	4.1	211.8	8.3	119.3	14.3	296
39	10.6	164	4.1	211.8	8.3	119.3	14.3	296
40	10.6	164	4.1	211.8	8.3	119.3	14.3	296

No.	Q1 mean temp	Q1 total rainfall	Q2 mean temp	Q2 total rainfall	Q3mean temp	Q3 total rainfall	Q4 mean temp	Q4 total rainfall
41	10.6	164	4.1	211.8	8.3	119.3	14.3	296
42	10.7	170	4.6	113.4	9.2	153.9	14.3	324.6
43	10.7	170	4.6	113.4	9.2	153.9	14.3	324.6
44	10.7	170	4.6	113.4	9.2	153.9	14.3	324.6
45	10.7	170	4.6	113.4	9.2	153.9	14.3	324.6
46	10.7	170	4.6	113.4	9.2	153.9	14.3	324.6
47								
48								
49								
50								
51								
52	9.3	303.7	3.2	231.6	7.2	109.2	14.1	195.8
53	9.3	303.7	3.2	231.6	7.2	109.2	14.1	195.8
54	9.3	303.7	3.2	231.6	7.2	109.2	14.1	195.8
55	9.3	303.7	3.2	231.6	7.2	109.2	14.1	195.8
56	9.3	303.7	3.2	231.6	7.2	109.2	14.1	195.8
57	9.3	303.7	3.2	231.6	7.2	109.2	14.1	195.8
58	9.8	273.6	3.6	159	7.5	141.8	13.9	216.6
59	9.8	273.6	3.6	159	7.5	141.8	13.9	216.6
60	9.8	273.6	3.6	159	7.5	141.8	13.9	216.6
61	9.8	273.6	3.6	159	7.5	141.8	13.9	216.6
62	9.8	273.6	3.6	159	7.5	141.8	13.9	216.6
63	13.8	623.2	8	440.3	12.2	287.9	19.4	233.8
64	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
65	13.8	623.2	8	440.3	12.2	287.9	19.4	233.8
66	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
67	13.8	623.2	8	440.3	12.2	287.9	19.4	233.8
68	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
69	13.8	623.2	8	440.3	12.2	287.9	19.4	233.8
70	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
71	14.7	315.4	7.4	197.5	12.6	200.5	21.4	191
72	14.7	315.4	7.4	197.5	12.6	200.5	21.4	191
73	14.7	315.4	7.4	197.5	12.6	200.5	21.4	191
74	14.7	315.4	7.4	197.5	12.6	200.5	21.4	191
75	14.7	315.4	7.4	197.5	12.6	200.5	21.4	191
76	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
77	14.7	315.4	7.4	197.5	12.6	200.5	21.4	191
78	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8

Table 30. Site details for field trials

No.	Year	Trial code	County	Region	Tillage	Straw removal	sow date	Variety	varietal rating	Soil type
1	2003	Bankhead-02-01	1	1	1	1	30-Sep-02	1	7	2
2	2003	Bankhead-02-01	1	1	1	1	30-Sep-02	12	6	2
3	2003	Bankhead-02-01	1	1	1	1	31-Oct-02	1	7	2
4	2003	Bankhead-02-01	1	1	1	1	31-Oct-02	12	6	2
5	2002	HGA-01-01	8	2	1	2	28-Oct-01	1	7	13
6	2002	HGA-01-01	8	2	1	2	06-Nov-01	1	7	13
7	2002	HGA-01-01	8	2	2	2	28-Oct-01	1	7	13
8	2002	HGA-01-01	8	2	2	2	06-Nov-01	1	7	13
9	2002	HGA-01-02	8	2	1	2	20-Oct-01	1	7	1
10	2002	HGA-01-02	8	2	1	2	06-Nov-01	1	7	1
11	2002	HGA-01-02	8	2	2	2	20-Oct-01	1	7	1
12	2002	HGA-01-02	8	2	2	2	06-Nov-01	1	7	1
13	2002	HGA-01-03	11	3	1	2	08-Sep-01	1	7	10
14	2002	HGA-01-03	11	3	1	2	26-Sep-01	1	7	10
15	2002	HGA-01-03	11	3	2	2	08-Sep-01	1	7	10
16	2002	HGA-01-03	11	3	2	2	26-Sep-01	1	7	10
17	2002	HGA-01-04	11	3	1	1	08-Sep-01	1	7	10
18	2002	HGA-01-04	11	3	1	1	26-Sep-01	1	7	10
19	2002	HGA-01-04	11	3	2	1	08-Sep-01	1	7	10
20	2002	HGA-01-04	11	3	2	1	26-Sep-01	1	7	10
21	2002	HGA-01-05	6	2	1	2	25-Sep-01	1	7	14
22	2002	HGA-01-05	6	2	1	2	11-Oct-01	1	7	14
23	2002	HGA-01-05	6	2	2	2	25-Sep-01	1	7	14
24	2002	HGA-01-05	6	2	2	2	11-Oct-01	1	7	14
25	2002	HGA-01-06	6	2	1	2		1	7	10
26	2002	HGA-01-06	6	2	1	2	02-Nov-01	1	7	10
27	2002	HGA-01-06	6	2	2	2	02-Nov-01	1	7	10
28	2002	HGA-01-07	14	3	1	1	28-Sep-01	1	7	13
29	2002	HGA-01-07	14	3	1	1	13-Oct-01	1	7	13
30	2002	HGA-01-07	14	3	2	1	28-Sep-01	1	7	13
31	2002	HGA-01-07	14	3	2	1	13-Oct-01	1	7	13
32	2002	HGA-01-08	14	3	1	1	26-Sep-01	1	7	15
33	2002	HGA-01-08	14	3	1	1	15-Oct-01	1	7	15
34	2002	HGA-01-08	14	3	2	1	26-Sep-01	1	7	15
35	2002	HGA-01-08	14	3	2	1	15-Oct-01	1	7	15

No.	Year	Trial code	County	Region	Tillage	Straw removal	sow date	Variety	varietal rating	Soil type
36	2003	HGA-02-01	8	2	1	2	30-Sep-02	1	7	3
37	2003	HGA-02-01	8	2	1	2	28-Oct-02	1	7	3
38	2003	HGA-02-01	8	2	2	2	30-Sep-02	1	7	3
39	2003	HGA-02-01	8	2	2	2	28-Oct-02	1	7	3
40	2003	HGA-02-02	8	2	1	2	20-Sep-02	4	5	3
41	2003	HGA-02-02	8	2	1	2	28-Oct-02	4	5	3
42	2003	HGA-02-02	8	2	2	2	20-Sep-02	4	5	3
43	2003	HGA-02-02	8	2	2	2	28-Oct-02	4	5	3
44	2003	HGA-02-03	11	3	1	1	10-Oct-02	1	7	16
45	2003	HGA-02-03	11	3	1	1	25-Sep-02	1	7	16
46	2003	HGA-02-03	11	3	2	1	10-Oct-02	1	7	16
47	2003	HGA-02-03	11	3	2	1	25-Sep-02	1	7	16
48	2003	HGA-02-04	6	2	1	2	01-Oct-02	8	7	5
49	2003	HGA-02-04	6	2	2	2	01-Oct-02	8	7	5
50	2003	HGA-02-05	6	2	1	1	17-Sep-02	9	7	6
51	2003	HGA-02-05	6	2	1	1	01-Oct-02	9	7	6
52	2003	HGA-02-05	6	2	2	1	17-Sep-02	9	7	6
53	2003	HGA-02-05	6	2	2	1	01-Oct-02	9	7	6
54	2003	HGA-02-06	12	3	1	2	07-Oct-02	3	6	7
55	2003	HGA-02-06	12	3	1	2	28-Feb-03	3	6	7
56	2003	HGA-02-06	12	3	2	2	28-Feb-03	3	6	7
57	2002	Markle Mains-01-01	3	1	1			1	7	6
58	2002	Markle Mains-01-02	3	1	1			1	7	6
59	2001	SAC-00-01	4	1	1	1	04-Sep-00	1	7	1
60	2001	SAC-00-01	4	1	1	1	04-Sep-00	2	8	1
61	2001	SAC-00-01	4	1	1	1	02-Nov-00	1	7	1
62	2001	SAC-00-01	4	1	1	1	02-Nov-00	2	8	1
63	2002	SAC-01-01	1	1	1	1	03-Oct-01	1	7	8
64	2002	SAC-01-01	1	1	1	1	03-Oct-01	2	8	8
65	2002	SAC-01-01	1	1	1	1	29-Oct-01	1	7	8
66	2002	SAC-01-01	1	1	1	1	29-Oct-01	2	8	8
67	2001	VEL-EFT 1	14	3	2	1	12-Sep-00	1	7	15
68	2001	VEL-EFT 1	14	3	2	1	04-Oct-00	1	7	15
69	2001	VEL-EFT 1	14	3	1	1	12-Sep-00	1	7	15
70	2001	VEL-EFT 1	14	3	1	1	04-Oct-00	1	7	15

No.	Year	Trial code	County	Region	Tillage	Straw removal	sow date	Variety	varietal rating	Soil type
71	2001	VEL-EFT 1	14	3	1	1	04-Oct-00	1	7	2
72	2001	VEL-EFT 1	14	3	1	1	04-Nov-00	1	7	2
73	2001	VEL-EFT 1	14	3	2	1	04-Oct-00	1	7	2
74	2001	VEL-EFT 1	14	3	2	1	04-Nov-00	1	7	2
75	2001	VEL-EFT 2	8	2	1	2	05-Oct-00	1	7	1
76	2001	VEL-EFT 2	8	2	2	2	05-Oct-00	1	7	1
77	2001	VEL-EFT 2	8	2	1		22-Sep-00	1	7	1
78	2001	VEL-EFT 2	8	2	1	2	04-Oct-00	1	7	1
79	2001	VEL-EFT 2	8	2	1	2	04-Oct-00	1	7	1
80	2001	VEL-EFT 2	8	2	2		11-Sep-00	9	7	1
81	2001	VEL-EFT 2	8	2	2	2	04-Oct-00	1	7	1
82	2001	VEL-EFT 2	8	2	2	2	04-Oct-00	1	7	1
83	2001	VEL-EFT 3	11	3	1	2	13-Sep-00	1	7	15
84	2001	VEL-EFT 3	11	3	1	2	13-Oct-00	1	7	15
85	2001	VEL-EFT 3	11	3	2	2	13-Sep-00	1	7	15
86	2001	VEL-EFT 3	11	3	2	2	13-Oct-00	1	7	15
87	2001	VEL-EFT 3	11	3	1	2	17-Sep-00	1	7	1
88	2001	VEL-EFT 3	11	3	1	2	14-Oct-00	1	7	1
89	2001	VEL-EFT 3	11	3	2	2	17-Sep-00	1	7	1
90	2001	VEL-EFT 3	11	3	2	2	14-Oct-00	1	7	1
91	2001	VEL-EFT 4	6	2	1	1	24-Sep-00	1	7	14
92	2001	VEL-EFT 4	6	2	1	1	28-Oct-00	1	7	14
93	2001	VEL-EFT 4	6	2	2	1	24-Sep-00	1	7	14
94	2001	VEL-EFT 4	6	2	2	1	28-Oct-00	1	7	14
95	2001	VEL-EFT 4	6	2	1	1	14-Oct-00	1	7	14
96	2001	VEL-EFT 4	6	2	1	1	28-Oct-00	1	7	14
97	2001	VEL-EFT 4	6	2	2	1	14-Oct-00	1	7	14
98	2001	VEL-EFT 4	6	2	2	1	14-Oct-00	1	7	14
99	2001	VEL-EFT 4	6	2	2	1	14-Oct-00	1	7	14
100	2001	VEL-EFT 4	6	2	2	1	28-Oct-00	1	7	14
101	2001	VEL-EFT 4	6	2	2	1	28-Oct-00	1	7	14
102	2001	VEL-EFT 4	6	2	2	1	28-Oct-00	1	7	14

No.	soil	soil pH	soil P	soil K	soil Mg	Rotation - 1	Rotation -2	Rotation -3	Rotation -4
1	1	6.2	1	1	4	2	1	1	5
2	1	6.2	1	1	4	2	1	1	5
3	1	6.2	1	1	4	2	1	1	5
4	1	6.2	1	1	4	2	1	1	5
5	2	7.3	3	1	1	14	1	10	1
6	2	7.3	3	1	1	14	1	10	1
7	2	7.3	3	1	1	14	1	10	1
8	2	7.3	3	1	1	14	1	10	1
9	3	8.2	1	1	1	1	11	1	7
10	3	8.2	1	1	1	1	11	1	7
11	3	8.2	1	1	1	1	11	1	7
12	3	8.2	1	1	1	1	11	1	7
13	5	8.0	3	2	1	10	1	7	2
14	5	8.0	3	2	1	10	1	7	2
15	5	8.0	3	2	1	10	1	7	2
16	5	8.0	3	2	1	10	1	7	2
17	5	7.6	1	3	2	1	10	1	7
18	5	7.6	1	3	2	1	10	1	7
19	5	7.6	1	3	2	1	10	1	7
20	5	7.6	1	3	2	1	10	1	7
21	5	8.0	2	2	1	10	1	7	2
22	5	8.0	2	2	1	10	1	7	2
23	5	8.0	2	2	1	10	1	7	2
24	5	8.0	2	2	1	10	1	7	2
25	5		2	2		1			
26	5		2	2		1			
27	5		2	2		1			
28	2	7.9	4	2		14	1	16	1
29	2	7.9	4	2		14	1	16	1
30	2	7.9	4	2		14	1	16	1
31	2	7.9	4	2		14	1	16	1
32	4	8.3	2	1		1	14	1	10
33	4	8.3	2	1		1	14	1	10
34	4	8.3	2	1	n/a	1	14	1	10
35	4	8.3	2	1		1	14	1	10
36	2	7.5	1	2	1	7	1	7	1
37	2	7.5	1	2	1	7	1	7	1

No.	soil	soil pH	soil P	soil K	soil Mg	Rotation - 1	Rotation -2	Rotation -3	Rotation -4
38	2	7.5	1	2	1	7	1	7	1
39	2	7.5	1	2	1	7	1	7	1
40	2	8.1	2	2	2	1	14	1	7
41	2	8.1	2	2	2	1	14	1	7
42	2	8.1	2	2	2	1	14	1	7
43	2	8.1	2	2	2	1	14	1	7
44	3	8	4	3	2	17	1	7	1
45	3	8	4	3	2	17	1	7	1
46	3	8	4	3	2	17	1	7	1
47	3	8	4	3	2	17	1	7	1
48	3	7.4	2	3	5	1	7	1	1
49	3	7.4	2	3	5	1	7	1	1
50	2	6.7	2	2		10	1	7	2
51	2	6.7	2	2		10	1	7	2
52	2	6.7	2	2		10	1	7	2
53	2	6.7	2	2		10	1	7	2
54	4	8.4	1	1	1	10	1	7	2
55	4	8.4	1	1	1	10	1	7	2
56	4	8.4	1	1	1	10	1	7	2
57	2	6.9	2	2	4	7	2	1	
58	2	6.9	2	2	4	7	2	1	
59	3	6.6	2	2	3	1	1	7	5
60	3	6.6	2	2	3	1	1	7	5
61	3	6.6	2	2	3	1	1	7	5
62	3	6.6	2	2	3	1	1	7	5
63	4	6.3	2	2	4	1	17	3	3
64	4	6.3	2	2	4	1	17	3	3
65	4	6.3	2	2	4	1	17	3	3
66	4	6.3	2	2	4	1	17	3	3
67	4	8.4	2	1	n/a	7	1	10	1
68	4	8.4	2	1	n/a	7	1	10	1
69	4	8.4	2	1	n/a	7	1	10	1
70	4	8.4	2	1	n/a	7	1	10	1

No.	soil	soil pH	soil P	soil K	soil Mg	Rotation - 1	Rotation -2	Rotation -3	Rotation -4
71	1	8.2	2	1	n/a	1	7	2	1
72	1	8.2	2	1	n/a	1	7	2	1
73	1	8.2	2	1	n/a	1	7	2	1
74	1	8.2	2	1	n/a	1	7	2	1
75	3	8.4	3	4	6	1	1	7	1
76	3	8.4	3	4	6	1	1	7	1
77	3	6.3	2	2	1	11	1	7	1
78	3	8.3	3	4	6	7	1	1	7
79	3	8.2	3	3	6	7	1	1	9
80	3	6.2	3	2	3	7	5	1	16
81	3	8.3	3	4	6	7	1	1	7
82	3	8.2	3	3	6	7	1	1	9
83	4	8.1	3	2	2	7	1	10	1
84	4	8.1	3	2	2	7	1	10	1
85	4	8.1	3	2	2	7	1	10	1
86	4	8.1	3	2	2	7	1	10	1
87	3	8	2	2	3	1	7	2	2
88	3	10	2	2	5	1	7	2	2
89	3	7	2	2	2	1	7	2	2
90	3	9	2	2	4	1	7	2	2
91	5					7	1	9	1
92	5					7	1	9	1
93	5					7	1	9	1
94	5					7	1	9	1
95	5					1	7	2	2
96	5					1	7	2	2
97	5					1	7	2	2
98	5					1	7	2	2
99	5					1	7	2	2
100	5					1	7	2	2
101	5					1	7	2	2
102	5					1	7	2	2

No.	Q1 mean temp	Q1 total rainfall	Q2 mean temp	Q2 total rainfall	Q3mean temp	Q3 total rainfall	Q4 mean temp	Q4 total rainfall
1	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
2	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
3	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
4	8.9	314.4	3.4	167.8	8.2	172.6	15.3	131
5	15.2	246.3	8.8	212.8	14.2	181.9	20.7	179.7
6	15.2	246.3	8.8	212.8	14.2	181.9	20.7	179.7
7	15.2	246.3	8.8	212.8	14.2	181.9	20.7	179.7
8	15.2	246.3	8.8	212.8	14.2	181.9	20.7	179.7
9	15.2	246.3	8.8	212.8	14.2	181.9	20.7	179.7
10	15.2	246.3	8.8	212.8	14.2	181.9	20.7	179.7
11	15.2	246.3	8.8	212.8	14.2	181.9	20.7	179.7
12	15.2	246.3	8.8	212.8	14.2	181.9	20.7	179.7
13	14.3	229.4	8.0	215.6	13.6	157.5	20.0	203.4
14	14.3	229.4	8.0	215.6	13.6	157.5	20.0	203.4
15	14.3	229.4	8.0	215.6	13.6	157.5	20.0	203.4
16	14.3	229.4	8.0	215.6	13.6	157.5	20.0	203.4
17	14.3	229.4	8.0	215.6	13.6	157.5	20.0	203.4
18	14.3	229.4	8.0	215.6	13.6	157.5	20.0	203.4
19	14.3	229.4	8.0	215.6	13.6	157.5	20.0	203.4
20	14.3	229.4	8.0	215.6	13.6	157.5	20.0	203.4
21	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
22	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
23	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
24	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
25	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
26	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
27	13.8	236.3	7.5	211.7	12.8	134.9	19.2	235.0
28	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
29	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
30	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
31	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
32	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
33	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
34	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
35	14.6	346.3	8.7	423.3	12.9	299.8	18.6	186.8
36	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
37	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1

No.	Q1 mean temp	Q1 total rainfall	Q2 mean temp	Q2 total rainfall	Q3mean temp	Q3 total rainfall	Q4 mean temp	Q4 total rainfall
38	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
39	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
40	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
41	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
42	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
43	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
44	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
45	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
46	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
47	11.6	310.1	5.0	257.7	9.9	106.7	17.8	115.1
48	9.6	250.6	3.8	211.8	8.8	120.1	16.0	153.4
49	9.6	250.6	3.8	211.8	8.8	120.1	16.0	153.4
50	10.4	267.2	4.2	205.6	9.4	137.2	16.9	140.8
51	10.4	267.2	4.2	205.6	9.4	137.2	16.9	140.8
52	10.4	267.2	4.2	205.6	9.4	137.2	16.9	140.8
53	10.4	267.2	4.2	205.6	9.4	137.2	16.9	140.8
54	11.2	459.6	5.0	351.7	9.5	196.0	16.3	195.7
55	11.2	459.6	5.0	351.7	9.5	196.0	16.3	195.7
56	11.2	459.6	5.0	351.7	9.5	196.0	16.3	195.7
57	10.7	170	4.6	113.4	9.2	153.9	14.3	324.6
58	10.7	170	4.6	113.4	9.2	153.9	14.3	324.6
59	8	636.7	2.2	4224.9	5.9	197.9	12.6	319.4
60	8	636.7	2.2	4224.9	5.9	197.9	12.6	319.4
61	8	636.7	2.2	4224.9	5.9	197.9	12.6	319.4
62	8	636.7	2.2	4224.9	5.9	197.9	12.6	319.4
63	10.5	256	3.8	152.5	8.5	199.4	14.4	321.9
64	10.5	256	3.8	152.5	8.5	199.4	14.4	321.9
65	10.5	256	3.8	152.5	8.5	199.4	14.4	321.9
66	10.5	256	3.8	152.5	8.5	199.4	14.4	321.9
67	13.8	623.2	8.0	440.3	12.2	287.9	19.4	233.8
68	13.8	623.2	8.0	440.3	12.2	287.9	19.4	233.8
69	13.8	623.2	8.0	440.3	12.2	287.9	19.4	233.8
70	13.8	623.2	8.0	440.3	12.2	287.9	19.4	233.8

No.	Q1 mean temp	Q1 total rainfall	Q2 mean temp	Q2 total rainfall	Q3mean temp	Q3 total rainfall	Q4 mean temp	Q4 total rainfall
71	13.8	623.2	8.0	440.3	12.2	287.9	19.4	233.8
72	13.8	623.2	8.0	440.3	12.2	287.9	19.4	233.8
73	13.8	623.2	8.0	440.3	12.2	287.9	19.4	233.8
74	13.8	623.2	8.0	440.3	12.2	287.9	19.4	233.8
75	14.7	474.1	8.0	330.2	13.0	229.2	21.5	153.9
76	14.7	474.1	8.0	330.2	13.0	229.2	21.5	153.9
77	14.7	474.1	8.0	330.2	13.0	229.2	21.5	153.9
78	14.7	474.1	8.0	330.2	13.0	229.2	21.5	153.9
79	14.7	474.1	8.0	330.2	13.0	229.2	21.5	153.9
80	14.7	474.1	8.0	330.2	13.0	229.2	21.5	153.9
81	14.7	474.1	8.0	330.2	13.0	229.2	21.5	153.9
82	14.7	474.1	8.0	330.2	13.0	229.2	21.5	153.9
83	13.6	399.9	6.9	239.5	12.3	214.4	20.5	178.0
84	13.6	399.9	6.9	239.5	12.3	214.4	20.5	178.0
85	13.6	399.9	6.9	239.5	12.3	214.4	20.5	178.0
86	13.6	399.9	6.9	239.5	12.3	214.4	20.5	178.0
87	13.6	399.9	6.9	239.5	12.3	214.4	20.5	178.0
88	13.6	399.9	6.9	239.5	12.3	214.4	20.5	178.0
89	13.6	399.9	6.9	239.5	12.3	214.4	20.5	178.0
90	13.6	399.9	6.9	239.5	12.3	214.4	20.5	178.0
91	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
92	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
93	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
94	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
95	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
96	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
97	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
98	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
99	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
100	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
101	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8
102	12.9	376.4	6.4	225.2	11.2	162.6	19.2	180.8