
**PROJECT REPORT No. 297**

# MAXIMISING CONTROL WITH FUNGICIDES OF *FUSARIUM* EAR BLIGHT (FEB) IN ORDER TO REDUCE TOXIN CONTAMINATION OF WHEAT

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# MAXIMISING CONTROL WITH FUNGICIDES OF *FUSARIUM* EAR BLIGHT (FEB) IN ORDER TO REDUCE TOXIN CONTAMINATION OF WHEAT

by

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## ABSTRACT

Fusarium ear blight (FEB) constitutes a disease complex involving toxin producing species (e.g. *Fusarium culmorum, F. graminearum, F. avenaceum* and *F. poae*) and non-toxin producing species (e.g. *Microdochium nivale* vars. *majus* and *nivale*). The chief toxins produced are trichothecenes, of which deoxynivalenol (DON) and nivalenol (NIV) are most commonly associated with FEB.

Field trials were carried out over three years at each of three sites in the UK. Wheat plots were inoculated at mid-anthesis with a mixture of toxin producing and non-toxin producing species. Fungicides were applied 2-3 days post inoculation (years 1 and 2) or pre- or post inoculation (year 3) to determine their efficacy in controlling disease, each of the fungal species and toxin accumulation in the grain. Disease was assessed visually and grain was analysed to determine the relative amounts of each fungal species present and also the amount of DON toxin.

Fungicides were differentially active against the toxin-producing and non toxin-producing species that cause FEB. Azoxystrobin had very high activity against *M. nivale* but only very limited activity against *Fusarium* species. In contrast, tebuconazole, metconazole and HGCA2 had high activity against the *Fusarium* species while being ineffective against *M. nivale*. The control of disease and toxin accumulation was generally related to fungicide dose for those compounds with activity against *Fusarium* species. Efficacy was reduced with reduced fungicide dose. Pre- and post inoculation fungicide application controlled disease to similar extents where application was very close to the time of inoculation (±2-3 days). Where *Fusarium* species predominated at a site, application of azoxystrobin generally did not markedly affect the level of disease or toxin accumulating in grain. However, where significant amounts of *M. nivale* were present, the application of azoxystrobin sometimes led to increases in the toxin content of grain.

Although the reduction in the DON content of grain was sometimes very large relative to the untreated plots, in no case did fungicide application lead to a level of DON below 0.75 ppm (mg/kg).

Lodging occurred on one site in the last year of the project. High levels of DON were detected in grain from the plots where lodging occurred, overwhelming the effect of fungicide application.

The use of double fan nozzles combined with a reduced tractor speed greatly improved coverage of wheat ears with fungicide and led to improved control of disease.

## SUMMARY

**Background**

*Fusarium* ear blight (FEB) of wheat is a potentially serious, although sporadic, disease of wheat in the UK. A number of *Fusarium* species have been reported to cause FEB but the major pathogens are *Fusarium culmorum, F. graminearum, F. avenaceum* and *F. poae* along with *Microdochium nivale* (varieties *majus* and *nivale*) (formerly *F. nivale*). As well as the loss of yield and quality, FEB is of particular importance because of the potential of the majority of the species to produce toxins within the grain that are harmful to humans and livestock.

**Objectives**

1) To determine the effect of fungicides on FEB caused by the major pathogens, on interactions between FEB pathogens, and on mycotoxin accumulation.

2) To optimise control of FEB and mycotoxin accumulation through improved fungicide application techniques.

**Methods**

Similar experiments were done in each of three years, 1998/1999, 1999/2000 and 2000/2001, on three sites in England. These were in the north-east at the Central Science Laboratory (CSL), in the west midlands at Harper Adams University College (HAUC) and in East Anglia at the Morley Research Centre (MRC). Three key aspects of fungicide activity against fusarium ear blight pathogens and toxin accumulation were investigated: (a) fungicide efficacy (yr1), (b) fungicide dose (yr 2) and (c) fungicide timing (yr 3).

Each experiment had four randomised blocks (minimum dimensions 1.5 m x 4 m) in which the effects of fungicide treatments (including untreated controls) were compared on single wheat cultivars at each site. HAUC: Equinox, (1998/99, 1999/00), Cadenza (2000/01); MRC: winter wheat Charger in all three years; CSL: Charger 1998/99, 1999/00) Chablis (spring wheat) (2000/01). The cultivars chosen had low resistance to ear blight.

In the first year (1998/1999), field plots were established at CSL, HAUC and MRC in which the crop was inoculated at mid-anthesis with a range of FEB pathogens (*F. culmorum, F. graminearum, F. avenaceum, F. poae and M. nivale*) and treated with selected fungicides (Table 1) at full dose. Disease was assessed and grain from field plots was harvested and assayed a range of yield characters and for species, fungal biomass (PCR) and toxins (GC-MS/ELISA).

Table 1. Treatment rate and timing of application for products used during the project.

| | | Rate and timing of fungicide application | | |
|---|---|---|---|---|
| Fungicide | Active ingredient | Year 1 | Year 2 | Year 3 |
| Untreated | - | - | - | - |
| HGCA 1 | test chemical | Full/pre-inoc | | |
| HGCA 2 | test chemical | Full/pre-inoc | Full and half/pre-inoc | Half and quarter/pre and post-inoc* |
| HGCA 3 (Twist) | trifloxystrobin | Full/pre-inoc | | |
| HGCA 4 | test chemical | | Full/pre-inoc* | |
| Plover | difenoconazole | Full/pre-inoc | | |
| Folicur | tebuconazole | Full/pre-inoc | Full and half/pre-inoc | Half and quarter/pre and post-inoc |
| Amistar | azoxystrobin | Full/pre-inoc | Full and half/pre-inoc | Half and quarter/pre and post-inoc |
| Caramba | metconazole | Full/pre-inoc | Full and half/pre-inoc | |
| Sportak 45 | prochloraz | Full/pre-inoc | | |
| Landmark | epoxiconazole + kresoxim-methyl | Full/pre-inoc | Full/pre-inoc | |
| Derosal[1] | carbendazim | Full/pre-inoc* | | |
| Bavistim[2] | carbendazim | Full/pre-inoc** | | |
| Opus | epoxiconazole | | Full and half/pre-inoc | |
| Amistar + Folicur | azoxystrobin + tebuconazole | | Both half/pre-inoc | Half and quarter/pre and post-inoc |
| Amistar + Caramba | azoxystrobin + metconazole | | Both half/pre-inoc | |
| Amistar + Opus | azoxystrobin + epoxiconazole | | Both half/pre-inoc | |

* applied only at CSL and MRC; ** applied only at HAUC

In the second year (1999/2000), the field trials were repeated at CSL, HAAC and MRC with selected fungicides and fungicide combinations at differing rates of application (Table 1). Disease was assessed and grains analysed as in year 1 except that most toxin analyses were carried out using ELISA.

In the final year (2000/2001), reduced rates of selected fungicides and fungicide mixtures were applied pre- or post inoculation to determine the relative efficacy of prophylactic and post-infection treatment (Table 1). Disease, yield and other analyses were carried out as in year 2.

Objective 1) was studied in detail following the finding of that there were significant differences in the profile of the Fusarium ear blight populations which established at the three sites (probably due to

agronomic and climatic factors). This necessitated the retention of all three sites throughout the study. A significant body of work relating to objective 2) was made available to the project from Bayer plc. that allowed efforts to concentrate upon the primary objective.

**Results and Discussion**

*Evaluation of fungicide efficacy against Fusarium ear blight and trichothecene mycotoxins accumulation in grain of winter wheat (year 1).*

The relative efficacies of the fungicides at all three sites were generally similar although significant differences in efficacy against different ear blight populations were observed. Visual disease assessment generally reflected differences in fungal colonisation of grain and toxin accumulation although on some sites significant differences in disease could not be observed while the level of fungal colonisation and mycotoxins present in harvested grain was found to differ significantly.

*Fusarium culmorum* was the main pathogen that developed and colonised the grain at all three sites. However, differences were observed in the composition of the fungal populations which established at the three sites. At CSL, moderate levels of *F. graminearum* and *F. poae* were also present along with low levels of *F. avenaceum* while at HAUC the levels of *F. graminearum* and *F. avenaceum* were low and *F. poae* was very low. At MRC moderate levels of *F. avenaceum* were found while both *F. graminearum* and *F. poae* were at very low levels. The level of both *M. nivale* varieties was very low at both CSL and MRC sites and was present at slightly higher levels at HAUC. Over all sites, three fungicides were most effective at reducing the level of *F. culmorum* (the major pathogen), these being metconazole, tebuconazole and HGCA2. At two sites (CSL and MRC) MBC fungicide also significantly reduced the colonisation of grain by *F. culmorum*.

Reduction in the amount of *F. culmorum* present in grain was generally associated with a reduced level of the predominant trichothecene, this being DON at the CSL and HAUC sites and NIV at MRC. The greater the reduction in the amount of *F. culmorum* colonising the grain, the lower the amount of trichothecene toxin present. The fungicides appeared to act similarly against DON and NIV-producing isolates of *F. culmorum*. This result is of significance because of the presence of both NIV and DON producing isolates in the UK.

The mean level of DON was reduced by fungicide treatment in all but two cases, although differences were not significant. Toxin content in grain following application of azoxystrobin or difenoconazole at the HAUC was higher than in grain from untreated plots. This site was the only one where either variety of *M. nivale* was present at reasonable levels. This is of significance in light of results obtained in the second year of trials (see below). Although moderate levels of *F. poae* were present at the CSL site, the level of type A trichothecenes (DAS, HT-2, T-2) was extremely low. Similarly, at the sites where this species was only present at extremely low levels, only trace levels of these toxins were detected.

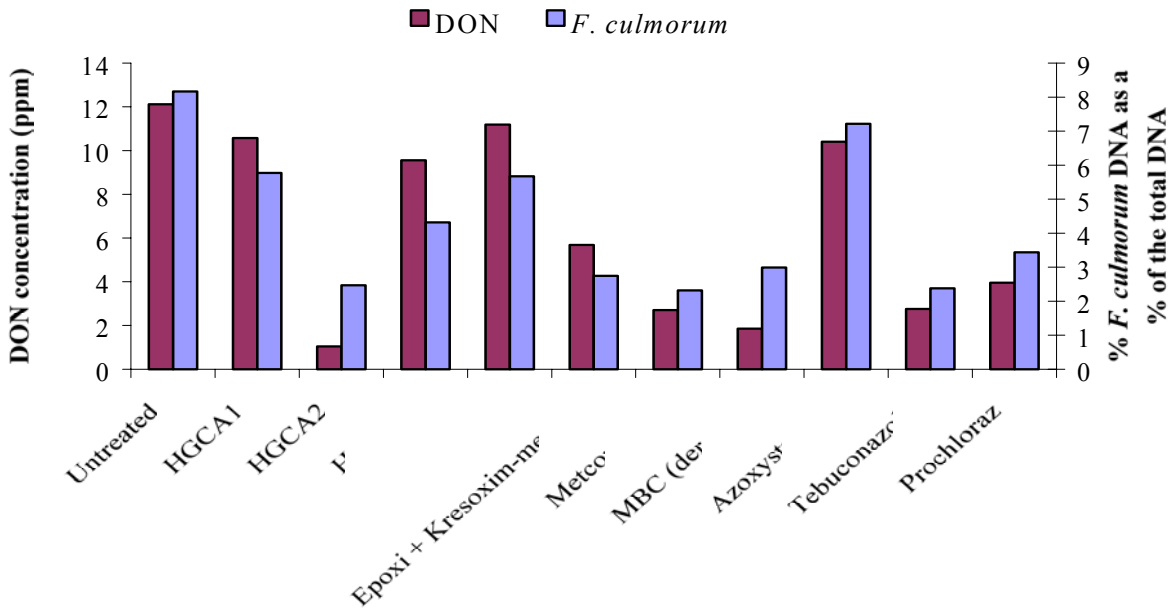



Figure1. Effect of fungicide treatment on levels of DON and *F. culmorum* at CSL in 1999.

The effect of the various fungicide treatments on yield parameters differed across the three sites. Yield was unaffected by any treatment at CSL while at HAUC yield was significantly increased by both HGCA2 and metconazole. In contrast, at MRC only two fungicides failed to effect a significant increase in yield (HGCA3 and difenoconazole). Thousand grain weight was not influenced by any treatment at CSL or MRC while at HAUC thousand grain weight was significantly increased by four fungicides (HGCA2, metconazole, tebuconazole and epoxiconazole + kresoxim-methyl). Specific weight was also significantly increased by three of these (HGCA2, metconazole and tebuconazole).

Overall, three fungicides (HGCA2, metconazole and tebuconazole) appeared to be most effective at reducing visual disease, fungal colonisation of grain and mycotoxin accumulation in grain. Whilst the effect on various yield parameters differed somewhat across sites, these fungicides also generally produced higher yield (t/ha) and thousand grain weight.

*Effect of fungicide dose and mixtures against Fusarium ear blight and trichothecene mycotoxin accumulation in grain of winter wheat (year2).*

At all three sites the great majority of treatments significantly reduced disease levels. The level of disease estimated by visual assessment during grain development at the three sites did not reflect the relative amounts of DON detected in harvested grain of the untreated plots. Disease levels were 47.5% (spikelets affected) at HAUC (GS 75), 1.6% (ear area affected) at CSL (GS 75) and 45% (ear area affected) at MRC (GS73) while DON levels in harvested grain were 7.33 ppm, 13.63 ppm and 2.59 ppm respectively. At individual sites the relationship between disease control achieved by each of the treatments and control of DON accumulation also differed markedly. At MRC and HAUC there was little relationship between

disease and DON accumulation. For example, at HAUC (GS85) least disease was observed where azoxystrobin (full dose) was applied and most disease where tebuconazole (full dose) was applied. The level of DON was 7.58 ppm for the former and only 4.61 ppm for the latter. At CSL the relationship between disease (GS85) and DON accumulation was closer but still weak across all treatments.

The composition of the fungal populations that developed at the three sites differed significantly. At HAUC and MRC, *F. avenaceum* was the predominant *Fusarium* species with the level of *F. graminearum* being moderate at HAUC and very low at MRC. The level of *F. graminearum* was similar at HAUC and CSL (Figure 2) being the predominant species present at the latter site. The level of *F. avenaceum* was similar at CSL and MRC (1.51 % and 1.57% respectively) and very high at HAUC (5.9%). The high level of *F. avenaceum* is of significance because this species does not produce DON or other trichothecene mycotoxins. The level of non-toxin producing *Microdochium nivale* varieties was relatively high at HAUC and MRC but was very low at CSL (Figure 2).

At HAUC the effect of fungicide treatments was significant only against *M. nivale* varieties (with the single exception of metconazole (full dose) against *F. culmorum*. All treatments involving azoxystrobin reduced either or both *M. nivale* varieties. The only other treatment to affect the level of fungal colonisation of grain was HGCA4 (full dose), which significantly reduced *M. nivale* var. *majus*. Similar activity of HGCA4 and all treatments involving azoxystrobin were observed at MRC. No treatment significantly reduced the level of any *Fusarium* species colonising the grain. In contrast, at CSL where the level of *Fusarium* species was far greater than that of *M. nivale* varieties, many of the compounds that had shown activity against *Fusarium* species in year 1 of the project significantly reduced the level of *F. graminearum* and/or *F. culmorum* in harvested grain. Azoxystrobin-containing treatments and HGCA4 also significantly reduced the level of *M. nivale* var. *majus* in grain, a result similar to those at the other two sites.

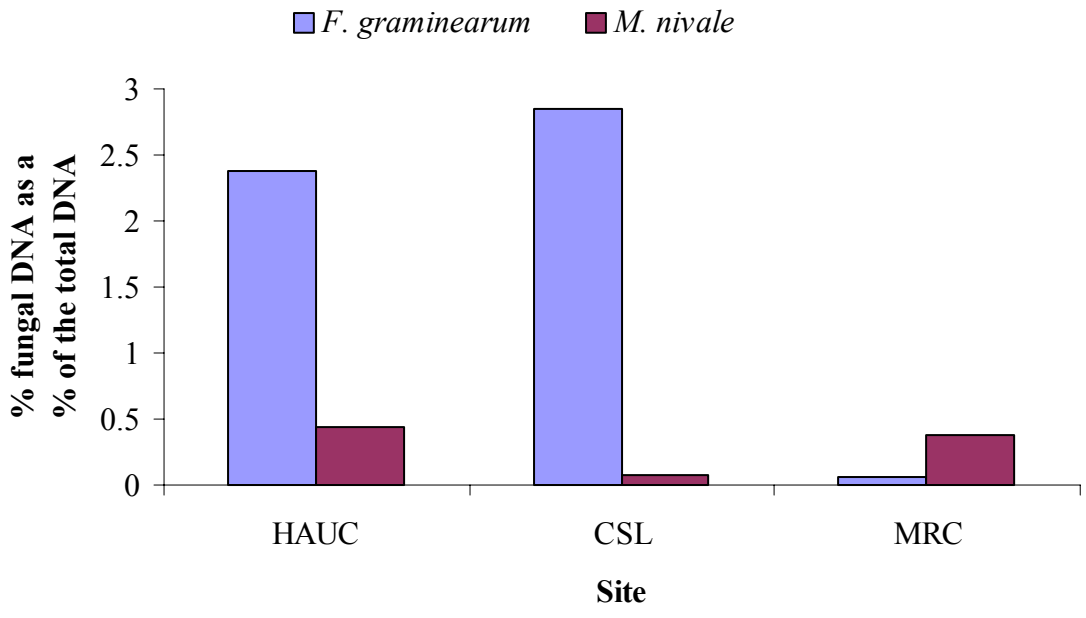



Figure 2. Species profile for *F. graminearum* and *M. nivale* at HAUC, CSL and MRC in 2000.

The level of DON in grain from the untreated plots differed widely across the three sites, with CSL having very high DON levels (13.63 ppm), HAUC intermediate levels (7.33 ppm) and MRC relatively low levels (2.59 ppm). It is envisaged that the EU will produce guidelines or action limits for DON levels in harvested grain of 0.75 ppm. This level is similar to those already in place in the Netherlands and Austria. It is notable that, even at MRC where the DON level was relatively low, no fungicide treatment reduced DON to below this level. The lowest DON contents were generally achieved at MRC where full dose tebuconazole and metconazole reduced levels to 1.73 ppm and 1.85 ppm, respectively. At CSL several (generally full dose) treatments also reduced DON levels over three-fold with the greatest reduction being achieved by full dose HGCA2 (1.91 ppm).

At CSL (the site with greatest level of trichothecene-producing *Fusarium* species), all treatments except azoxystrobin (full and half dose) significantly reduced the level of DON in harvested grain. In all cases the reduction was greater for full dose than half dose applications. The least DON was present where full or half dose HGCA2 had been applied. At HAUC and MRC, no treatment significantly reduced the level of DON in harvested grain although the level was generally less where full, rather than half, dose had been applied. Most strikingly, at HAUC the level of DON in grain from plots treated with azoxystrobin (half dose) (14.91 ppm) was significantly greater than in grain from untreated plots (7.33 ppm), while at MRC both full and half dose azoxystrobin applications (5.11 ppm and 6.33 ppm respectively) resulted in a significantly greater level of DON than in untreated grain (2.59 ppm). It is probable that the differing efficacies of the fungicides with respect to accumulation of DON in grain can be explained in the context of the differing composition of the fungal populations at the three sites.

Where significant levels of trichothecene-producing species, such as *F. graminearum* or *F. culmorum* were present treatments such as tebuconazole, metconazole, HGCA2 and epoxiconazole as well as epoxiconazole + kresoxim methyl significantly reduced disease and DON accumulation. The use of full dose treatment resulted in a greater reduction of DON than half dose. Azoxystrobin appeared to have little, or no, activity against *Fusarium* species but in combination with tebuconazole, metconazole or epoxiconazole did reduce disease and DON levels in grain. In contrast, where significant levels of non-trichothecene producing species (*M. nivale*) were predominant, compounds such as tebuconazole, metconazole, HGCA2 and epoxiconazole had very limited effect on reducing fungal colonisation of grain. Azoxystrobin had high activity against both varieties of *M. nivale* and generally significantly reduced levels of these species, even where they comprised only a small proportion of the fungal FEB population. At HAUC and MRC, where *M. nivale* comprised a significant proportion of the fungal population, the application of treatments that included azoxystrobin not only led to a significant reduction in *M. nivale* but also often to a significant increase in the level of DON in harvested grain (Figure 3). Experiments carried out by partners in this project, as part of other projects, has revealed a competitive interaction between *F. culmorum* and *M. nivale* varieties. It is most probable that this increase in DON was a consequence of the control of *M. nivale* (and possibly also of other non toxin-producing fungi) which led to an increase in the colonisation of wheat ears

by *Fusarium* species to fill the vacant niche. However, it is also possible that the fungicide has a direct influence on toxin production by the fungi.

The effect of treatment and dose against FEB on yield parameters was variable across the sites. At CSL (*Fusarium* species predominant) all treatments significantly increased thousand grain weight (TGW). Thousand-grain weight was increased significantly by most treatments at MRC, particularly those including azoxystrobin while at HAUC only two treatments resulted in increased TGW (azoxystrobin + tebuconazole and HGCA4). There was no relationship between TGW and DON observed at any site. The majority of treatments at MRC (significant levels of *M. nivale*) also significantly increased yield whereas at HAUC (intermediate between CSL and MRC with respect to fungal population) no treatment resulted in a significant increase in yield. Again, no relationship was observed between yield and DON content of grain.

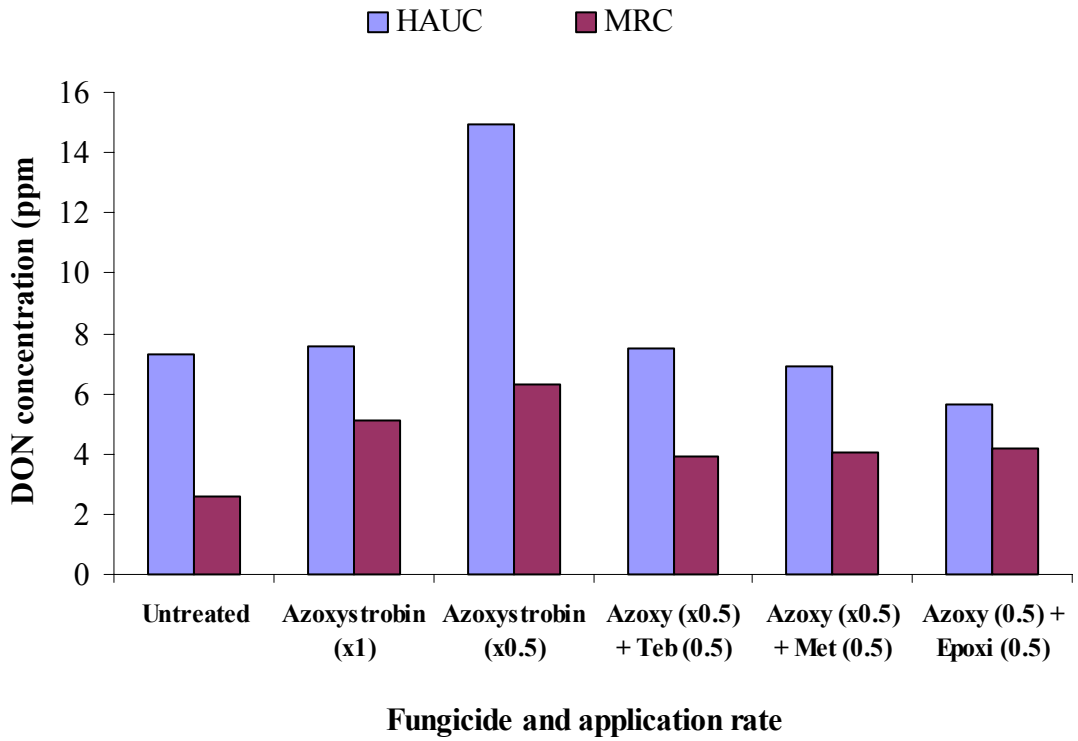



Figure 3. Effect of azoxystrobin treatments on DON concentration in grain at HAUC and MRC in 2000.

*Effect of timing of fungicide application and fungicide dose against Fusarium ear blight and trichothecene mycotoxin accumulation in grain of winter wheat (year 3).*

Experiments were carried out to examine the effectiveness of fungicides applied either before or after inoculation with ear blight pathogens.

Low levels of disease developed on two of the sites (HAUC and CSL) while at MRC disease levels were high. At CSL, where treatments were applied 3 days pre- and 3 days post inoculation, most treatments reduced disease and DON levels whether they were applied pre- or post-inoculation. While the higher dose generally reduced disease more than the 0.25 dose, there appeared to be little effect of fungicide dose on

DON content with 0.25 and 0.5 dose applications being largely similar in effect. Pre and post-inoculation treatments resulted in similar average DON levels (1.13 ppm and 1.14 ppm respectively). Considering only single products (0.25 or 0.5 dose), the average DON accumulation for pre and post-inoculation treatments was least where tebuconazole (mean 1.02 ppm) was applied and greatest for azoxystrobin treatments (1.23 ppm) with HGCA2 (1.11 ppm) being intermediate. Only two treatments resulted in a reduction in colonisation by trichothecene-producing species (*F. culmorum*), these being (tebuconazole (0.5 dose) applied pre-inoculation and HGCA2 (0.5 dose) applied post inoculation.

At HAUC where treatments were applied 2 days pre- and 2 days post inoculation visual disease was reduced by only two treatments, (tebuconazole (0.5 dose) pre-inoculation and azoxystrobin + tebuconazole (0.5+0.5 dose) pre-inoculation), at the time of the first assessment. By the time of the second assessment, no differences were observed in disease levels across treatments. Only two treatments significantly reduced the colonisation of grain by *F. culmorum* (tebuconazole (0.5 dose) and azoxystrobin + tebuconazole (0.25+0.25 dose) both applied post-inoculation. No treatment significantly reduced the amount of DON in grain although the lowest levels were for plots treated with tebuconazole (0.5 dose) post-inoculation and azoxystrobin + tebuconazole (0.5+0.5 dose) treated post-inoculation. In general the overall level of DON was less where treatments were applied post inoculation (mean 1.66 ppm) than where applied pre-inoculation (1.94 ppm). Considering only single products (0.25 or 0.5 dose), the average DON accumulation was least for applications of tebuconazole (mean 1.43 ppm) and greatest for azoxystrobin treatments (2.29 ppm).

Severe lodging was observed in the central portion of two rows of the MRC site. The amount of fungus and DON that accumulated in the grain of the plots in this region were significantly higher than in grain from the non-lodged plots. This variability was reflected in the lack of statistical evidence for any treatment having an effect on either fungal colonisation or DON accumulation. However, high levels of fungus and DON were observed even in non-lodged plots indicating that disease pressure at this site was very high. This factor may also have contributed to the failure of any treatment to significantly influence DON accumulation or fungal colonisation.

**Conclusions and implications for levy payers**

1. Fungicides are differentially active against the toxin-producing and non toxin-producing species that cause FEB. Azoxystrobin has very high activity against *M. nivale* but appears to have only very limited activity against *Fusarium* species. In contrast, tebuconazole, metconazole and HGCA2 have high activity against the *Fusarium* species while being ineffective against *M. nivale* varieties. Results indicate that a fungicide mixture is necessary to effectively control mixed populations of ear blight pathogens.

2. Evidence from this project shows that the use of full dose fungicides applied very close to mid-anthesis, using compounds with high activity against *Fusarium* ear blight species can significantly reduce

disease, increase yield and reduce accumulation of DON. Fungicides with greatest efficacy against DON producing isolates also have most effect against NIV producing isolates.

3. Where high levels of disease occur chiefly due to *Fusarium* species, both full and half dose fungicide treatments with active ingredients such as tebuconazole, metconazole and HGCA2 significantly reduce disease and toxin levels. However, using a lower fungicide dose reduces efficacy of control of disease and toxin accumulation relative to the full dose and will not control infections effectively under high disease pressure.

4. Where disease is due to *Fusarium* species and *M. nivale* is absent (or nearly so) higher levels of disease are associated with greater amounts of DON accumulating in grain.

5. Where high levels of disease occur and both *Fusarium* species and *M. nivale* varieties are present, application of azoxystrobin can result in significantly reduced disease but significantly higher DON levels. The effect on DON accumulation appears to be more marked when the dose of azoxystrobin is reduced. The increase in DON content of grain may even occur where azoxystrobin is applied in mixture with compounds active against *Fusarium* species.

6. Fungicides were generally most effective when applied post inoculation.

7. Where disease levels are low, half and quarter dose application of tebuconazole or HGCA2 may significantly reduce disease and toxin levels when applied up to 3 days either side of mid-anthesis.

8. Lodging of the crop can lead to large increases in the toxin content of grain, overwhelming any effects of fungicide application to the ear.

9. Use of double fan nozzles combined with reduced traverse speed of fungicide application can improve efficacy of disease control.

# TECHNICAL DETAILS

## 1 Introduction

*Fusarium* ear blight (FEB) of wheat is a potentially serious, although sporadic, disease of wheat in the UK. Evidence from the MAFF disease survey indicated that a significant proportion of the UK 1998 winter wheat crop was affected by FEB (Judith Turner, unpublished). FEB is becoming of increasing concern internationally with significant losses occurring in Canada and the USA in recent years. For example in Minnesota in 1993 yield losses in wheat and barley were estimated to be 33% with a value of 1 billion dollars (Dill-Macky & Jones, 1997). A number of *Fusarium* species have been reported to cause FEB but the major pathogens are *Fusarium culmorum, F. graminearum, F. avenaceum* and *F. poae* along with *Microdochium nivale* (varieties *majus* and *nivale*) (formerly *F. nivale*) (Parry *et al.*, 1995). Although *Fusarium graminearum* is the predominant pathogen worldwide, *F. culmorum* tends to predominate in cooler maritime regions such as the UK (Parry *et al.*, 1995). FEB forms part of a disease cycle on wheat and all the above pathogens may also cause seedling blight and brown foot rot. Increased levels of FEB have been associated with reduced tillage practices, and also with increased production of maize, both of which permit build-up of inoculum (Dill-Macky, 1997).

As well as the loss of yield and quality, FEB is of particular importance because of the potential of the majority of the species to produce toxins within the grain that are harmful to humans and livestock. The chief toxins of concern are trichothecenes (including deoxynivalenol, nivalenol, diacetoxyscirpenol and T-2 toxin), produced by *F. culmorum, F. graminearum, F. poae* and *F. sporotrichioides* which are immunosuppressive and potent inhibitors of protein synthesis (Joffe, 1986). In a recent UK survey of winter wheat *F. poae* was the species most commonly isolated from diseased glumes (Polley & Turner, 1995). This species is of significance because it is one of the most important producers of mycotoxins among *Fusarium* species infecting cereal grains (Joffe, 1986). *Fusarium avenaceum* produces a range of other mycotoxins (moniliformin and enniatins) that differ in their effect on plant hosts and human and animal consumers (Joffe, 1986) while *M. nivale* varieties are not known to produce any mycotoxins.

Control of FEB is difficult for a number of reasons. There are few sources of host resistance available and these are generally found in exotic germplasm and are difficult to transfer to agronomically adapted varieties. Fungicidal control of FEB is also problematic and there have been reports where application of fungicides has resulted in increased production of trichothecene mycotoxins (D'Mello *et al.,* 1996; Gareis & Ceynowa, 1994). Thus, whatever the species causing disease, it is vital that measures taken to control FEB also reduce levels of mycotoxins.

The symptoms caused by each of the *Fusarium* species, as well as those of the *M. nivale* varieties, are generally indistinguishable, making accurate disease diagnosis impossible. Even attempts to evaluate the relative proportion of each species in plant samples by isolation into axenic culture only reveals

what can be grown out of the plant rather than what is within the plant. The inability to detect, identify and quantify individual *Fusarium* species within plant tissues has seriously hindered the study and control of FEB. For example, the relationship between visual disease and yield loss differs markedly between species and it is thus important that the FEB species present are accurately diagnosed in order to determine the efficacy of any control measures (Turner & Jennings, 1997; Doohan, 1998). Differences in the ability of the FEB pathogen species to produce mycotoxins has obvious implications for the potential for mycotoxin contamination of the grain. It is essential to ensure that all the fungal species involved in this disease complex are effectively controlled and that suppression of one does not merely lead to it being replaced by another. This is of particular concern as certain fungicides may differentially control toxin-producing and non-producing species enabling greater infection of ears by toxin-producing species (Liggitt *et al.,* 1997; Nicholson *et al.,* unpublished).

Techniques are required to enable each of the FEB pathogens to be detected and quantified separately in plant tissue, even where they are present as part of a mixture of pathogens. An array of such techniques, including novel molecular and monoclonal assays for the study of FEB have been produced recently. A unique set of highly specific and sensitive polymerase chain reaction (PCR)-based assays have been developed at the JIC for the detection and quantification of *Fusarium* species and *Microdochium* varieties causing FEB (Nicholson *et al.*, 1996; Parry & Nicholson, 1996; Nicholson *et al*., 1998, Turner *et al.*, 1998). These assays have been designed to provide an integrated system for the study of FEB to enable the relative amount of each pathogen species present to be determined without having to isolate into culture.

Given the complex nature of FEB, a clear understanding of the factors (including fungicides) affecting disease development, yield and quality loss and mycotoxin accumulation will only be gained through rigorous, detailed and controlled studies. The information gained from such studies may then be applied to develop efficient strategies to control all of the pathogens responsible for FEB along with their associated mycotoxins.

This project aims to identify the relative efficacies of a range of fungicides against toxin-producing and non toxin-producing species associated with FEB. Further to this, the effect of reducing fungicide dose and altering application time were also to be investigated. It was originally intended to carry out studies to determine the effect of different application techniques on control of FEB. A significant body of work had already been carried out by Bayer plc. who were willing to make this information available to the project. This permitted all three sites to participate in the fungicide efficacy trials. This was most fortunate as the three sites (CSL, HAUC and MRC) provided contrasting FEB populations and this, in turn, had significant implications for the control of FEB and the associated mycotoxins.

Field plots were established at three sites (CSL, HAAC and MRC). Plots were inoculated at mid-anthesis (GS 65) with a conidial mixture of the FEB pathogens of most relevance in the UK (*F. culmorum, F. graminearum, F. avenaceum, F. poae* and *M. nivale*) to establish a crop under high and,

most importantly, uniform disease pressure. Mist irrigation was applied during, and for selected periods after anthesis, to promote disease development and mycotoxin accumulation. Plants were treated with selected fungicides according to the manufacturers recommendations.

Efficacy of control was evaluated using a range of criteria. Ears were assessed for visual disease prior to ripening. At harvest, plot yields were determined where possible and 1000 grain weight was measured. Molecular diagnostic assays (species-specific quantitative PCR) was used determine how colonisation of grain by each of the pathogens was affected by particular fungicides. These assays provide the means to clearly reveal where fungicidal application alters the balance of species present or leads to the replacement of one pathogen by another.

Mycotoxin accumulation was determined in grain of the relevant material to determine the effect of species/fungicide/environment on mycotoxin accumulation and relate this to fungal biomass and disease levels. In the first year, quantification of mycotoxins was determined at CSL using gas chromatography/mass spectroscopy (GC-MS) and high performance liquid chromatography (HPLC) techniques to provide detailed information on the profile of toxins present. In subsequent years the prevalent mycotoxin at all sites was deoxynivalenol. Commercially available monoclonal antibodies to deoxynivalenol were used to determine the effect of fungicides on mycotoxin accumulation in the grain and to relate this to toxin production per unit of fungus in the grain.

## 2 Materials and Methods

### 2.1 Field experiments

Trials were carried out over a three year period (1998-2001) at three sites in England. These were in the north-east at the Central Science Laboratory (CSL), in the West Midlands at Harper Adams University College (HAUC) and in East Anglia at the Morley Research Centre (MRC). Each experiment consisted of a randomised block design with four replicates in which the effects of fungicide treatments were compared on single wheat cultivars at each site. The cultivars chosen have poor resistance to FEB pathogens.

### 2.2 Evaluation of fungicide efficacy against FEB pathogens and mycotoxin accumulation (year 1).

Winter wheat crops of Charger were established at CSL and MRC, and Equinox at HAUC in 1998/1999.

#### *2.2.1 Plot inoculation*

Plots were artificially inoculated at mid-anthesis (GS65) at HAUC (09/6/99) and MRC (14/6/99). Inoculation was delayed at CSL until GS73 (24/6/99), due to the closed flowering phenotype preventing accurate estimation of the growth stage. Inoculation was a mixed conidial suspension of consisting of *F. culmorum* (CSL - cc41, 45, 53, 70, 66; HAUC - cc70, cc53, Fc47/1, Fc, FWW/95; MRC - Fu42, 491b/1,339/1, 418/17), *F. graminearum* (CSL - cc20, 113, 120, 145, 148; HAUC - cc113, 145; MRC -

cc19, 20, 113, 120), *F. poae* (CSL - cc4, 9, 12, 14, 50; HAUC - Fp 97/05, Fp W71), *F. avenaceum* (CSL - cc32, 35, 37, 39, 40; HAUC - Fa 27, Fa 88; MRC - Fu34, 239, C2781, 430c/1), *M. nivale* var. *majus* (CSL - cc114, 115, 193; HAUC - 80/1, 30/3; MRC - 11.2, Mn21, Mn4/1,Mn10/1, Mn 29/1) and *M. nivale* var. *nivale* (CSL - cc224; HAUC - 94/1, 30/1, 117/1). At MRC *F. graminearum* isolates failed to sporulate in year 1, as a result they were not inoculated. Each species was applied at $10^5$ spores/ml in an application volume of 500, 333 and 200 l water/ha at CSL, HAUC and MRC respectively. Mist irrigation of plots started immediately after inoculation and lasted for 5, 7 and 12 days at CSL, HAUC and MRC respectively.

*2.2.2 Fungicide application*

Fungicides were applied 2 days post-inoculation at CSL (26/6/99) and HAUC (11/6/99), and 3 days post-inoculation at MRC (17/6/99). All fungicides (Table 1) were applied at full rate in 200 l water/ha

Table 1. *Fungicides tested in 1999 at CSL, HAUC and MRC against Fusarium ear blight pathogens*

| Fungicide | Dose (l or kg/ha) | Active ingredient | active ingredient (g/l or kg/ha) | Formulation |
|---|---|---|---|---|
| Untreated | - | - | - | - |
| HGCA 1 | 2.0 | ? | ? | ? |
| HGCA 2 | 0.8 | ? | ? | ? |
| HGCA 3 (Twist) | 2.0 | trifloxystrobin | 125 | EC |
| Plover | 0.3 | difenoconazole | 250 | EC |
| Folicur | 1.0 | tebuconazole | 250 | EW |
| Amistar | 1.0 | azoxystrobin | 250 | SC |
| Caramba | 1.5 | metconazole | 60 | SL |
| Sportak 45 | 0.9 | prochloraz | 450 | EC |
| Landmark | 1.0 | epoxiconazole + kresoxim-methyl | 125 + 125 | SC |
| Derosal[1] | 0.2 | carbendazim | 500 | SC |
| Bavistim[2] | 0.4 | carbendazim | 500 | SC |

[1] Applied at CSL and MRC [2] Applied at HAUC

*2.2.3 Disease assessment*

Plots were assessed for disease symptoms at two growth stages, GS 73-75 and GS 80-85. The protocol for assessment varied between sites as detailed below.

CSL - The % of the total ear area affected was assessed on 50 ears per plot. Discrete lesions on a single glume were assessed as 3%. More than one lesion on a spikelet or a bleached spikelet was assessed as 5%.

HAUC - Disease was assessed as % spikelets infected for 25 ears per plot.

MRC - The % ear area affected was assessed on 50 ears per plot

**2.3 Effect of fungicide dose and mixtures on FEB pathogens and mycotoxin accumulation (Year 2).**

Winter wheat crops of Charger were established at CSL and MRC, and Equinox at HAUC in 1999/2000.

*2.3.1 Plot inoculation*

At all 3 sites plots were artificially inoculated at GS65 (11/6/00 at CSL; 18/6/00 at HAUC; 12/6/00 at MRC). Plot inoculation and mist irrigation was as described in year 1, with the exception that *F. graminearum* (isolates cc19, 20, 113, 120) were applied at MRC.

*2.3.2 Fungicide application*

Fungicides were applied 2 days post-inoculation at CSL (13/6/00) and HAUC (20/6/00), and 3 days post-inoculation at MRC (15/6/00). Fungicides were applied in 200 l water/ha as detailed in Table 2.

*2.3.3 Disease assessment*

Disease assessments were carried out as detailed in year 1.

Table 2. *Treatments applied in 2000 at CSL, HAUC and MRC against Fusarium ear blight pathogens*

| Fungicide | Dose (l or kg/ha) | Active ingredient | active ingredient (g/l or kg/ha) | Formulation |
|---|---|---|---|---|
| Untreated | - | - | - | - |
| Folicur | 1.0 | tebuconazole | 250 | EW |
| Folicur | 0.5 | tebuconazole | 125 | EW |
| Caramba | 1.5 | metconazole | 60 | SL |
| Caramba | 0.75 | metconazole | 30 | SL |
| Amistar | 1.0 | azoxystrobin | 250 | SC |
| Amistar | 0.5 | azoxystrobin | 125 | SC |
| Landmark | 1.0 | epoxiconazole + kresoxim-methyl | 125 + 125 | SC |
| Opus | 1.0 | epoxiconazole | 125 | SC |
| Opus | 0.5 | epoxiconazole | 62.5 | SC |
| Amistar + Folicur | 0.5 + 0.5 | azoxystrobin + tebuconazole | 125 + 125 | SC + EW |
| Amistar + Caramba | 0.5 + 0.75 | azoxystrobin + metconazole | 125 + 30 | SC + SL |
| Amistar + Opus | 0.5 + 0.5 | azoxystrobin + epoxiconazole | 125 + 62.5 | SC + SC |
| HGCA 2 | 0.8 | - | - | - |
| HGCA 2 | 0.4 | - | - | - |
| HGCA 4 | 1.0 | - | - | - |

**2.4 Effects of fungicide timing and dose on FEB pathogens and mycotoxin accumulation (Year 3).**

Crops of winter wheat varieties Cadenza and Charger, and spring wheat variety Chablis were established at HAUC, MRC and CSL respectively in 2000/2001.

*2.4.1 Plot inoculation*

At all CSL and MRC plots were artificially inoculated at GS65 (29/6/01 at CSL; 18/6/01 at MRC). At HAUC GS 65 was missed due to retention of anthers so plots were inoculated at GS 70 (27/6/01). Plot inoculation and mist irrigation was as described in year 2. Spore levels inoculated were reduced to more closely mimic levels of FEB seen naturally.

*2.4.2 Fungicide application*

Fungicides were applied either pre- or post- inoculation as detailed in Table 3. At HAUC spray timing was 2 days pre- (25/6/01) or 2 days post-inoculation (29/6/01), at CSL spray timing was 3 days pre- (26/6/01) or 3 days post-inoculation (2/7/01) and at MRC spray timing was 6 days pre- (12/6/01) or 4 days post-inoculation (22/6/01). All fungicides were applied in 200 l water/ha.

Table 3. Treatments applied in 2001 at CSL, HAUC and MRC against Fusarium ear blight

| Fungicide | Timing of fungicide application | Dose (l or kg/ha) | Active ingredient | Active ingredient (g/l or kg/ha) | Formulation |
|---|---|---|---|---|---|
| Untreated | | - | | - | - |
| Amistar | Pre-inoculation | 0.5 | azoxystrobin | 125 | SC |
| Amistar | Pre-inoculation | 0.25 | azoxystrobin | 62.5 | SC |
| Folicur | Pre-inoculation | 0.5 | tebuconazole | 125 | EW |
| Folicur | Pre-inoculation | 0.25 | tebuconazole | 62.5 | EW |
| Amistar + Folicur | Pre-inoculation | 0.5 + 0.5 | azoxystrobin + tebuconazole | 125 + 125 | SC + EW |
| Amistar + Folicur | Pre-inoculation | 0.25 + 0.25 | azoxystrobin + tebuconazole | 62.5 + 62.5 | SC + EW |
| HGCA 2[1] | Pre-inoculation | 0.4 | - | - | - |
| HGCA 2[1] | Pre-inoculation | 0.2 | - | - | - |
| Amistar | Post-inoculation | 0.5 | azoxystrobin | 125 | SC |
| Amistar | Post-inoculation | 0.25 | azoxystrobin | 62.5 | SC |
| Folicur | Post-inoculation | 0.5 | tebuconazole | 125 | EW |
| Folicur | Post-inoculation | 0.25 | tebuconazole | 62.5 | EW |
| Amistar + Folicur | Post-inoculation | 0.5 + 0.5 | azoxystrobin + tebuconazole | 125 + 125 | SC + EW |
| Amistar + Folicur | Post-inoculation | 0.25 + 0.25 | azoxystrobin + tebuconazole | 62.5 + 62.5 | SC + EW |
| HGCA 2[1] | Post-inoculation | 0.4 | - | - | - |
| HGCA 2[1] | Post-inoculation | 0.2 | - | - | - |

[1] Applied only at CSL and MRC

*2.4.3 Disease assessment*

Disease assessments were carried out as detailed in year 1.

**2.5 Yield assessment**

In all three years plot yield, moisture content, thousand grain weight (TGW) and specific weight assessed for each plot at harvest. Samples (500 g) of grain from each plot were sent to John Innes Institute for PCR diagnostics and 500 g of grain was sent to CSL for mycotoxin analysis.

**2.6 Molecular diagnosis and quantification of fungal pathogens**

*2.6.1 Tissue preparation and DNA extraction*

Grain was freeze dried and ground to a fine powder in a ball mill (e.g. Glen Creston) prior to DNA extraction. Milled grain was added to 30 ml CTAB buffer (Nicholson & Parry, 1996) in 50 ml centrifuge tubes. Tubes were shaken well and incubated at 65$^{o}$C for 1-2 h with shaking at intervals. Following incubation, 10 ml of 5 M potassium acetate and 5 ml of chloroform/isoamyl alcohol (24:1) were added to each sample. The tubes were mixed by inversion, placed at -20$^{o}$C for 30 min and centrifuged at 3,000 rpm for 15 min. The aqueous phase was removed to a fresh tube and two volumes of ethanol (100%) were added followed by centrifugation as above to precipitate the DNA. The pellet was washed in a 70% solution of cold ethanol and dissolved in TE buffer (10 mM Tris HCL, 1 mM EDTA)at 0.1 µl mg$^{-1}$ dry weight of plant material. DNA was quantified by SybrGreen fluorescence (see below) and concentration figures used to prepare sub-samples at fixed concentrations (typically 40 ng µl$^{-1}$). DNA samples were stored at 4$^{o}$C until use (or frozen for long-term storage).

*2.6.2 DNA Quantification*

DNA was quantified according to the method described by Hopwood *et al*., (1997). Aliquots of each DNA sample were added to a solution containing 100 ppm SYBR Green (Flowgen) and assayed using a Fluroskan II plate reader (INC Biomedicals Ltd, UK), which measured emission at 538 nm after excitation at 485 nm. The DNA concentration was ascertained by comparison with a serial dilution (0.0-1.8 ng l$^{-1}$) of DNA (Hind III cut DNA) included on each plate. A standard curve relating DNA concentrations to excitation/emission figures was prepared ($r^2$ typically 0.99) and applied to excitation/emission figures from 1 µl of each DNA sample in 100 µl working dilution of SybrGreen. Duplicate readings were taken for each sample and mean concentrations determined. All samples were diluted to a fixed concentration (typically 40 ng µl$^{-1}$) on the basis of the above quantification prior to PCR.

*2.6.3 PCR amplification*

Competitive PCR for, *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. poae*, *M. n.* var. *majus* and *M. nivale* var. *nivale* was performed as described by Nicholson *et al* (1996) using relevant primer pairs for each species (Doohan *et al*., 1998; Nicholson *et al*., 1996, 1997; Parry & Nicholson, 1996; Turner *et al*., 1998). Amplification reactions were performed in volumes of 50 µl containing 200 ng DNA extracted from infected grain. The reaction buffer consisted of 100 µm each of dATP, dCTP, dGTP and dTTP, 100 nm each of forward and reverse primer for PCR reactions, and 0.8 units of Taq polymerase in 10 mM Tris-HCL (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCL, 100 µg/ml gelatine and 0.05% each of Tween 20 and Nonidet P-40 and the selected concentration of competitor template for the target species. Samples were applied to a preheated PCR block and denatured at 95$^{o}$C for 2 min prior to cycling. DNA was amplified using 'touchdown' PCR (Don *et al.*, 1991) to ensure specificity of product amplification. The annealing temperature was 66°C for the first 5 cycles and 64°C for the next 5 cycles, followed by 25 cycles at 62°C. The temperature cycle consisted of denaturation (95$^{o}$C) for 30 s, annealing (as described above) for 20 s and extension (72$^{o}$C) for 45 s with maximal ramping rates between temperatures. A final extension step of 5 min was incorporated followed by cooling to 10$^{o}$C until recovery of samples.

Following amplification, PCR products were separated by electrophoresis through 2% agarose gel. Gels were stained with ethidium bromide, viewed under UV light on a 'Gel Doc 1000' system (Bio-Rad) and analysed using Molecular Analyst software (Bio-Rad) to estimate the relative degree of amplification of the fungal and competitor PCR product in each sample. The relationship was then determined, for each dilution series, between the PCR product ratios and the amount of fungal DNA added to the reaction. This generated a standard curve, by reference to which the amount of fungal DNA, of the relevant species, in plant samples was estimated.

**2.7 Trichothecene analysis**

In the first year a full trichothecene analysis was carried out using GC/MS.

*2.7.1 Toxin extraction*

Grain samples were ground to flour and 20 g sub-samples shaken with 100 ml acetonitrile:water (84:16, v:v) for 120 minutes on a wrist action shaker. Each sample was then homogenised in an Ultra Turrax blender for 1 minute and filtered through Whatman No. 4 filter paper. Approximately 10 ml of each filtrate was passed through a Mycosep #225 clean-up column (Romer Labs Inc). This process was repeated a second time and 10 ml of the cleaned up extract removed to a clean vial. The 10 ml of cleaned up extract was evaporated to dryness under nitrogen at 50˚C.

*2.7.2 Spiked samples*

To 20 g of the ground sample 200 µl of 20 µg/ml mixed trichothecene standard solution was added. The solvent was allowed to evaporate before extraction proceeded.

*2.7.3 Derivatisation*

The dry residue was re-suspended in 50 µl of Tri-Sil/TBT, mixed for 30 seconds in a vortex mixer and heated to 80˚C for 30 minutes. On cooling 500 µl of hexane and 1 ml of phosphate buffer were added to the vial and mixed for 30 sec. The phases were allowed to separate and the hexane fraction transferred to a vial containing a small amount of sodium sulphate. An aliquot of extract was then transferred to an auto sampler vial.

*2.7.4 GC/MS Analysis*

Analysis of the extract was by gas chromatography/mass spectroscopy (GC/MS) using selected ion monitoring for deoxynivalenol (DON), nivalenol (NIV), 3-O-acetyldeoxynivalenol (3AcDON), 15-O-acetyldeoxynivalenol (15AcDON), fusarenon X (FUS X), T2-toxin (T2), HT2-toxin (HT2) and diacetoxyscirpenol (DAS).

**2.8 DON analysis by ELISA**

In 2000 and 2001 DON was assayed by ELISA using monoclonal antibody kits (DON FAST) supplied by R-Biopharm Rhone Ltd. The DON content of grain was analysed according to the manufacturer's instructions.

### 2.9 Statistical analyses

Data were analysed using Minitab (version 13). Effects of treatments on disease, yield variates, toxin levels and DNA quantity were determined by factorial analysis of variance. The data were transformed where relevant to obtain normality of distribution and variance. DNA quantities were analysed as pg fungal DNA $ng^{-1}$ total DNA following $log_{10}$ transformation. All treatments were compared to the untreated controls using Dunnett's test. Effects were considered to be significant where $P \leq 0.05$.

## 3 Evaluation of fungicide efficacy against FEB pathogens and mycotoxin accumulation.

### 3.1 Background

The effect of application of full rates of a range of fungicides applied shortly after inoculation of ears with conidia of several FEB pathogens was assessed at three sites, HAUC, CSL and MRC. The efficacy of treatment was determined by reference to inoculated but untreated plots at each site. The effect of fungicides was measured as reduced visual disease, reduced colonisation of grain by each fungal species, reduced trichothecene mycotoxin content of grain and yield parameters, including plot yield (t/ha), thousand grain weight (TGW) and specific weight (SPWT).

### 3.2.Disease control

*3.2.1 HAUC*

Disease (%spikelets infected) on the ears from untreated inoculated plots was 14.2% and 49.4% at GS75 and 85 (Figure 1). At GS75 no treatment differed significantly from the untreated plots with respect to disease severity. Disease was least where HGCA2, tebuconazole or metconazole had been applied. Two treatments, azoxystrobin and difenoconazole resulted in increased disease relative to the untreated plots but the differences were not statistically significant. Similarly, at GS85 no treatment differed significantly from the untreated plots. Disease was least on plots treated with HGCA2 and greatest where prochloraz had been applied. No treatment resulted in disease levels greater than those on the untreated plots.

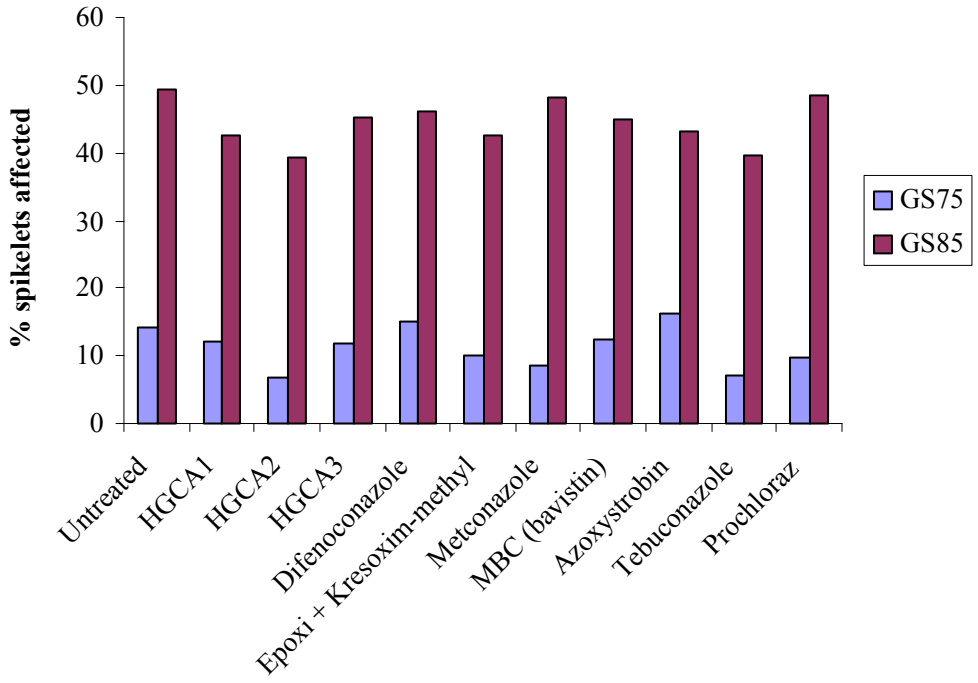



Figure 1. The effect of fungicide treatment on disease levels assessed at HAUC (GS 75 and 85) (1999). Bars with a value are significantly different from the untreated.

*3.2.2 CSL*

Disease (% total ear area affected) on the ears from untreated inoculated plots was 4.2% and 34.18% at GS 75 and 85 (Figure 2). At GS75 no treatment differed significantly from the untreated plots with respect to disease severity. Disease was least where tebuconazole, HGCA2 or metconazole had been applied. The majority of the other treatments also reduced disease. Two treatments difenoconazole and azoxystrobin resulted in increased disease relative to the untreated plots, however these differences were not statistically significant. At GS 85 the majority of treatments differed significantly from the untreated plots. Disease was least on plots treated with HGCA2, MBC, metconazole and tebuconazole. Only HGCA1 and difenoconazole failed to significantly reduce disease. No treatment resulted in increased disease relative to the untreated plots.

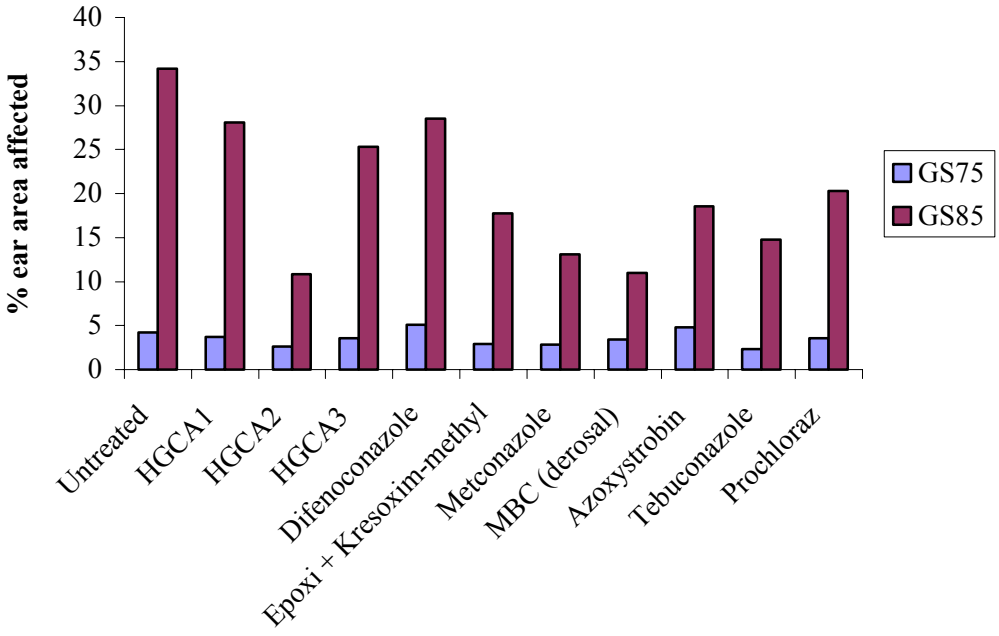



Figure 2. The effect of fungicide treatment on disease levels assessed at CSL (GS 75 and 85) (1999). Bars with a value are significantly different from the untreated.

*3.2.3 MRC*

Disease (% ear area affected) on the ears from untreated inoculated plots was 19.9% at GS 85 (Figure 3). Disease was significantly reduced where metconazole, tebuconazole, epoxiconazole + kresoxim-methyl, HGCA2, prochloraz or HGCA3 had been applied. No treatment resulted in increased disease relative to the untreated plots.

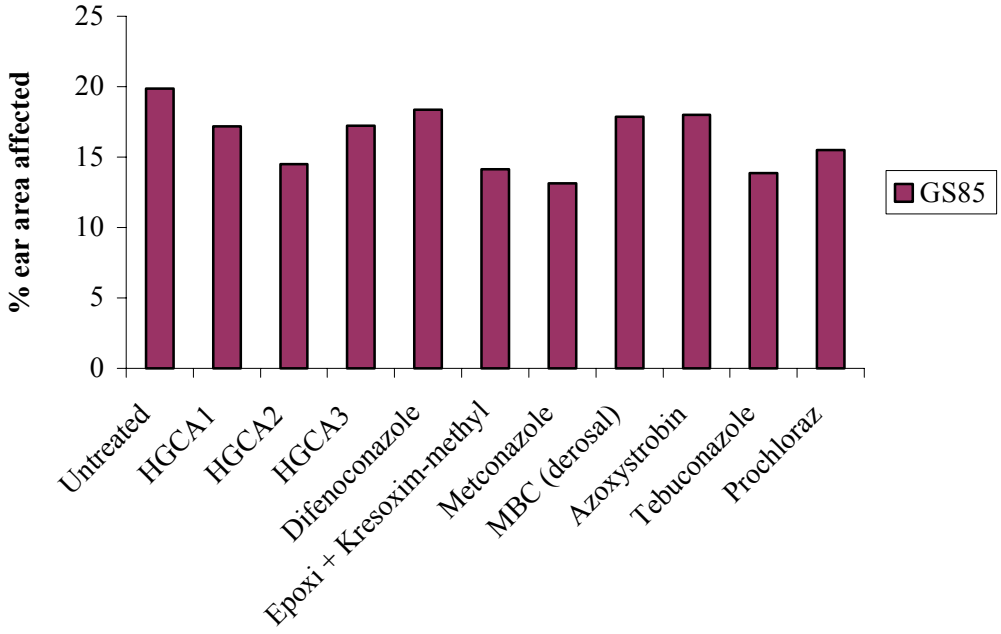



Figure 3. The effect of fungicide treatment on disease levels assessed at MRC (GS 85) (1999). Bars with a value are significantly different from the untreated.

### 3.3 Fungal species

*3.3.1 HAUC*

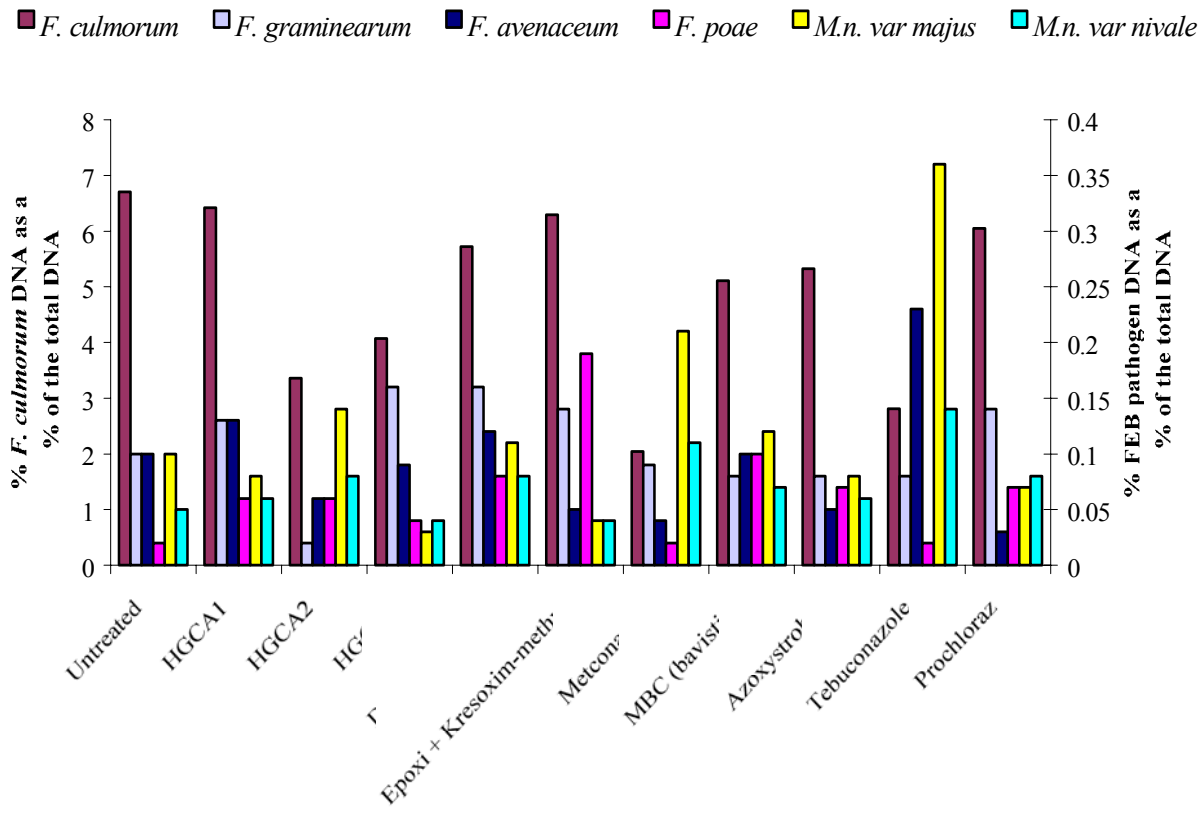
The predominant species present in grain was *F. culmorum* although *F. graminearum, F. avenaceum, F. poae* and both the *M. nivale* varieties were also present, however these were at relatively low levels (Figure 4). The amount of *F. culmorum* was reduced relative to that in the grain from untreated plots (6.7% fungal DNA) by several of the treatments including metconazole (2.0%), tebuconazole (2.8%), HGCA2 (3.4%) although only the reduction by metconazole was statistically significant. The level of *F. graminearum* was significantly reduced relative to that of the untreated plots (0.1%) only by HGCA2 (0.02%). No treatment significantly reduced *F. avenaceum, F. poae* or either variety of *M. nivale*. *F. poae* and *M. nivale* var. *nivale* increased significantly relative to the untreated plots following sprays of epoxiconazole + kresoxim-methyl and tebuconazole respectively.

*3.3.2 CSL*

The predominant species present in grain was *F. culmorum* although *F. graminearum* and *F. poae* were also present at moderate levels. *Fusarium avenaceum,* was present at low levels while both *M. nivale* varieties were present only at very low levels (Figure 5). The amount of *F. culmorum* was reduced relative to that in the grain from untreated plots (3.4% fungal DNA) by several of the treatments including HGCA2 0.2%), MBC (0.3%) tebuconazole (0.5%), metconazole (1.0%) and prochloraz (1.1%). The level of *F. graminearum* was significantly reduced relative to that of the untreated plots (0.6%) only by HGCA2 (0.4%). Both azoxystrobin (0.7%) and HGCA3 (0.6%) had a higher level of *F.*

*graminearum* present than the untreated although the increase was not significant. Both HGCA2 (0.3%) and tebuconazole (0.3%) significantly reduced the level of *F. avenaceum* present in grain relative to that from untreated plots. No treatment significantly reduced *F. poae* or either variety of *M. nivale*.

Figure 4. The effect of fungicide treatment on FEB pathogens (as determined by PCR analysis) at HAUC (1999).




*3.3.3 MRC*

The predominant species present in grain at this site was *F. culmorum* although *F. avenaceum* was also present at moderate levels. *Fusarium graminearum, F. poae* and both *M. nivale* varieties were present at very low levels (Figure 6). The amount of *F. culmorum* was significantly reduced relative to that in the grain from untreated plots (8.2% fungal DNA) by several of the treatments including metconazole (2.3%), tebuconazole (2.4%), HGCA2 (2.5%,), epoxiconazole + kresoxim-methyl (2.8%) and MBC (3.0%). The level of *F. avenaceum* was significantly reduced relative to that of the untreated plots (1.3%) only by HGCA2 (0.4%) and tebuconazole (0.6%). No treatment significantly reduced *F. graminearum, F. poae* or either variety of *M. nivale* probably because of the very low levels present.

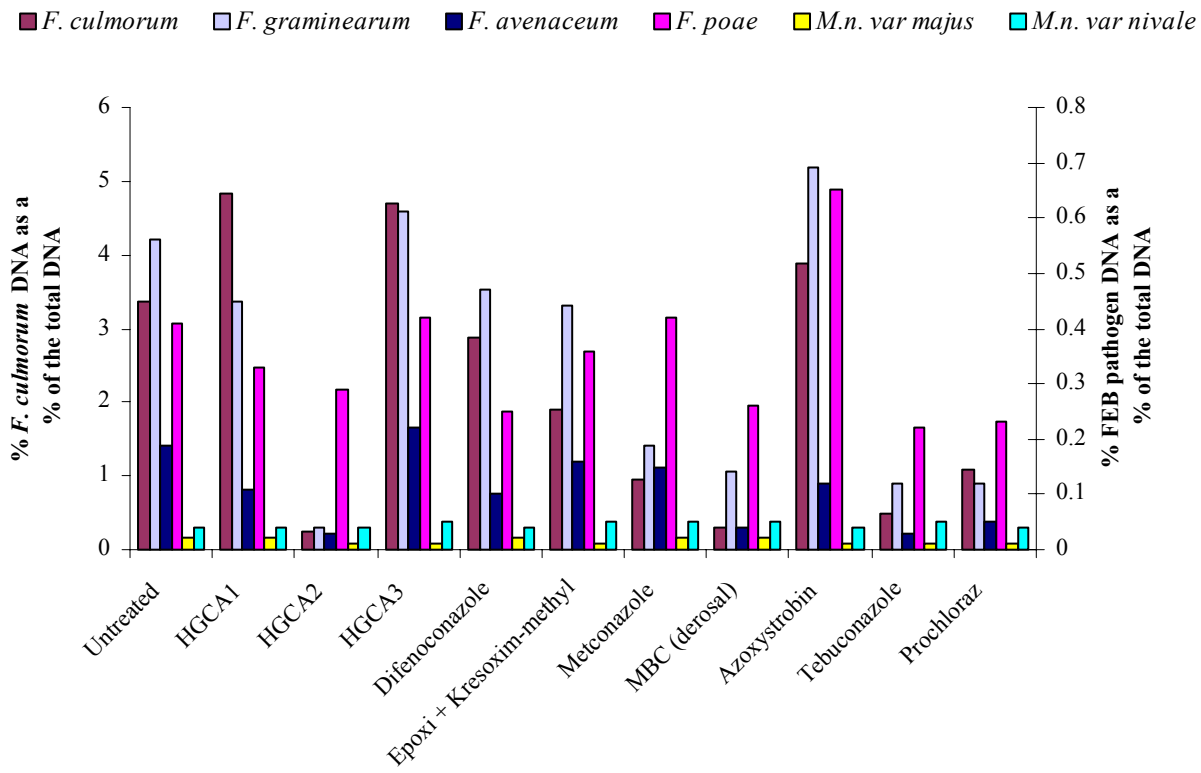



Figure 5. The effect of fungicide treatment on FEB pathogens (as determined by PCR analysis) at CSL (1999).

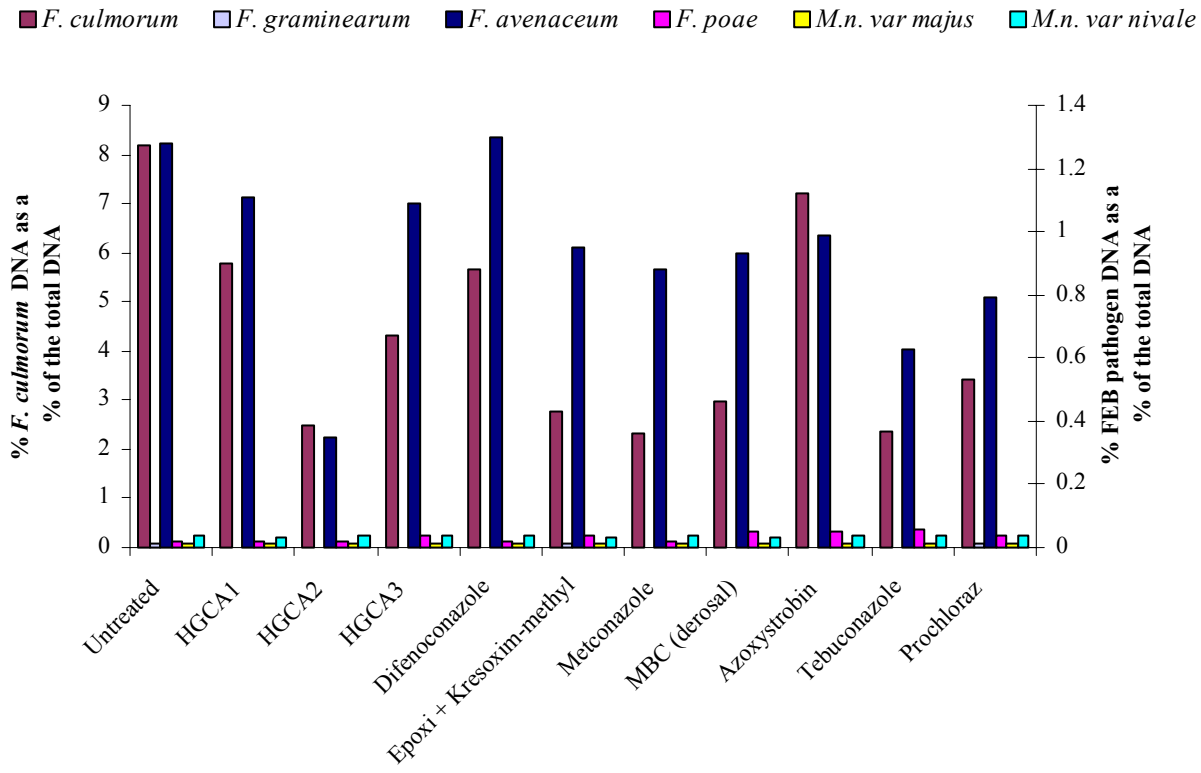



Figure 6. The effect of fungicide treatment on FEB pathogens (as determined by PCR analysis) at MRC (1999).

### 3.4 Trichothecene mycotoxins

*3.4.1 HAUC*

DON was the predominant trichothecene detected in the grain (9.29 ppm in the untreated) although significant amounts of NIV were also present (1.39 ppm in the untreated) (Figure 7). Very low levels of 3 and 15-acetylDON, DAS, HT-2 and T2 were present while the level of fusarenone X was below the detection limit. The level of DON was significantly reduced by HGCA2, metconazole and tebuconazole. The level of DON was higher than that in the untreated following treatment with azoxystrobin and HGCA1 although in neither case was the increase significant. No treatment significantly influenced the level of NIV but the lowest levels were those following treatment with tebuconazole, metconazole and HGCA2 and the highest levels were in plots treated with azoxystrobin and HGCA1. No treatment significantly affected the level of any of the other trichothecene mycotoxins assayed, probably due to the very low levels present.

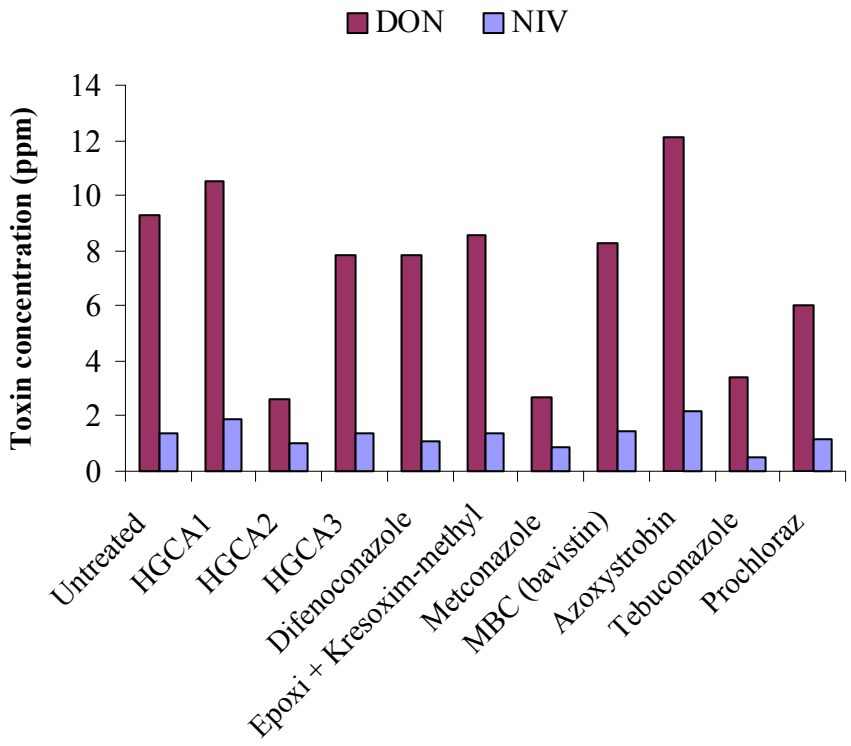



Figure 7. The effect of fungicide treatment on deoxynivalenol and nivalenol concentration in grain at HAUC (1999).

*3.4.2 CSL*

DON was the predominant trichothecene detected in the grain (12.12 ppm in the untreated) although significant amounts of NIV were also present (1.29 ppm in the untreated) (Figure 8). Low levels of 3 and 15-acetyl DON and very low levels of HT-2 and T2 were present while the levels of DAS and fusarenone X were below the detection limit. The level of DON was significantly reduced by HGCA2, MBC, metconazole, tebuconazole, prochloraz and epoxiconazole + kresoxim-methyl. No treatment resulted in a higher level of DON than that in the untreated plots. No treatment significantly influenced the level of NIV but the lowest levels were those following MBC, HGCA2 and tebuconazole. No

treatment significantly affected the level of any of the other trichothecene mycotoxins assayed, probably due to the very low levels present.

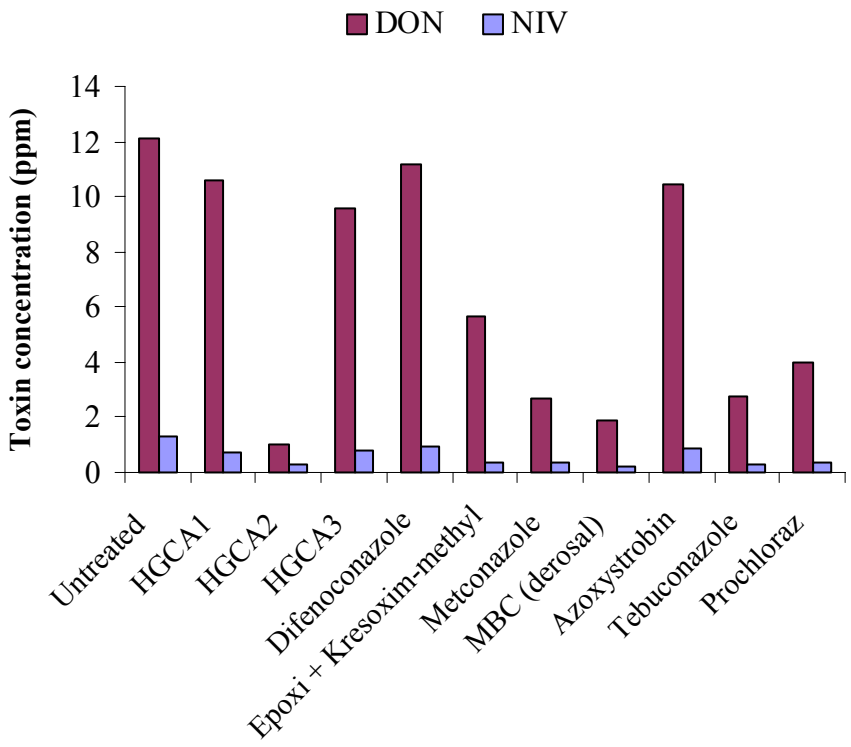



Figure 8. The effect of fungicide treatment on deoxynivalenol and nivalenol concentration in grain at CSL (1999).

*3.4.3 MRC*

Unlike the other two sites, NIV was the predominant trichothecene detected in the grain (2.53 ppm in the untreated) although significant amounts of DON were also present (0.44 ppm in the untreated) (Figure 9). Very low levels of 3 and 15-acetyl DON, HT-2 and T2 were present while the level of fusarenone X and DAS was below the detection limit. The level of NIV was significantly reduced by MBC, epoxiconazole + kresoxim-methyl, prochloraz and HGCA2. No treatment significantly influenced the level of DON but the lowest levels were those following epoxiconazole + kresoxim-methyl, MBC and prochloraz. No treatment significantly affected the level of any of the other trichothecene mycotoxins assayed, probably due to the very low levels present.

**3.5 Yield parameters**

*3.5.1 HAUC*

Yield was assessed by three measures; tonnes/hectare at 15% moisture (t/ha), thousand grain weight (TGW) and specific weight as kg per hectalitre (SPWT) (Table 4). The untreated plots produced 6.8 t/ha. While all treatments, except MBC, resulted in increased yield the increase was significant only for metconazole and HGCA2. The weight of 1000 grains for the untreated was 44.04g and all treatments resulted in a higher TGW (Table 4). The increase was significant for metconazole, tebuconazole,

HGCA2 and epoxiconazole + kresoxim-methyl. Specific weight was also greater than the untreated for all treatments with the increase being significant for HGCA2, metconazole, and tebuconazole (Table 4).

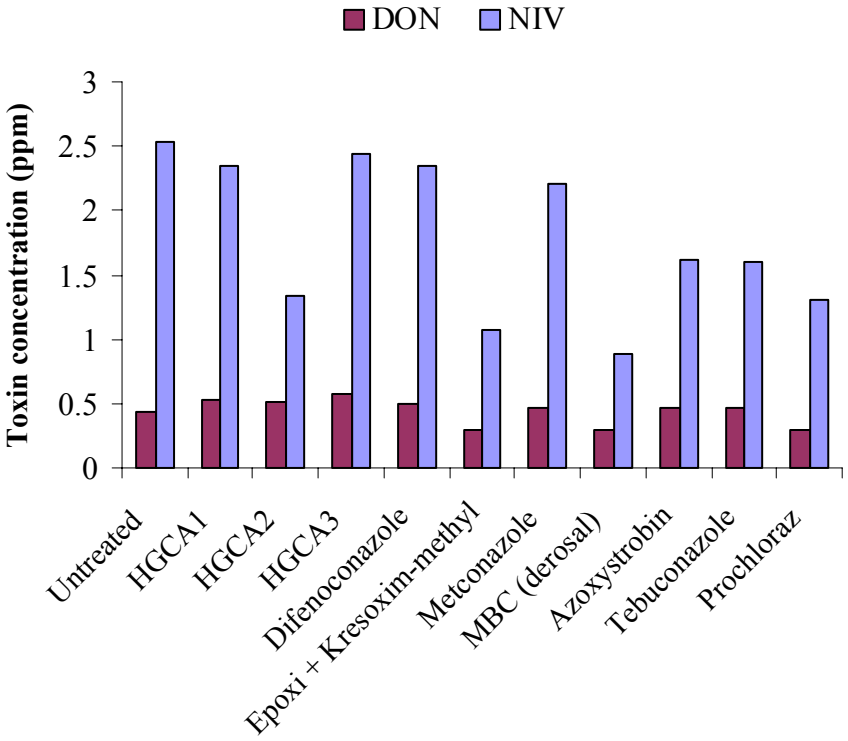



Figure 9. The effect of fungicide treatment on deoxynivalenol and nivalenol concentration in grain at MRC (1999).

Table 4. Effects of fungicide application on yield parameters at HAUC (1999).

| Fungicide | Yield (t/ha) | TGW (g) | Specific weight (kg/hl) |
|---|---|---|---|
| Untreated | 6.80 | 44.04 | 55.25 |
| HGCA1 | 6.92 | 47.38 | 57.43 |
| HGCA2 | 8.15 | 52.52 | 63.40 |
| HGCA3 | 7.15 | 49.32 | 59.05 |
| Difenoconazole | 7.22 | 47.55 | 56.90 |
| Epoxiconazole + Kresoxim-methyl | 7.91 | 50.45 | 58.75 |
| Metconazole | 8.27 | 53.00 | 62.88 |
| MBC (Bavistin) | 6.67 | 45.82 | 55.65 |
| Azoxystrobin | 6.96 | 46.63 | 55.93 |
| Tebuconazole | 7.96 | 52.72 | 61.85 |
| Prochloraz | 7.48 | 48.65 | 58.88 |

*3.5.2 CSL*

Yield was assessed by TGW (Table 5). The TGW for untreated plots was 44.59g. All treatments resulted in increased TGW but the increase was not statistically significant for any treatment. The highest TGW was achieved following treatment with epoxiconazole + kresoxim-methyl, azoxystrobin, MBC and HGCA3.

Table 5. Effects of fungicide application on yield parameters at CSL (1999).

| Fungicide | TGW (g) |
|---|---|
| Untreated | 44.59 |
| HGCA1 | 44.81 |
| HGCA2 | 46.35 |
| HGCA3 | 47.35 |
| Difenoconazole | 44.67 |
| Epoxiconazole + Kresoxim-methyl | 47.79 |
| Metconazole | 45.28 |
| MBC (Bavistin) | 47.63 |
| Azoxystrobin | 47.77 |
| Tebuconazole | 46.74 |
| Prochloraz | 46.60 |

*3.5.3 MRC*

Yield was assessed by two measures; tonnes/hectare at 15% moisture (t/ha) and TGW. The untreated plots produced 9.54 t/ha (Table 6). All treatments except HGCA3 and difenoconazole resulted in significantly increased yield. The weight of 1000 grains for the untreated was 44.71g but no treatment resulted in a significantly higher TGW (Table 6).

Table 6. Effects of fungicide application on yield parameters at MRC (1999).

| Fungicide | Yield (t/ha) | TGW (g) |
|---|---|---|
| Untreated | 9.54 | 44.71 |
| HGCA1 | 10.10 | 46.24 |
| HGCA2 | 10.72 | 47.06 |
| HGCA3 | 10.04 | 44.11 |
| Difenoconazole | 9.88 | 44.71 |
| Epoxiconazole + Kresoxim-methyl | 10.60 | 46.86 |
| Metconazole | 10.72 | 46.36 |
| MBC (derosal) | 10.37 | 46.43 |
| Azoxystrobin | 10.18 | 44.61 |
| Tebuconazole | 10.78 | 46.40 |
| Prochloraz | 10.36 | 44.37 |

**3.6 Discussion**

The relative efficacy of fungicides at the three sites were generally similar although some interesting differences were observed. Visual disease assessment generally reflected differences in fungal colonisation of grain and toxin accumulation although on some sites and at some times after inoculation

significant differences in disease could not be observed while the level of fungal colonisation and mycotoxins present in harvested grain was found to differ significantly. HGCA2, metconazole and tebuconazole reduced disease symptoms most overall although MBC was also highly effective at CSL.

Differences were observed in the fungal populations at the three sites although *Fusarium culmorum* was the main pathogen that developed and colonised the grain at all sites. At CSL, moderate levels of *F. graminearum* and *F. poae* were also present along with low levels of *F. avenaceum* while at HAUC the levels of *F. graminearum* and *F. avenaceum* were low and *F. poae* was very low. At MRC moderate levels of *F. avenaceum* were found while both *F. graminearum* and *F. poae* were at very low levels. This latter finding provides evidence that the pathogens that colonised the grain were those inoculated rather than arising from natural inoculum at the site (neither species was included in the inoculum). The level of both *M. nivale* varieties was very low at both CSL and MRC sites being present at slightly higher levels at HAUC. Over all sites, three fungicides were most effective at reducing the level of *F. culmorum* (the major pathogen), these being metconazole, tebuconazole and HGCA2. At two sites (CSL and MRC) MBC fungicide also significantly reduced the colonisation of grain by *F. culmorum*. No effect was observed at HAUC. The reasons for this difference are not known although the product used at the latter site (Bavistin) differed from that at CSL and MRC (Derosal).

Reduction in the amount of *F. culmorum* present in grain was generally associated with a reduced level of the predominant trichothecene, this being DON at the CSL and HAUC sites and NIV at MRC. The greater the reduction in the amount of *F. culmorum* colonising the grain, the lower the amount of trichothecene toxin present. The fungicides appear to act similarly against DON and NIV producing isolates of *F. culmorum*. This result is of significance because of the presence of both NIV and DON producing isolates in the UK. The mean level of DON was greater than the untreated in only two cases, albeit non significant. At HAUC, the mean DON content was higher following application of azoxystrobin or difenoconazole than for the untreated. This site was the only one where either variety of *M. nivale* was present at reasonable levels. This is of significance in light of results obtained in the second year of trials. Although moderate levels of *F. poae* were present at the CSL site, the level of type A trichothecenes (DAS, HT-2, T-2) was extremely low. The levels of these mycotoxins was also extremely low at HAUC and MRC where this species was only present at extremely low levels.

The effect of the various fungicides on yield parameters differed across the three sites. Yield was unaffected by any treatment at CSL while at HAUC yield was significantly increased by both HGCA2 and metconazole. In contrast, at MRC only two fungicides failed to effect a significant increase in yield (HGCA3 and difenoconazole). Thousand grain weight was not influenced by any treatment at CSL or MRC while at HAUC thousand grain weight was significantly increased by four fungicides (HGCA2, metconazole, tebuconazole and epoxiconazole + kresoxim-methyl). Specific weight was also significantly increased by three of these (HGCA2, metconazole and tebuconazole).

Overall, three fungicides (HGCA2, metconazole and tebuconazole) appeared to be most effective at reducing visual disease, fungal colonisation of grain and mycotoxin accumulation in grain. While the effect on various yield parameters differed somewhat across sites these fungicides also generally produced higher yield (t/ha) and thousand grain weight.

# 4 Effect of fungicide dose and mixtures against Fusarium ear blight and deoxynivalenol accumulation in grain of winter wheat.

## 4.1 Background

The results from the first year of trials had indicated that application of full dose of three fungicides, HGCA2, metconazole and tebuconazole could reduce the level of disease, colonisation by *Fusarium* species and accumulation of trichothecenes in grain of wheat. Results from other studies had demonstrated the efficacy of azoxystrobin against *M. nivale* another important pathogen associated with FEB in the UK. A further fungicide, epoxiconazole had not been assessed in the first year of trials and this was included to determine whether it has activity against FEB. Trials were undertaken to determine the effect of reduced fungicide dose on disease control and to assess whether the combination of compounds active against *Fusarium* species with azoxystrobin (active against *M. nivale*) could provide control of the whole spectrum of FEB pathogens encountered under UK growing conditions. A full list of treatments is given in Table 2.

## 4.2 Disease control

### *4.2.1 HAUC*

Disease (% spikelets infected) on the ears from untreated inoculated plots was 47.5% and 62.5% at GS 75 and GS 85 respectively (Figure 10). At GS75 no treatment differed significantly from the untreated plots with respect to disease severity although the lowest level of disease was where tebuconazole (0.5 dose) or azoxystrobin (0.5 dose) + epoxiconazole (0.5 dose) had been applied and the majority of other treatments also reduced disease. In contrast, at GS85 the majority of treatments significantly reduced disease relative to that on the untreated plots (Figure 10). Only, tebuconazole (1 and 0.5 dose), metconazole (1 dose), HGCA2 (0.5 dose) and epoxiconazole (0.5 dose) did not significantly reduce disease relative to the untreated plots.

### *4.2.2 CSL*

Disease (% total ear area affected) on the ears from untreated inoculated plots was 1.6% and 30.4% at GS 75 and GS 85 respectively (Figure 11). At GS75 all treatment differed significantly from the untreated plots with respect to disease severity. The lowest level of disease followed treatment with tebuconazole, metconazole and. At GS85 all the treatments, except azoxystrobin (0.5 dose) significantly reduced disease relative to that on the untreated plots (Figure 11).

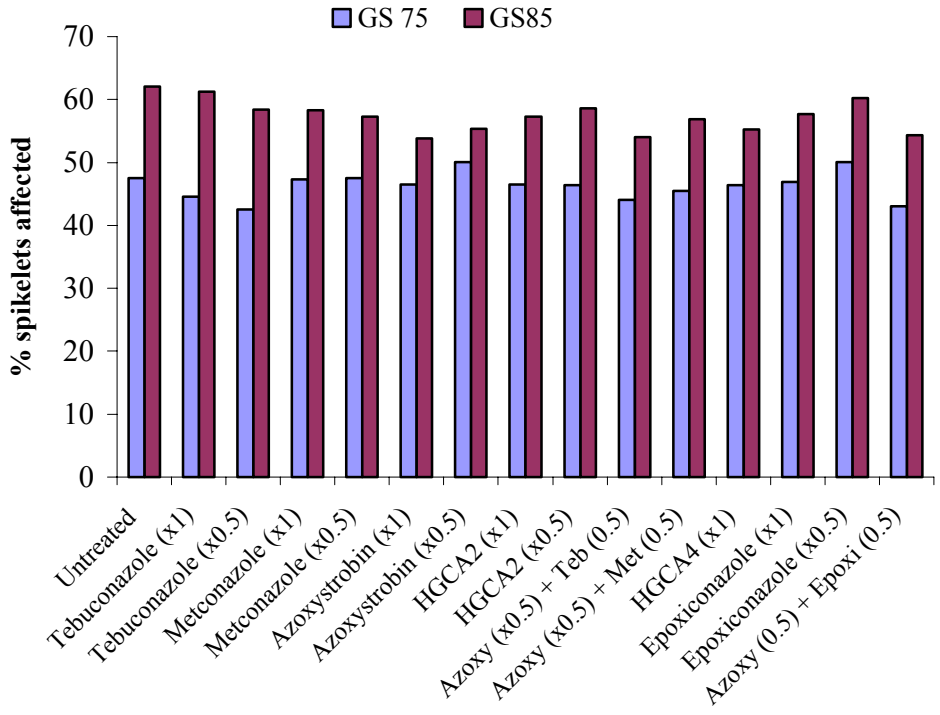



Figure 10. The effect of fungicide treatment on disease levels assessed at HAUC (GS 75 and 85) (2000).

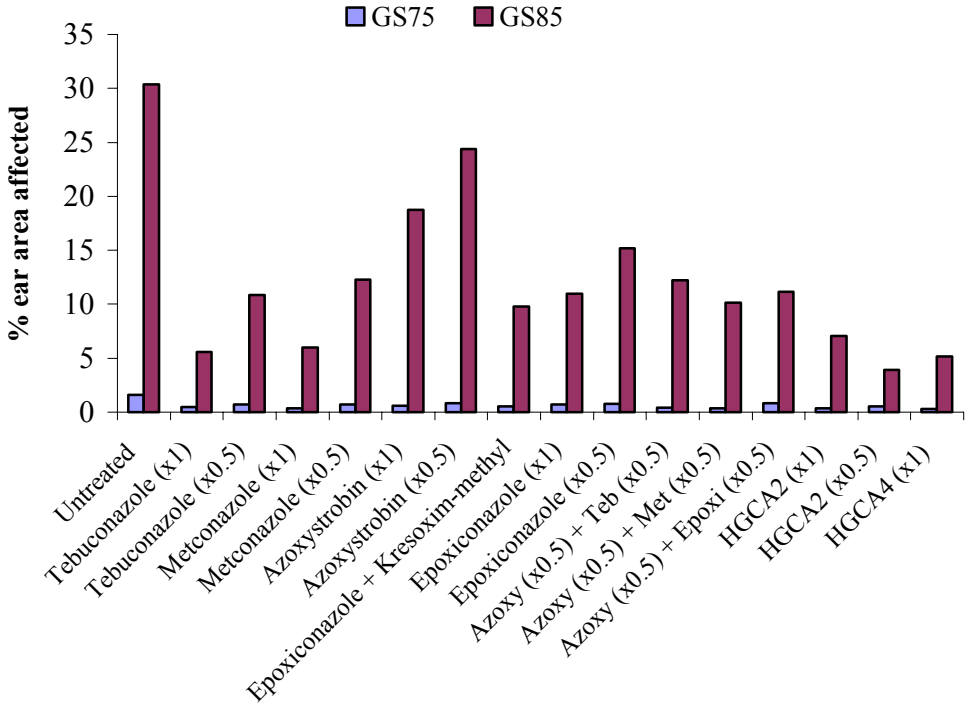



Figure 11. The effect of fungicide treatment on disease levels assessed at CSL (GS 75 and 85) (2000).

*4.2.3 MRC*

Disease (% ear area affected) on the ears from untreated inoculated plots was 45% at GS85 (Figure 12). At this time all treatments had significantly less disease than the untreated plots with the lowest level of disease where azoxystrobin + epoxiconazole (0.5+0.5 dose), epoxiconazole + kresoxim-methyl (1 dose), HGCA4 (1 dose), and azoxystrobin + tebuconazole (0.5+0.5 dose) had been applied.

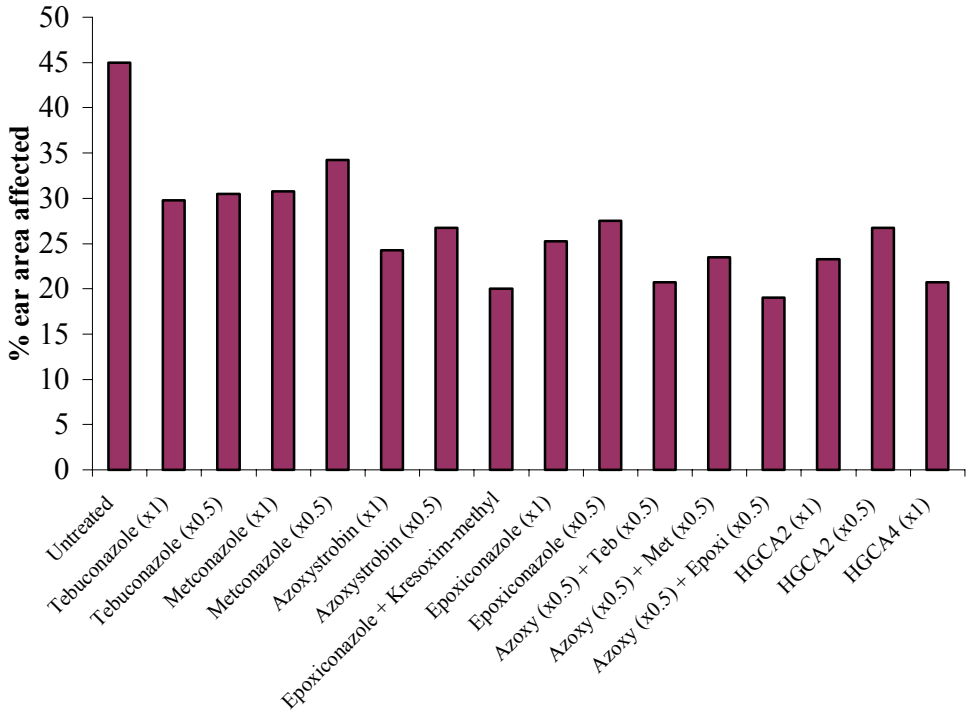



Figure 12. The effect of fungicide treatment on disease levels assessed at MRC (GS 85) (2000).

**4.3 Fungal species**

*4.3.1 HAUC*

The predominant species present in grain at this site was *F. avenaceum* (5.9% fungal DNA) although *F. graminearum* (2.38%) was also present at moderate levels. Both *M. nivale* var. *majus* and var. *nivale* were present at low levels (0.29% and 0.15% respectively). The level of *F. culmorum* (0.12%) was much less than that of *F. avenaceum* and *F. graminearum* while *F. poae* was absent from most plots (Figure 13). No treatment significantly influenced the amount of the predominant *Fusarium* species (*F. avenaceum* and *F. graminearum*) in the grain although the level of *F. avenaceum* was least where full dose tebuconazole or HGCA2 had been applied while the lowest amount of *F. graminearum* was present in plots treated with full dose metconazole. The amount of *F. culmorum* was reduced relative to that in the grain from untreated plots by a single treatment, metconazole (0.03%). The level of *M. nivale* var. *majus* was significantly reduced relative to that of the untreated plots by several treatments, the majority of which included azoxystrobin either alone or in mixture (azoxystrobin (0.5 dose), azoxystrobin + tebuconazole (0.5+0.5 dose), azoxystrobin + metconazole (0.5+0.5 dose), azoxystrobin

+ epoxiconazole (0.5+0.5 dose) and HGCA4 (1 dose)). Four treatment significantly reduced *M. nivale* var. *nivale*, all of which included azoxystrobin either alone or in mixture (azoxystrobin (1 and 0.5 dose), azoxystrobin + tebuconazole (0.5+0.5 dose), and azoxystrobin + epoxiconazole (0.5+0.5 dose).

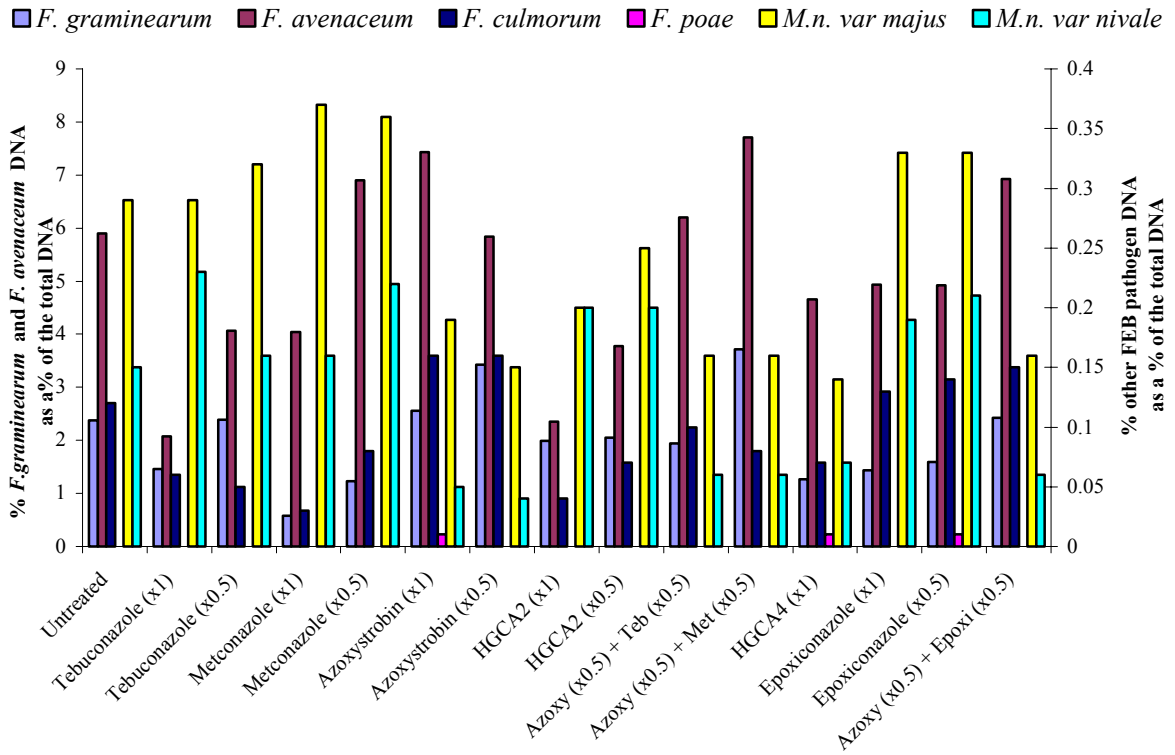



Figure 13. The effect of fungicide treatment on FEB pathogens (as determined by PCR analysis) at HAUC (2000).

*4.3.2 CSL*

The predominant species present in grain at this site was *F. graminearum* (2.85% fungal DNA) although *F. avenaceum* (1.51%) and *F. culmorum* (0.57%) were also present at moderate levels. Both *M. nivale* var. *majus* and *F. poae* were present at very low levels (0.07% and 0.03% respectively) while *M. nivale* var. *nivale* was negligible (0.01%) (Figure 14). The majority of treatments significantly reduced the amount of the predominant *Fusarium* species. The level of *F. graminearum* was least where HGCA4, HGCA2 (1 and 0.5 dose), metconazole (1 dose) and tebuconazole (1 dose) had been applied. Only five treatments failed to significantly reduce the level of *F. graminearum* in grain relative to that from untreated plots (azoxystrobin (1 dose), epoxiconazole + kresoxim-methyl (1 dose), azoxystrobin + epoxiconazole (0.5+0.5 dose), metconazole (0.5 dose) and epoxiconazole (0.5 dose)). Six treatments significantly reduced the level of *F. culmorum* relative to that in grain from untreated plots. These were HGCA2 (1 and 0.5 dose), tebuconazole (1 and 0.5 dose), metconazole (1 dose) and HGCA4. The level of *F. avenaceum* was significantly reduced relative to that of untreated plots only by metconazole (1 dose). All treatments containing azoxystrobin, even in mixtures, significantly reduced the level of *M. nivale* var. *majus*, as did HGCA4 (Figure 14).

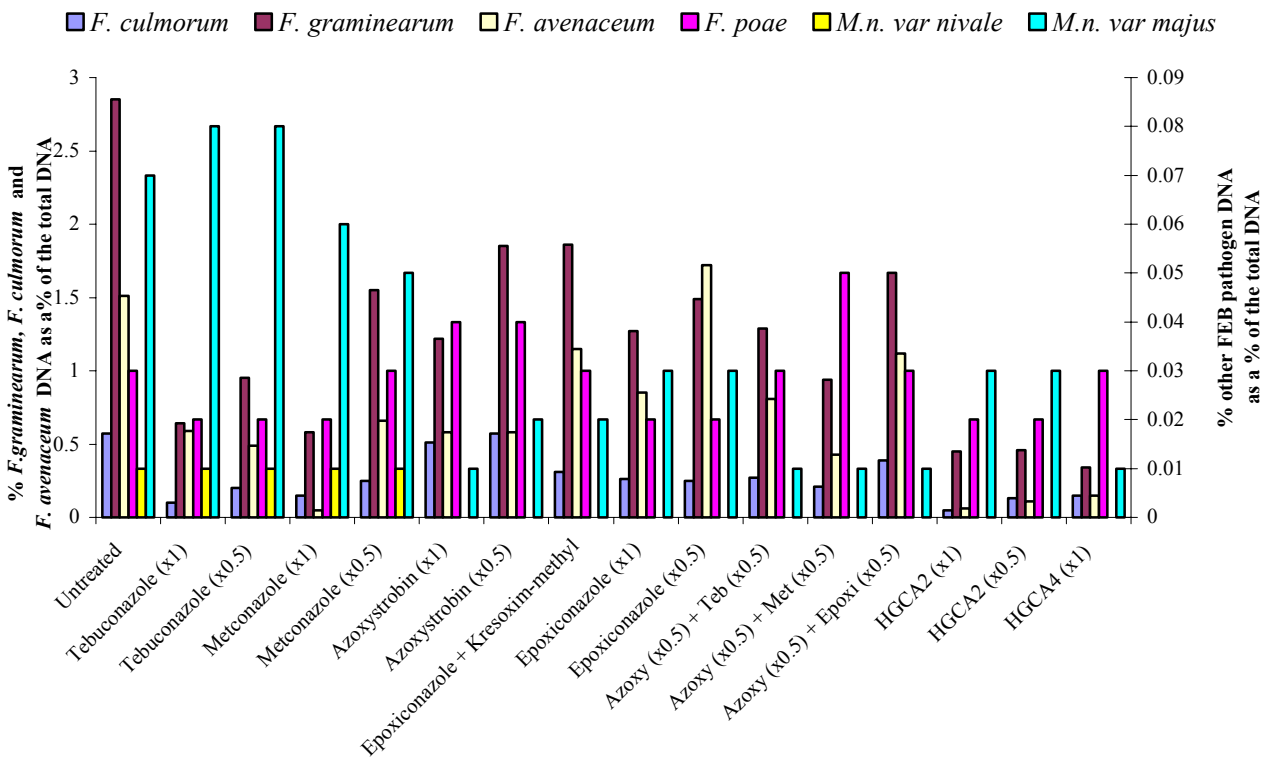



Figure 14. The effect of fungicide treatment on FEB pathogens (as determined by PCR analysis) at CSL (2000).

*4.3.3 MRC*

The predominant *Fusarium* species present in grain at this site was *F. avenaceum* (1.57% fungal DNA) although *F. culmorum* (0.18%) was also present at moderate levels (Figure 15). The level of *F. graminearum* (0.12%) was much less than that of *F. avenaceum* while *F. poae* was absent from most plots (Table 4.6). The level of *M. nivale* var. *majus* was moderate (0.33%) while that of *M. nivale* var. *nivale* was low (0.05%). No treatment significantly influenced the amount of any of the *Fusarium* species in the grain although the level of *F. avenaceum* was least where full or half dose HGCA2 had been applied while the lowest amount of *F. culmorum* was present in plots treated with tebuconazole (1 and 0.5 dose), HGCA2 (1 dose) or metconazole (1 dose). The level of *M. nivale* var. *majus* was significantly reduced relative to that of the untreated plots by all treatments that included azoxystrobin either alone or in mixture (azoxystrobin (1 and 0.5 dose), azoxystrobin + tebuconazole (0.5+0.5 dose), azoxystrobin + metconazole (0.5+0.5 dose), azoxystrobin + epoxiconazole (0.5+0.5 dose) and also by HGCA4 (1 dose)). Three treatments significantly reduced *M. nivale* var. *nivale*, these again included azoxystrobin either alone or in mixture (azoxystrobin (1 and 0.5 dose), azoxystrobin + metconazole (0.5+0.5 dose)).

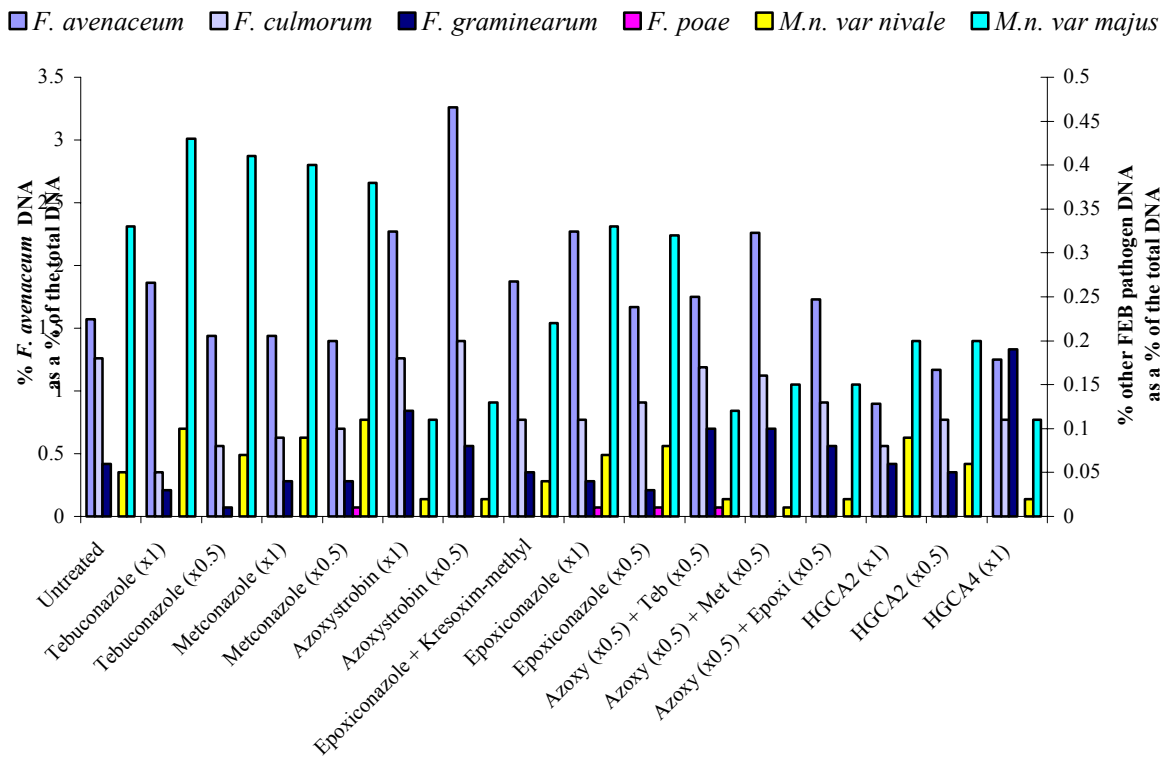



Figure 15. The effect of fungicide treatment on FEB pathogens (as determined by PCR analysis) at MRC (2000).

### 4.4 Deoxynivalenol content of harvested grain

*4.4.1 HAUC*

The level of DON in the grain was 7.3 ppm in the untreated (Figure 16). No treatment significantly reduced the amount of DON in the grain although the mean level was less for most treatments. Notably, the level of DON was significantly increased relative to that of the untreated plots where azoxystrobin (0.5 dose) had been applied. The level of DON was also greater where azoxystrobin (1 dose) or azoxystrobin + tebuconazole (0.5+0.5 dose) had been applied but this was not statistically significant.

*4.4.2 CSL*

The level of DON in the grain was very high (13.6 ppm) in the untreated (Figure 17). The great majority of treatments significantly reduced the amount of DON in the grain with the least DON being where HGCA2 (1 and 0.5 dose), HGCA4, epoxiconazole (1 dose) or tebuconazole (1 dose) had been applied. Only two treatments failed to significantly reduce the level of DON accumulating in the grain (azoxystrobin (1 and 0.5 dose).

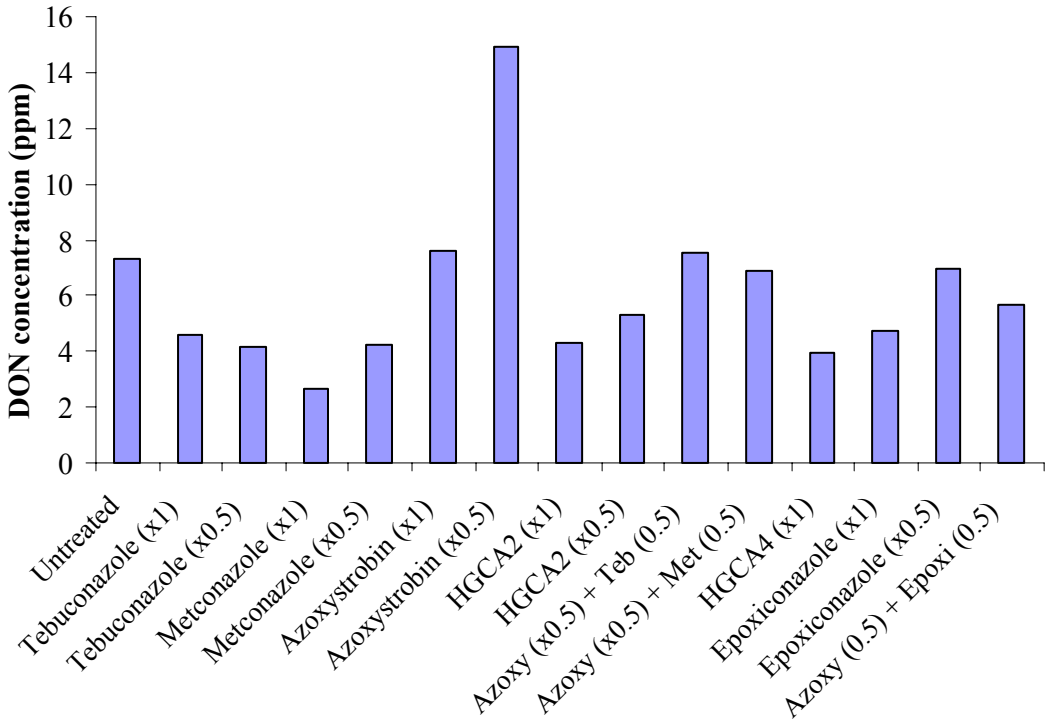



Figure 16. The effect of fungicide treatment on DON concentration in grain from HAUC (2000).

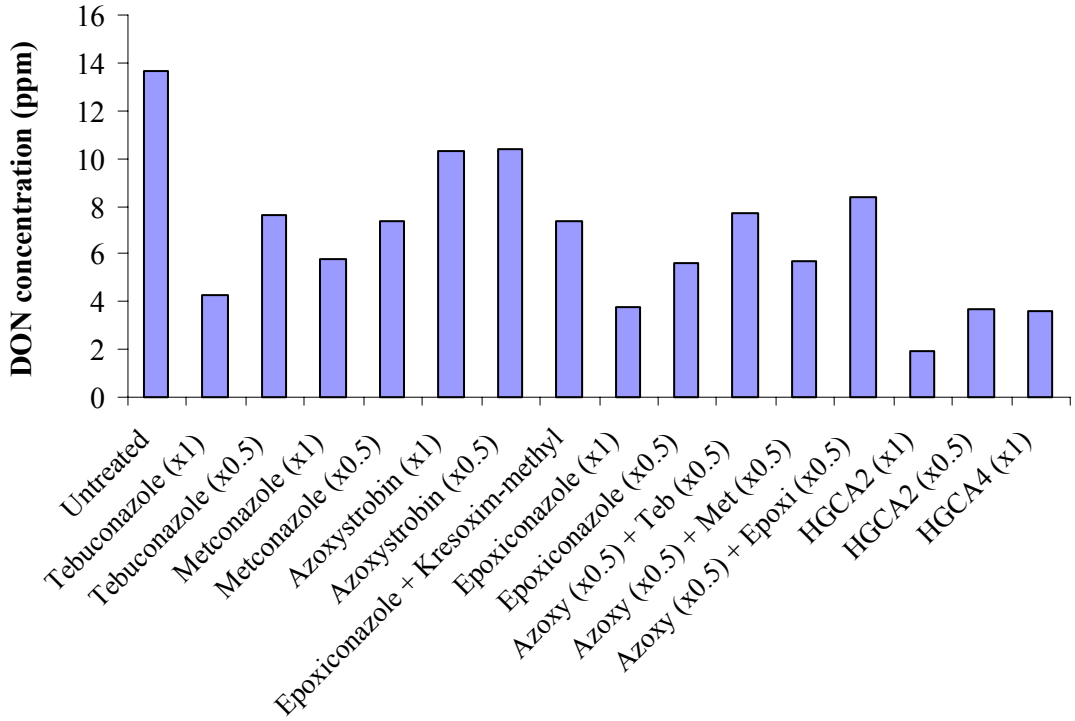



Figure 17. The effect of fungicide treatment on DON concentration in grain from CSL (2000).

*4.4.3 MRC*

The level of DON in the grain of the untreated plots (2.59 ppm) (Figure 18) was low in comparison with the other two sites (HAUC, 7.33 ppm; CSL 13.63 ppm). No treatment significantly reduced the amount of DON in the grain although the mean level was less for tebuconazole (1 dose), metconazole (1 dose), HGCA2 (1 dose) and epoxiconazole + kresoxim-methyl (1 dose). Notably, the level of DON was significantly increased relative to that of the untreated plots where azoxystrobin had been applied at either full or half dose. The level of DON was also greater where azoxystrobin + metconazole (0.5+0.5 dose), azoxystrobin + epoxiconazole (0.5+0.5 dose), azoxystrobin + tebuconazole (0.5+0.5 dose) or HGCA4 (1 dose) had been applied but these were not statistically significant.

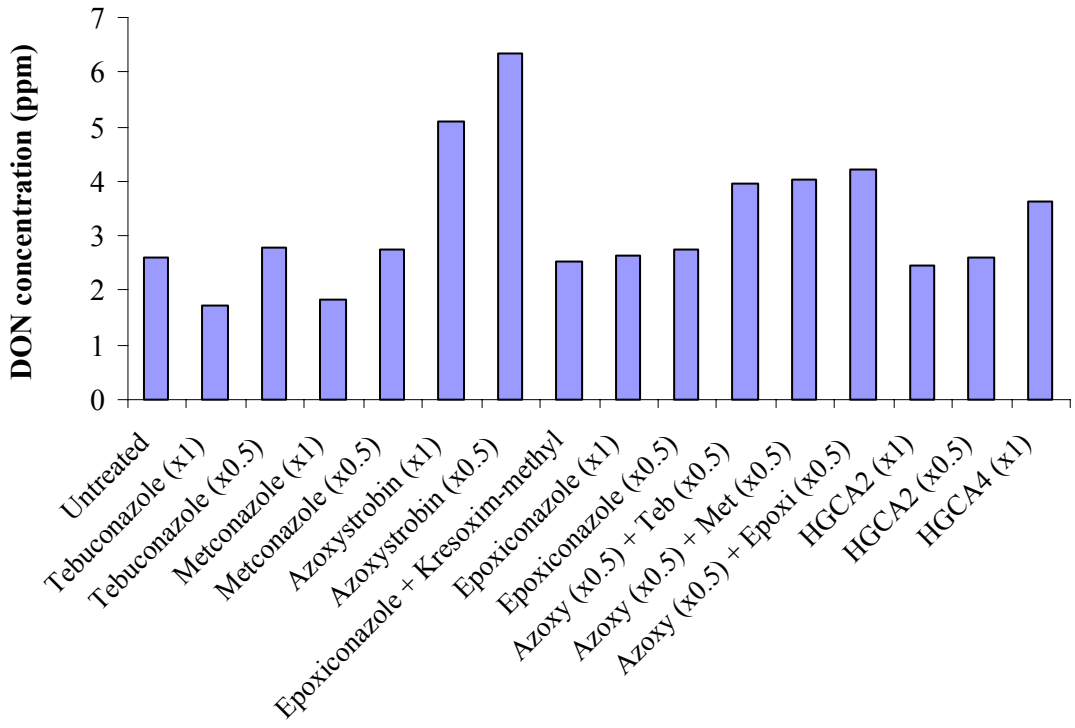



Figure 18. The effect of fungicide treatment on DON concentration in grain from MRC (2000).

**4.5 Yield parameters**

*4.5.1 HAUC*

Yield was assessed by three measures; tonnes/hectare at 15% moisture (t/ha), thousand grain weight (TGW) and specific weight as kg per hectalitre (SPWT) (Table 7). The untreated plots produced 6.83 t/ha. While all treatments except metconazole (0.5 dose) resulted in a higher yield the increase was not significant for any treatment. The weight of 1000 grains for the untreated was 48.84g and all treatments resulted in a higher TGW. The increase was significant for azoxystrobin + tebuconazole (0.5+0.5 dose) and HGCA4. Specific weight was also greater than the untreated for all treatments with the increase again being significant for azoxystrobin + tebuconazole (0.5+0.5 dose) and HGCA4.

Table 7. Effects of fungicide application on yield parameters at HAUC (2000).

| Fungicide | Yield (t/ha) | TGW (g) | Specific weight (kg/hl) |
|---|---|---|---|
| Untreated | 6.83 | 48.84 | 63.85 |
| Tebuconazole (x1) | 7.10 | 52.99 | 66.78 |
| Tebuconazole (x0.5) | 7.48 | 50.31 | 65.93 |
| Metconazole (x1) | 7.47 | 53.60 | 66.95 |
| Metconazole (x0.5) | 6.83 | 51.39 | 65.48 |
| Azoxystrobin (x1) | 7.35 | 53.02 | 65.33 |
| Azoxystrobin (x0.5) | 7.50 | 51.76 | 64.15 |
| HGCA2 (x1) | 7.66 | 53.12 | 66.75 |
| HGCA2 (x0.5) | 7.14 | 51.44 | 66.25 |
| Azoxystrobin (x0.5) + Tebuconazole (0.5) | 7.64 | 56.01+ | 68.3+ |
| Azoxystrobin (x0.5) + Metconazole (0.5) | 7.62 | 53.26 | 66.43 |
| HGCA4 (x1) | 7.49 | 55.63+ | 68+ |
| Epoxiconazole (x1) | 7.76 | 52.90 | 66.50 |
| Epoxiconazole (x0.5) | 7.07 | 50.57 | 64.58 |
| Azoxystrobin (0.5) + Epoxiconazole (0.5) | 7.67 | 53.63 | 66.05 |

*4.5.2 CSL*

Yield was assessed by TGW. The untreated plots had a TGW of 43.8 g (Table 8). All treatments resulted in a higher TGW than the untreated with the increase being greatest for HGCA2 (0.5 dose) and azoxystrobin (0.5 dose) + metconazole (0.5 dose).

Table 8. Effects of fungicide application on thousand grain weight (TGW) at CSL (2000).

| Fungicide | TGW |
|---|---|
| Untreated | 43.78 |
| Tebuconazole (x1) | 47.90 |
| Tebuconazole (x0.5) | 47.88 |
| Metconazole (x1) | 49.22 |
| Metconazole (x0.5) | 48.38 |
| Azoxystrobin (x1) | 49.48 |
| Azoxystrobin (x0.5) | 48.73 |
| Epoxiconazole + Kresoxim-methyl | 48.11 |
| Epoxiconazole (x1) | 48.35 |
| Epoxiconazole (x0.5) | 47.82 |
| Azoxy (x0.5) + Teb (x0.5) | 47.96 |
| Azoxy (x0.5) + Met (x0.5) | 50.11 |
| Azoxy (x0.5) + Epoxi (x0.5) | 48.58 |
| HGCA2 (x1) | 49.16 |
| HGCA2 (x0.5) | 50.26 |
| HGCA4 (x1) | 49.97 |

*4.5.3 MRC*

Yield was assessed by tonnes/hectare at 15% moisture (t/ha), TGW and SPWT (Table 9). The untreated plots produced 8.2 t/ha and all treatments except tebuconazole (0.5 dose) and metconazole (1 and 0.5 dose) resulted in a significantly higher yield than the untreated. The increase was greatest for HGCA4, epoxiconazole + kresoxim-methyl (1 dose) and azoxystrobin + tebuconazole (0.5+0.5 dose). The TGW

for the untreated was 37.52 g and many treatments resulted in a significantly higher TGW. The increase was greatest for epoxiconazole + kresoxim-methyl (1 dose) and was also significant for all treatments involving azoxystrobin (Table 9). SPWT was significantly greater than the untreated for three treatments, HGCA4, azoxystrobin + epoxiconazole (0.5+0.5 dose) and azoxystrobin + tebuconazole (0.5+0.5 dose).

Table 9. Effects of fungicide application on yield parameters at MRC (2000).

| Fungicide | Yield (t/ha) | TGW (g) | Specific weight (kg/hl) |
|---|---|---|---|
| Untreated | 8.20 | 37.52 | 71.94 |
| Tebuconazole (x1) | 8.85 | 39.53 | 72.98 |
| Tebuconazole (x0.5) | 8.68 | 37.49 | 72.41 |
| Metconazole (x1) | 8.61 | 38.41 | 71.96 |
| Metconazole (x0.5) | 8.48 | 38.61 | 72.09 |
| Azoxystrobin (x1) | 9.09 | 40.74 | 73.45 |
| Azoxystrobin (x0.5) | 9.03 | 40.23 | 72.98 |
| Epoxiconazole + Kresoxim-methyl | 9.56 | 41.9 | 73.97 |
| Epoxiconazole (x1) | 9.09 | 40.25 | 73.04 |
| Epoxiconazole (x0.5) | 8.94 | 38.54 | 71.36 |
| Azoxystrobin (x0.5) + Tebuconazole (x0.5) | 9.48 | 41.66 | 74.07 |
| Azoxystrobin (x0.5) + Metconazole (x0.5) | 9.08 | 39.95 | 73.46 |
| Azoxystrobin (x0.5) + Epoxiconazole (x0.5) | 9.34 | 40.69 | 74.21 |
| HGCA2 (x1) | 9.03 | 40.92 | 73.57 |
| HGCA2 (x0.5) | 8.95 | 38.02 | 73.21 |
| HGCA4 (x1) | 9.67 | 40.68 | 74.31 |

**4.6 Discussion**

At all three sites the great majority of treatments significantly reduced disease levels. The level of disease estimated during grain development at the three sites did not reflect the relative amounts of DON detected in harvested grain of the untreated plots. Disease levels at GS 85 were 63%, 30% and 45% at HAUC, CSL and MRC respectively, while DON levels in harvested grain were 7.33 ppm, 13.63 ppm and 2.59 ppm respectively. At individual sites the relationship between disease control achieved by each of the treatments and control of DON accumulation also differed markedly. At MRC and HAUC there was little relationship between disease and DON accumulation. For example, at HAUC (GS85) least disease was observed where azoxystrobin (1 dose) was applied and most disease where tebuconazole (1 dose) was applied. The level of DON was 7.58 ppm for the former and only 4.61 ppm for the latter. At CSL the relationship between disease (GS85) and DON accumulation was closer but still weak across all treatments.

The fungal populations that developed at the three sites differed significantly. At HAUC and MRC, *F. avenaceum* was the predominant *Fusarium* species with the level of *F. graminearum* being moderate at HAUC and very low at MRC. The level of *F. graminearum* was similar at HAUC and CSL (2.38% and 2.85% respectively) being the predominant species present at the latter site. The level of *F. avenaceum*

was similar at CSL and MRC (1.51 % and 1.57% respectively) and very high at HAUC (5.9%). The high level of *F. avenaceum* is of significance because this species does not produce DON or other trichothecene mycotoxins. The level of non-toxin producing *Microdochium nivale* varieties was relatively high at HAUC and MRC but was very low at CSL.

At HAUC the effect of fungicide treatments was significant only against *M. nivale* varieties (with the single exception of metconazole (1 dose) against *F. culmorum*. All treatments involving azoxystrobin reduced either or both *M. nivale* varieties. The only other treatment to affect the level of fungal colonisation of grain was HGCA4 (1 dose), which significantly reduced *M. nivale* var. *majus*. Similar activity of HGCA4 and all treatments involving azoxystrobin were observed at MRC. As at the HAUC site, no treatment significantly reduced the level of any *Fusarium* species colonising the grain. In contrast, at CSL where the level of *Fusarium* species was far greater than that of *M. nivale* varieties, many of the compounds that had shown activity against *Fusarium* species in year 1 of the project significantly reduced the level of *F. graminearum* and/or *F. culmorum* in harvested grain. Azoxystrobin containing treatments and HGCA4 also significantly reduced the level of *M. nivale* var. *majus* in grain, a result similar to those at the other two sites. At HAUC and CSL the full dose of azoxystrobin reduced the level of *F. graminearum* colonising the grain to a greater extent than the half dose while the effect of reduced dose against *F. culmorum* appeared to be less pronounced. The effect of azoxystrobin dose against *M. nivale* was minimal at all sites indicating a dose independent efficacy against *M. nivale*.

At CSL (the site with greatest level of trichothecene-producing *Fusarium* species), all treatments except azoxystrobin (1 and 0.5 dose) significantly reduced the level of DON in harvested grain. In all cases the reduction was greater for full dose than half dose applications. The least DON was present where full or half dose HGCA2 had been applied. At HAUC and MRC, no treatment significantly reduced the level of DON in harvested grain although the level was generally less where full, rather than half, dose had been applied. Most strikingly, at HAUC the level of DON in grain from plots treated with azoxystrobin (0.5 dose) (14.91 ppm) was significantly greater than in grain from untreated plots (7.33 ppm) while at MRC both full and half dose azoxystrobin (5.11 ppm and 6.33 ppm respectively) resulted in a significantly greater level of DON than in untreated grain (2.59 ppm). It is proposed that the differing efficacies of the tested products with respect to accumulation of DON in grain can be understood in the context of the fungal populations at the three sites.

Where significant levels of trichothecene-producing species, such as *F. graminearum* or *F. culmorum,* are present treatments such as tebuconazole, metconazole, HGCA2 and epoxiconazole as well as epoxiconazole + kresoxim-methyl significantly reduced disease and DON accumulation. The use of full dose treatment resulted in a greater reduction of DON than half dose. Azoxystrobin appears to have little, or no, activity against *Fusarium* species but in combination with tebuconazole, metconazole or epoxiconazole can result in reduced disease and DON levels in grain. In contrast, where significant levels of non trichothecene-producing species (e.g. *M. nivale*) were prevalent, compounds such as tebuconazole, metconazole, HGCA2 and epoxiconazole have very limited effect on reducing fungal

colonisation of grain by the *Fusarium* species and accumulation of DON in grain. Azoxystrobin has high activity against both varieties of *M. nivale* and generally significantly reduced levels of these species, even where they comprised only a small proportion of the fungal FEB population. On sites, such as HAUC and MRC, where *M. nivale* comprised a significant proportion of the fungal population the application of treatments that included azoxystrobin led to a significant reduction in *M. nivale* but also often to a significant increase in the level of DON in harvested grain. Experiments carried out by partners in this project, as part of other projects, has revealed a competitive interaction between *F. culmorum* and *M. nivale* varieties. It is most probable that this increase in DON is a consequence of the control of *M. nivale* (and possibly also of other non toxin-producing fungi) that leads to an increase in the colonisation of wheat ears by trichothecene-producing *Fusarium* species. However, it is conceivable that the fungicide has a direct influence on toxin production by the fungi although no evidence to support this has been obtained by the partners to this project.

The effect of treatment against FEB on yield parameters was variable across the sites. At CSL (*Fusarium* species predominant) TGW was significantly increased by all treatments. TGW was also increased significantly by most treatments at MRC, particularly those including azoxystrobin while at HAUC only two treatments resulted in increased TGW (Azoxystrobin + tebuconazole and HGCA4). There was no relationship between TGW and DON observed at any site. The majority of treatments at MRC (significant levels of *M. nivale*) also significantly increased yield whereas at HAUC (intermediate between CSL and MRC with respect to fungal population) no treatment resulted in a significant increase in yield. Again, no relationship was observed between yield and DON content of grain.

The level of DON in grain of the untreated plots differed widely across the three sites with CSL having very high DON levels (13.63 ppm), HAUC intermediate levels (7.33 ppm) and MRC relatively low levels (2.59 ppm). It is envisaged that the EU will produce guidelines or action limits for DON levels in harvested grain of 0.75 ppm. This level is similar to those already in place in the Netherlands and Austria. It is notable that, even at MRC where the DON level was relatively low, no fungicide treatment reduced DON to below this level. The lowest DON contents were generally achieved at MRC where full dose tebuconazole and metconazole reduced levels to 1.73 ppm and 1.85 ppm, respectively. At CSL several (generally full dose) treatments also reduced DON levels over three fold with the greatest reduction being achieved by full dose HGCA2 (1.91 ppm).

# 5 Effect of timing of fungicide application and fungicide dose against Fusarium ear blight and trichothecene mycotoxin accumulation in grain of winter wheat.

## 5.1 Background

Results from the first two years of trials had indicated that application of full or half dose of three fungicides, HGCA2, metconazole and tebuconazole could reduce the level of disease, colonisation by *Fusarium* species and accumulation of trichothecenes in grain of wheat when applied to the crop 2-3 days following inoculation. The effects of full dose were generally found to be greater than those for half dose. Results had also demonstrated the efficacy of azoxystrobin against *M. nivale* another important pathogen associated with FEB in the UK. The effect of reduced dose of this compound against *M. nivale* was less pronounced than that for the fungicides active against the true *Fusarium* species.

The current study was undertaken to determine the effect of timing of fungicide application on disease control to assess whether efficacy could be improved by earlier application. In order to reflect better the fungicides doses currently being used by growers in the UK, the current study examined both half and quarter dose treatments. The combination of a compound active against *Fusarium* species (tebuconazole) with one active against *M. nivale* (azoxystrobin) was also tested with the reduced dose application to determine whether such treatments could provide control of the whole spectrum of FEB pathogens encountered under UK growing conditions.

## 5.2 Disease control

### *5.2.1 HAUC*

Disease (% spikelets infected) on the ears from untreated inoculated plots was very low, being 7.0% at GS 85 (Figure 19). At GS 85 no treatment differed significantly from the untreated plots with respect to disease severity although the lowest level of disease was where tebuconazole (0.5 dose) (pre – inoculation) or tebuconazole + azoxystrobin (0.5 + 0.5 dose) (pre- and post-inoculation) had been applied.

### *5.2.2 CSL*

Disease (% ear area affected) was low on this site. Disease on the ears from untreated inoculated plots was 0.3% and 5.5% at GS 75 and GS 85 respectively (Figure 20). At GS75 no treatment differed significantly from the untreated plots with respect to disease severity. In contrast, at GS85 the majority of treatments significantly reduced disease relative to that on the untreated plots (Figure 20). The lowest level of disease was where tebuconazole (0.5 dose) was applied either pre- or post inoculation.

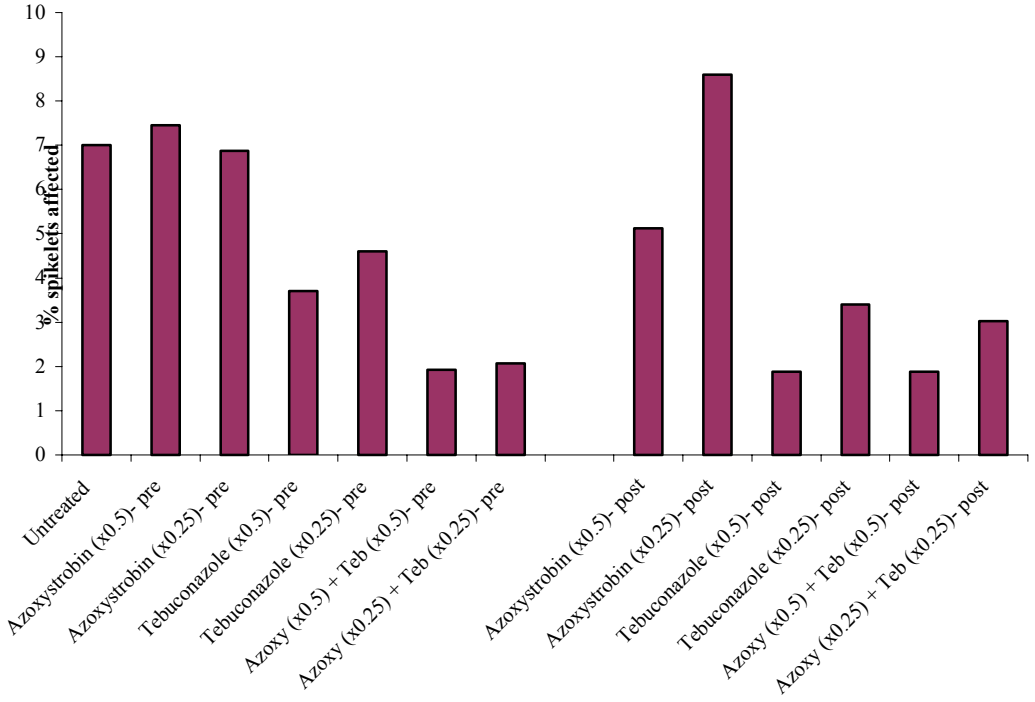



Figure 19. The effect of fungicide treatment on disease levels assessed at HAUC (GS 85) (2001).

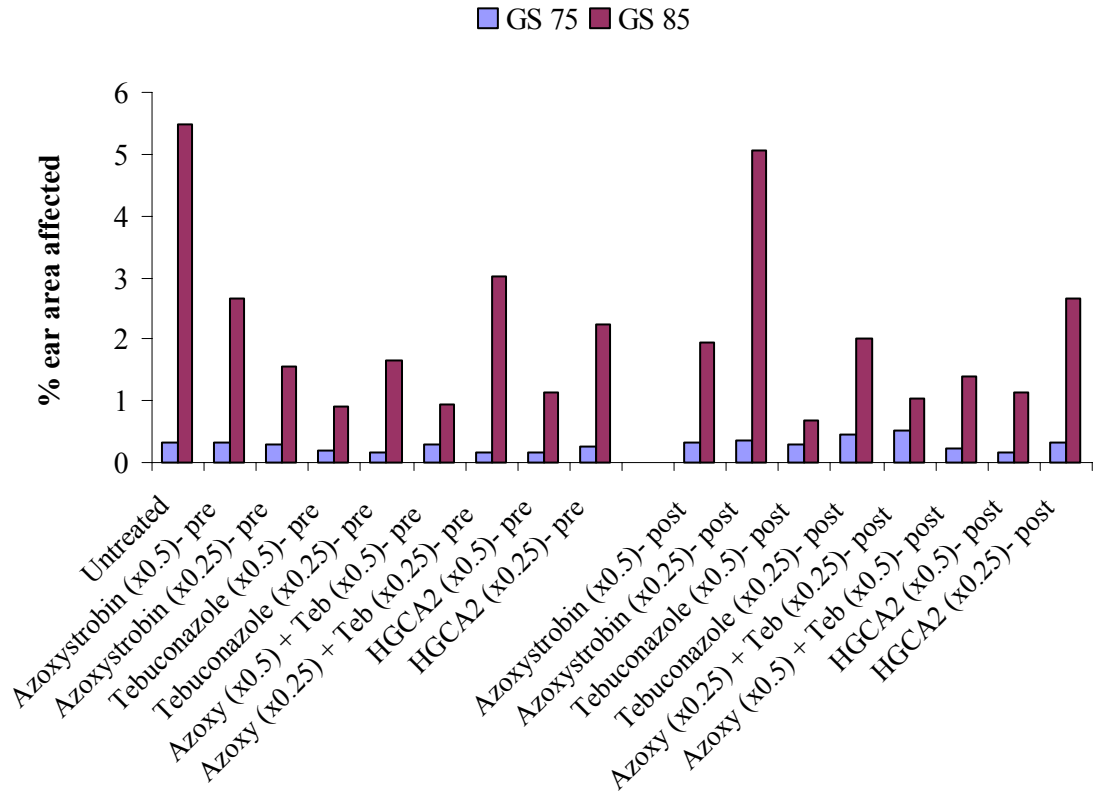



Figure 20. The effect of fungicide treatment on disease levels assessed at CSL (GS 75 and 85) (2001).

*5.2.3 MRC*

A high level of disease developed on this site. Disease (%spikelets infected) on the ears from untreated inoculated plots was 45.5% at GS 75 (Figure 21). At this time all treatments differed significantly from the untreated plots with respect to disease severity with the lowest level of disease where azoxystrobin + tebuconazole (0.5+0.5 dose or 0.25+0.25 dose) had been applied post inoculation. Post inoculation application of HGCA2 at 0.25 or 0.5 dose also markedly reduced disease at this stage (Figure 21). By the time of the second assessment the disease level on the untreated control plots was 62.5%. Most treatments significantly reduced disease with respect to the untreated. Post inoculation treatments appeared to be more effective than pre-inoculation with the greatest reduction being observed where azoxystrobin + tebuconazole (0.5+0.5 or 0.25+0.25 dose) was applied post inoculation. Notably no treatment involving HGCA2 significantly reduced disease.

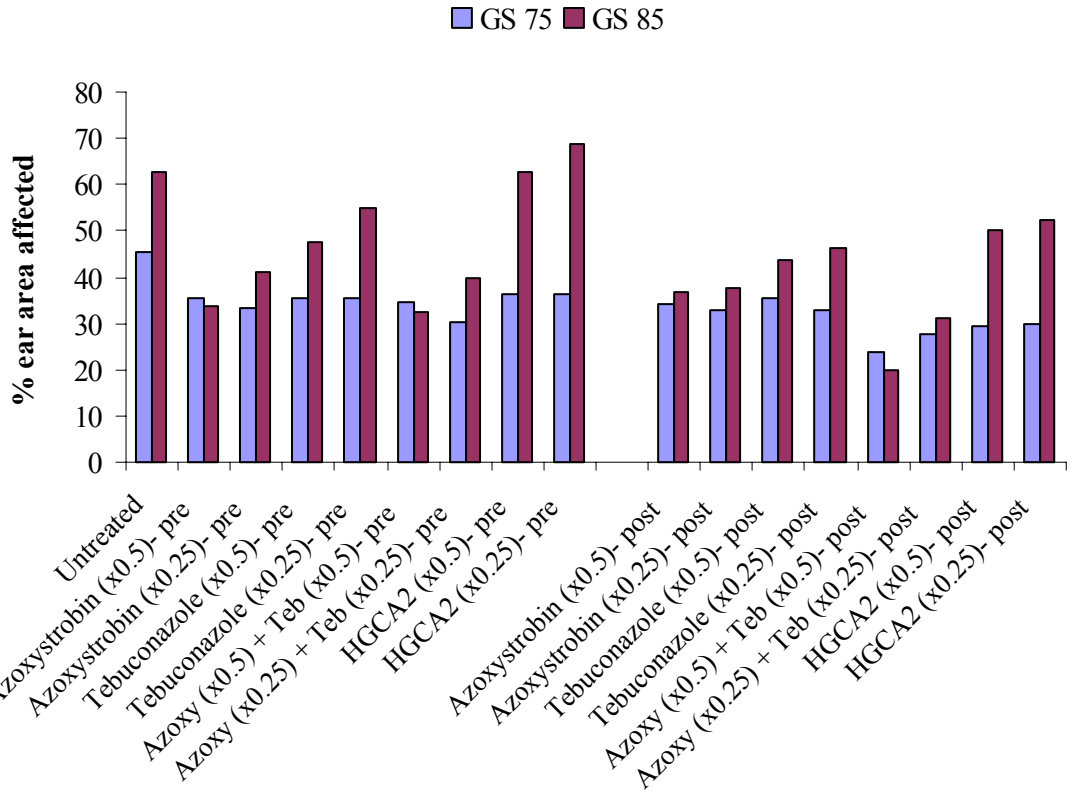



Figure 21. The effect of fungicide treatment on disease levels assessed at MRC (GS 75 and 85) (2001).

**5.3 Fungal species**

*5.3.1 HAUC*

The predominant species present in grain from the untreated plots at this site was *F. culmorum* (5.8% fungal DNA) although *F. avenaceum* (1.68%) was also present at moderate levels. *Fusarium graminearum* was present at only very low levels (0.04%), and *F. poae* and both *M. nivale* var. *majus* and var. *nivale* were absent (Figure 22). Only two treatments significantly influenced the amount of any *Fusarium* species (*F. culmorum*) in the grain. The two treatments were tebuconazole (0.5 dose) and tebuconazole +azoxystrobin (0.5 + 0.5 dose).

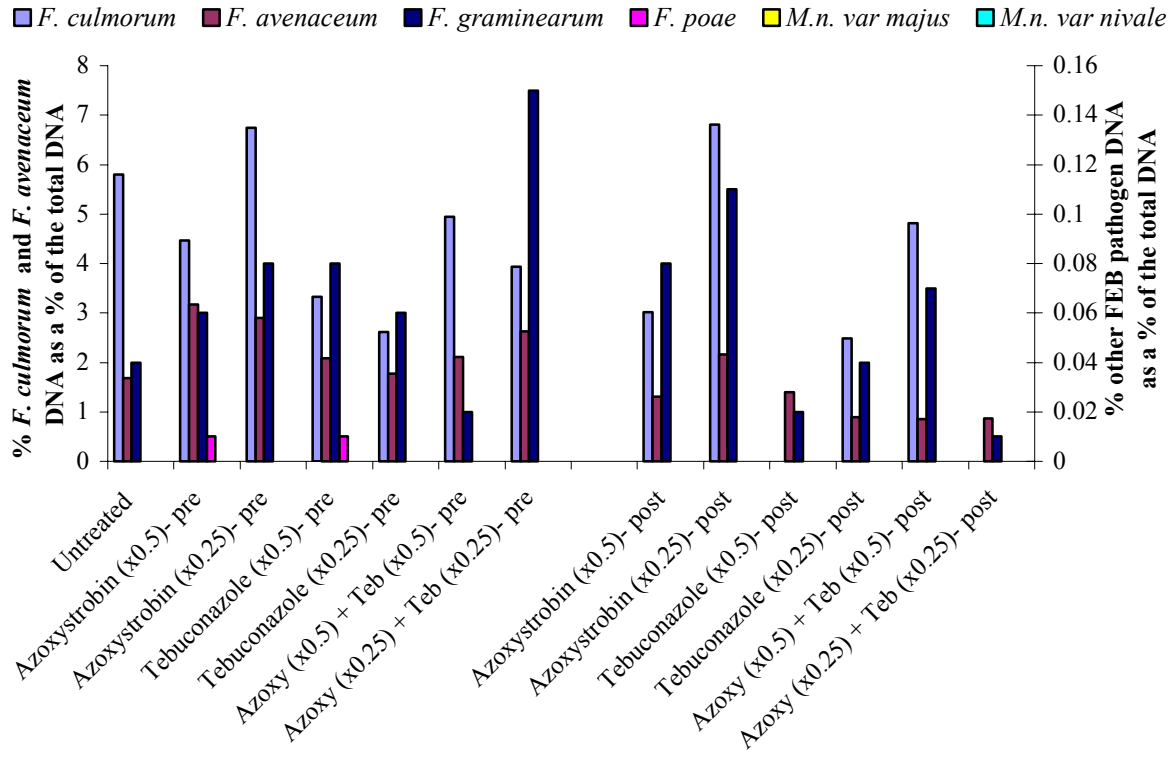



Figure 22. The effect of fungicide treatment on FEB pathogens (as determined by PCR analysis) at HAUC (2001).

*5.3.2 CSL*

Only *F. culmorum* (1.03% fungal DNA) was present in significant amounts in the grain from untreated plots. *F. graminearum, F. avenaceum* and *F. poae* were present but at only very low levels and the two *M. nivale* varieties were absent (Figure 23). Although all treatments reduced the amount of *F. culmorum* present the reduction was only significant where tebuconazole (0.5 dose) or HGCA2 (0.5 dose) were applied pre- or post- inoculation.

*5.3.3 MRC*

Two *Fusarium* species (*F. culmorum* and *F. avenaceum*) were predominant in grain from the untreated plots, with low levels of *F. graminearum* also occurring (0.43% fungal DNA). The levels of *F. avenaceum* (14.7%) and *F. culmorum* (12.95%) were very high (Figure 24). The level of *F. poae* was very low and *M. nivale* vars. *majus* and *nivale* were absent. There was a very high level of variability observed in the amount of both *F. culmorum* and *F. avenaceum* within and between treatments. For example the mean for *F. culmorum* in the azoxystrobin (0.5 dose) pre inoculation treatment was 32.31% while that for HGCA2 (0.25 dose) post inoculation was only 5.52%. In spite of this, none of the differences in the levels of fungal DNA were statistically significant.

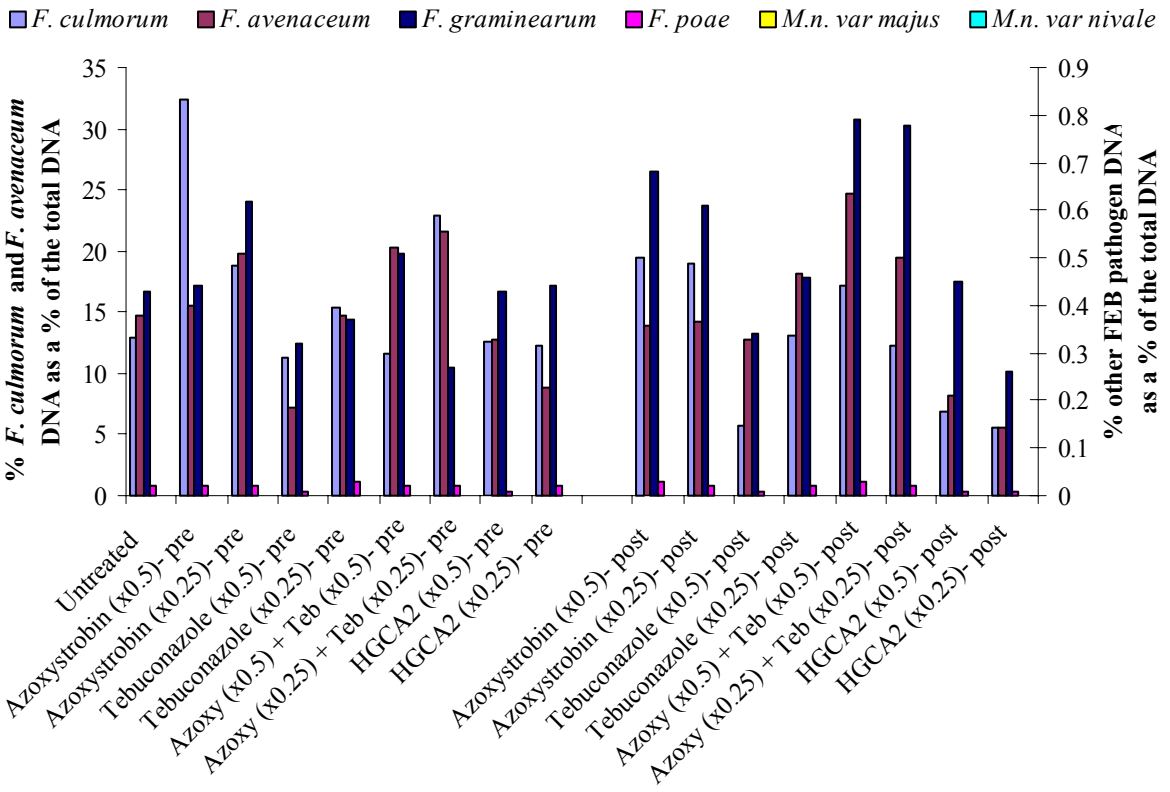



Figure 23. The effect of fungicide treatment on FEB pathogens (as determined by PCR analysis) at CSL (2000).

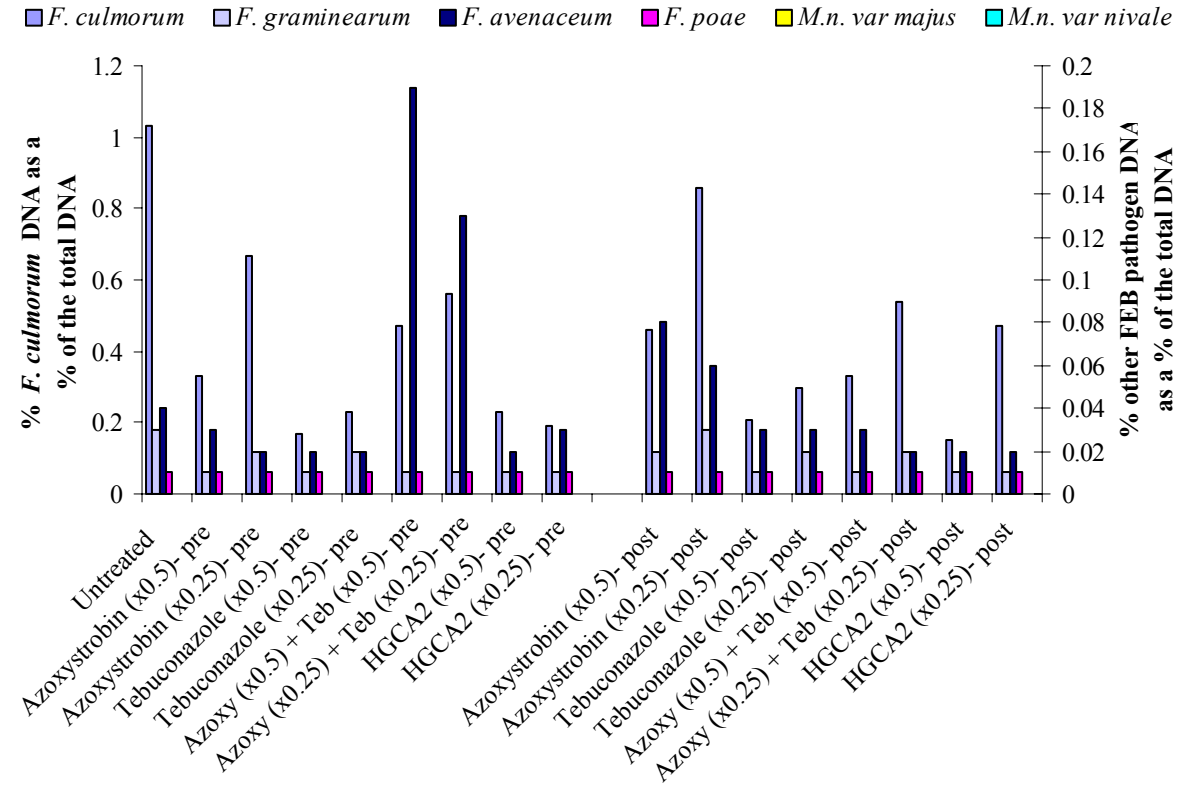



Figure 24. Effect of fungicide treatment on FEB pathogens at MRC (2001).

### 5.4 Deoxynivalenol content of harvested grain

*5.4.1 HAUC*

The level of DON in the grain was 2.13 ppm in the untreated (Figure 25). No treatment significantly reduced the amount of DON although the mean level was less for most treatments. The level of DON was greater, relative to that of the untreated plots, where azoxystrobin (0.25 dose) and azoxystrobin (0.5 dose) had been applied post inoculation. The level of DON was also greater where azoxystrobin (0.5 dose) or azoxystrobin + tebuconazole (0.5 + 0.5 dose) had been applied pre-inoculation but in no case was the increase statistically significant.

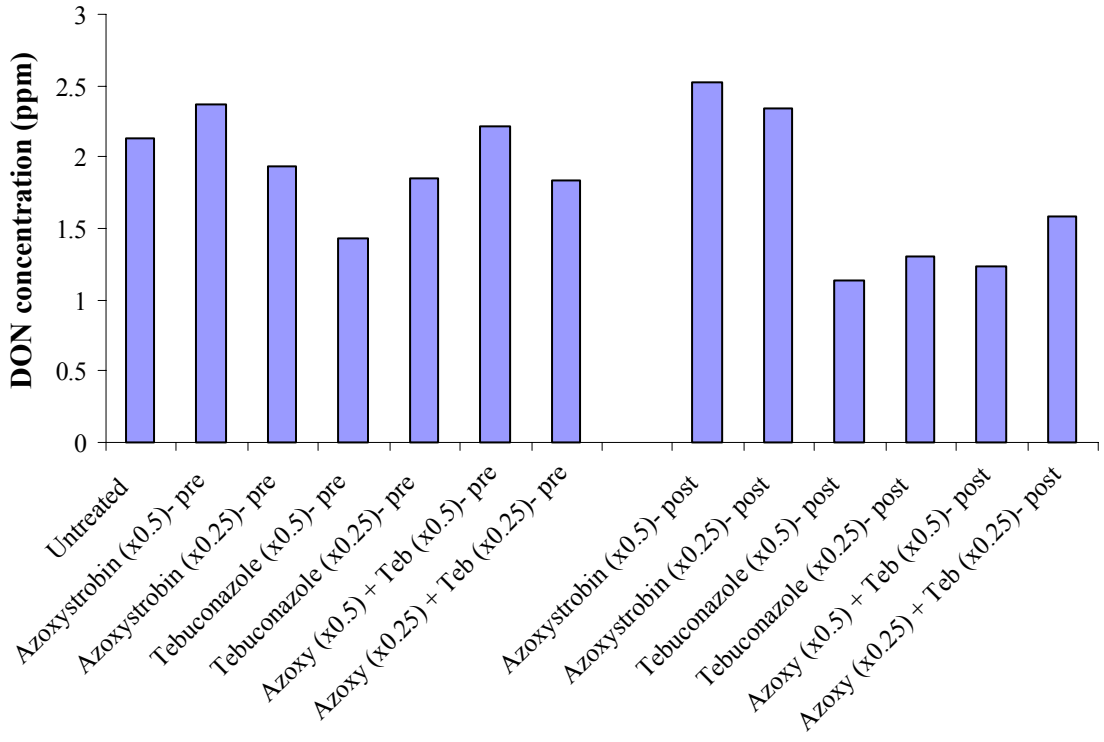



Figure 25. The effect of fungicide treatment on DON concentration in grain from HAUC (2001).

*5.4.2 CSL*

The level of DON in the grain from untreated plots was low (2.66 ppm) relative to the previous trials at this site (Figure 26). This level was high, however, given the low level of disease observed at the site. The great majority of treatments significantly reduced the amount of DON in the grain with the least DON being where HGCA2 (0.5 or 0.25 dose) was applied pre-inoculation, azoxystrobin + tebuconazole (0.5+0.5 dose) post inoculation or tebuconazole (0.5 dose) pre-inoculation. Only two treatments, both applied post inoculation, failed to significantly reduce the level of DON that accumulated in the grain (azoxystrobin (0.25 dose) and HGCA2 (0.5 dose).

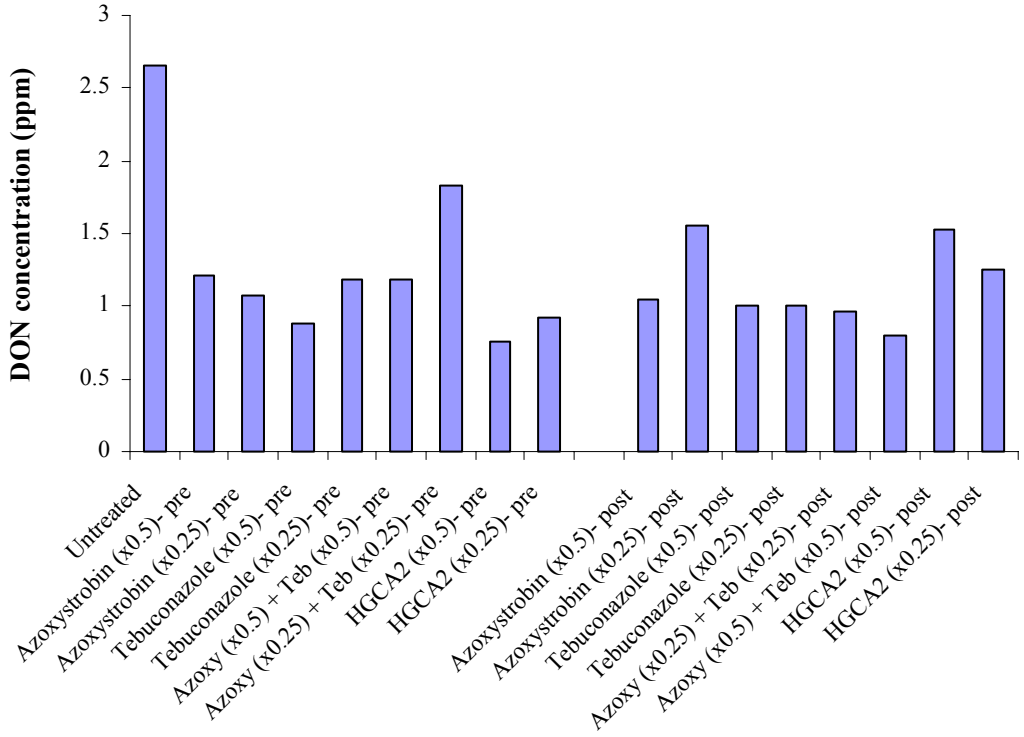



Figure 26. The effect of fungicide treatment on DON concentration in grain from CSL (2001).

*5.4.3 MRC*

The level of DON in the grain of the untreated plots (15.13 ppm) was very high, being greater than that observed on any site in any of the three years. No treatment significantly influenced the level of DON in the grain although levels were higher following some treatments most notably azoxystrobin (0.25 or 0.5 dose) pre inoculation (Figure 27). The lowest level of DON detected in the grain was where HGCA2 (0.25 and 0.5 dose) post inoculation but again, these were not statistically significant from that of the untreated controls.

**5.5 Yield parameters**

*5.5.1 HAUC*

Yield was assessed as tonnes/hectare at 15% moisture (t/ha), thousand grain weight (TGW) and specific weight as kg per hectalitre (SPWT) (Table 10). The untreated plots produced 8.92 t/ha. While all treatments resulted in a higher yield the increase was not significant for any treatment. The weight of 1000 grains for the untreated was 52.85g and no treatments resulted in a significantly higher TGW, the greatest being 54.7g following treatment with azoxystrobin (0.5 dose) post inoculation. Similarly, no treatment significantly influenced specific weight although the greatest SPWT was obtained where azoxystrobin + tebuconazole (0.25+0.25 dose) was applied post inoculation.

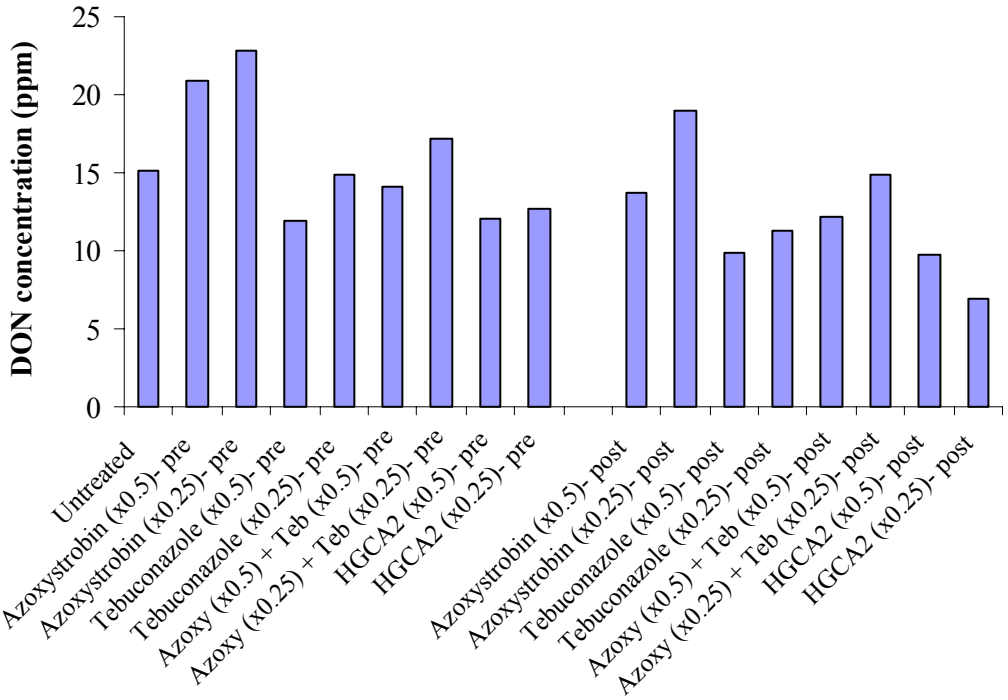



Figure 27. The effect of fungicide treatment on DON concentration in grain from MRC (2001).

Table 10. Effects of fungicide application on yield parameters at HAUC (2001).

| Fungicide | Yield (t/ha) | TGW (g) | Specific weight (kg/hl) |
|---|---|---|---|
| Untreated | 8.92 | 52.85 | 74.35 |
| Azoxystrobin (x0.5)- pre | 9.64 | 52.50 | 73.53 |
| Azoxystrobin (x0.25)- pre | 9.10 | 52.05 | 73.48 |
| Tebuconazole (x0.5)- pre | 9.59 | 52.05 | 74.55 |
| Tebuconazole (x0.25)- pre | 9.45 | 52.10 | 75.15 |
| Azoxy (x0.5) + Teb (x0.5)- pre | 9.33 | 52.30 | 74.00 |
| Azoxy (x0.25) + Teb (x0.25)- pre | 9.30 | 51.80 | 74.30 |
| | | | |
| Azoxystrobin (x0.5)- post | 9.34 | 54.70 | 74.40 |
| Azoxystrobin (x0.25)- post | 9.30 | 51.90 | 74.10 |
| Tebuconazole (x0.5)- post | 9.49 | 52.70 | 74.50 |
| Tebuconazole (x0.25)- post | 9.67 | 53.05 | 74.95 |
| Azoxy (x0.5) + Teb (x0.5)- post | 9.54 | 52.15 | 75.25 |
| Azoxy (x0.25) + Teb (x0.25)- post | 9.77 | 53.85 | 75.40 |

*5.5.2 CSL*

Yield was assessed by TGW. The untreated plots produced TGW of 48.68 g (Table 11). No treatment significantly influenced TGW. Some treatments resulted in a higher TGW than the untreated with the increase being greatest for azoxystrobin + tebuconazole (0.5 and 0.25 dose) applied pre-inoculation.

Table 11. Effects of fungicide application on yield parameters at CSL (2001).

| Fungicide | TGW (g) |
|---|---|
| Untreated | 48.68 |
| Azoxystrobin (x0.5)- pre | 48.96 |
| Azoxystrobin (x0.25)- pre | 49.45 |
| Tebuconazole (x0.5)- pre | 49.34 |
| Tebuconazole (x0.25)- pre | 48.87 |
| Azoxy (x0.5) + Teb (x0.5)- pre | 50.13 |
| Azoxy (x0.25) + Teb (x0.25)- pre | 50.27 |
| HGCA2 (x0.5)- pre | 49.22 |
| HGCA2 (x0.25)- pre | 48.30 |
| | |
| Azoxystrobin (x0.5)- post | 47.95 |
| Azoxystrobin (x0.25)- post | 47.99 |
| Tebuconazole (x0.5)- post | 48.68 |
| Tebuconazole (x0.25)- post | 49.43 |
| Azoxy (x0.25) + Teb (x0.25)- post | 49.38 |
| Azoxy (x0.5) + Teb (x0.5)- post | 49.58 |
| HGCA2 (x0.5)- post | 48.74 |
| HGCA2 (x0.25)- post | 49.51 |

*5.5.3 MRC*

Yield was assessed by three measures; tonnes/hectare at 15% moisture (t/ha), TGW and SPWT (Table 12). The untreated plots produced 9.1 t/ha and all treatments resulted in a higher yield than the untreated although in no case was the increase statistically significant. The increase was greatest for HGCA2 at 0.25 and 0.5 dose applied post inoculation. The TGW for the untreated was 41.53 g and all treatments resulted in a higher TGW although in no case was the increase statistically significant (Table 12). Specific weight was greater than the untreated (64.2 kg/hl) for all but two treatments (azoxystrobin 0.25 and 0.5 dose pre-inoculation), however the increase was significant only for HGCA2 (0.25 and 0.5 dose) applied post inoculation.

Table 12. Effects of fungicide application on yield parameters at MRC (2001).

| Fungicide | Yield | TGW | Specific weight |
|---|---|---|---|

| | (t/ha) | (g) | (kg/hl) |
|---|---|---|---|
| Untreated | 9.10 | 41.53 | 64.20 |
| Azoxystrobin (x0.5)- pre | 9.69 | 43.27 | 63.27 |
| Azoxystrobin (x0.25)- pre | 9.21 | 42.39 | 63.44 |
| Tebuconazole (x0.5)- pre | 9.38 | 44.07 | 66.25 |
| Tebuconazole (x0.25)- pre | 9.18 | 43.21 | 65.27 |
| Azoxy (x0.5) + Teb (x0.5)- pre | 9.78 | 44.44 | 65.41 |
| Azoxy (x0.25) + Teb (x0.25)- pre | 9.26 | 44.62 | 65.54 |
| HGCA2 (x0.5)- pre | 9.71 | 44.93 | 66.85 |
| HGCA2 (x0.25)- pre | 9.14 | 45.17 | 65.27 |
| | | | |
| Azoxystrobin (x0.5)- post | 9.77 | 42.45 | 65.45 |
| Azoxystrobin (x0.25)- post | 9.54 | 43.24 | 64.94 |
| Tebuconazole (x0.5)- post | 9.48 | 45.59 | 67.24 |
| Tebuconazole (x0.25)- post | 9.29 | 43.13 | 66.86 |
| Azoxy (x0.5) + Teb (x0.5)- post | 9.53 | 45.15 | 67.49 |
| Azoxy (x0.25) + Teb (x0.25)- post | 9.80 | 45.58 | 65.93 |
| HGCA2 (x0.5)- post | 10.13 | 45.22 | 67.99 |
| HGCA2 (x0.25)- post | 10.15 | 43.84 | 68.31 |

**5.6 Discussion**

Low levels of disease developed on two of the sites (HAUC and CSL) while at MRC disease levels were high. At CSL, where treatments were applied 3 days pre- and 3 days post inoculation, most treatments reduced disease and DON levels, whether applied pre- or post-inoculation. While the higher dose generally reduced disease more than the 0.25 dose, there appeared to be little effect of fungicide dose on DON content with 0.25 and 0.5 dose applications being largely similar in effect. Pre and post-inoculation treatments resulted in similar DON levels. Considering only single products (0.25 or 0.5 dose), the average DON accumulation was least where tebuconazole (mean 1.02 ppm) was applied and greatest for azoxystrobin treatments (1.23 ppm) with HGCA2 (1.11 ppm) being intermediate. Only two treatments resulted in less trichothecene producing species (*F. culmorum*) accumulating in the grain (tebuconazole (0.5 dose) pre-inoculation and HGCA2 (0.5 dose) post inoculation. Thus the reduction in disease and DON levels was not accompanied by a reduction in the amount of fungus able to colonise the grain.

At HAUC no differences were observed in disease levels across treatments. Only two treatments significantly reduced the colonisation of grain by *F. culmorum* (tebuconazole (0.5 dose) post-inoculation and azoxystrobin + tebuconazole (0.25+0.25 dose) post-inoculation. No treatment significantly reduced the amount of DON in grain although the lowest levels were in grain from plots treated with tebuconazole (0.5 dose) or azoxystrobin + tebuconazole (0.5+0.5 dose) post-inoculation. In general, the overall level of DON was less where treatments were applied post inoculation (mean 1.66 ppm) than where applied pre-inoculation (mean 1.94 ppm). Considering only single products (0.25 or 0.5 dose), the average DON accumulation was least where tebuconazole (mean 1.43 ppm) was applied and greatest for azoxystrobin treatments (2.29 ppm).

Severe lodging was observed in the central portion of two rows of the MRC site. The amount of fungus and DON that accumulated in the grain of the plots in this region were significantly higher than those from the non-lodged plots. This variability was reflected in the lack of statistical evidence for any treatment having an effect on either fungal colonisation or DON accumulation. However, high levels of fungus and DON were observed even in non-lodged plots indicating that disease pressure at this site was very high. These factors may have contributed largely to the failure of any treatment to significantly influence DON accumulation or fungal colonisation at this site.

# 6 Optimising control of FEB and mycotoxin accumulation through improved fungicide application techniques.

## 6.1 Background

The project originally intended to use track sprayer equipment at HAUC to study the effect of differing application techniques on the efficacy of control of FEB by selected fungicides. The field site experiments detailed above (Chapters 3-5) revealed highly significant site effects making it imperative that all three sites be used in each year to provide robust data on fungicide efficacy. Bayer plc had contracted ITCF to carry out extensive field and laboratory studies of fungicide distribution using differing nozzles. Additional tests were carried out to determine the effects of single and twin fan nozzles on FEB disease and yield parameters. Bayer plc offered to share their data with the project to permit the use of the HAUC site in all three years of the project.

## 6.2 Deposition studies

*6.2.1 Conventional application.*

Conventional single fan application was found to result in highly uneven distribution of fungicide upon wheat ears. The vast majority of fungicide is deposited on the side of the ear facing into the wind. On the windward side of the ear 39% of the upper third of the ear becomes covered while the central and lower portions are 36% and 30% covered respectively. On the leeward side only 2.1%, 1.6 and 1% of the ear becomes covered in the upper, central and lower portions respectively.

*6.2.2 Nozzle variation*

Wheat was sprayed at a speed of 7.5 km/h with nozzles set at a height to provide triple coverage. Application using a single fan nozzle (110$^{o}$, 3.5 bars, 277 l/ha) resulted in 7% coverage of the ear and 7.75% coverage of the collar. Application using double fan nozzles (80$^{o}$, 3.5 bars, 273 l/ha) provided greater coverage of both the ear and collar regions and resulted in 11.15% coverage of the ear and 11.65% coverage of the collar. Results of application using air nozzles (6 bars, 317 l/ha) were intermediate, resulting in 8.95% coverage of the ear and 6.3% coverage of the collar.

*6.2.3 Application speed*

Laboratory tests were carried out with double fan nozzles (110$^{o}$, 3.5 bars) by application to water sensitive paper presented to the sprayer as a "model" ear. When the application speed was 7.5 km/h (204 l/ha), deposition to the front of the ears was 25 droplets per cm$^2$ while that to the sides and back was 15 droplets per cm$^2$. When the application speed was reduced to 4 km/h (382 l/ha) deposition rates increased markedly providing much improved coverage of the "model" ear. Deposition on the front, left and right of the ears was 40 droplets per cm$^2$ while that to the back was 25 droplets per cm$^2$.

*6.2.4 Field assessment of nozzle performance*

Field plots of wheat were inoculated with conidia of FEB species. Plots were then either untreated or sprayed with full dose tebuconazole using either single or double fan nozzles. The untreated plots had 49% of spikelets infected. Disease on plots treated using single fan nozzles was 19.3% while double fan treated plots had only 10.6% disease. The percentage of infected grains (assessed visually) was 28.7% for the untreated and 13% and 11.6% for plots treated with single and double fan nozzles respectively. Yield was similarly improved by application using the double fan nozzles being 6.39 t/ha, 6.01 and 4.48 t/ha for double fan, single fan and the untreated respectively.

**6.3 Discussion**

Results clearly demonstrate that the single fan nozzle produces uneven distribution of fungicide to the ear. The use of double fan nozzles improves the level and distribution of coverage. A further improvement was provided by reducing the speed of traverse from 7.5 to 4 km/h. The reduction in traverse speed results in a greater application volume. When tested under field conditions the use of double fan nozzles reduced the level of disease markedly with respect to that achieved using single fan nozzles (from 19.3% to 10.6%). Overall, the results indicate that the use of double fan nozzles and reduced traverse speeds increases fungicide coverage of the wheat ears and can result in reduced FEB disease.

## 7 Conclusions

1. Fungicides are differentially active against the toxin-producing and non toxin-producing species that cause FEB. Azoxystrobin has very high activity against *M. nivale* but appears to have only very limited activity against *Fusarium* species. In contrast, tebuconazole, metconazole and HGCA2 have high activity against the *Fusarium* species while being ineffective against *M. nivale* varieties.

2. Full dose application, very close to mid-anthesis, of compounds with high activity against *Fusarium* species can significantly reduce disease, increase yield and reduce accumulation of DON. Fungicides with greatest efficacy against DON producing isolates also have most effect against NIV producing isolates.

3. Where high levels of disease occur chiefly due to *Fusarium* species, and high levels of DON accumulate in grain, both full and half dose fungicide (tebuconazole, metconazole and HGCA2) significantly reduce disease and toxin levels. The lower fungicide dose reduces efficacy of control of disease and toxin accumulation relative to the full dose.

4. Where high levels of disease occur and both *Fusarium* species and *M. nivale* varieties are present, reduction in disease and toxin levels by tebuconazole, metconazole and HGCA2 may not be significant although greater control of disease and toxin accumulation is generally achieved by the higher dose.

5. Where disease is due to *Fusarium* species and *M. nivale* is absent (or nearly so) higher levels of disease are associated with greater amounts of DON accumulating in grain irrespective of the fungicide applied.

6. Where high levels of disease occur and both *Fusarium* species and *M. nivale* varieties are present, application of azoxystrobin can result in significantly reduced disease but significantly higher DON levels. The effect on DON accumulation appears to be more marked when half dose azoxystrobin is applied than when full dose is applied. The increase in DON content of grain may even occur where azoxystrobin is applied in mixture with *Fusarium* active compounds.

7. Where disease levels are low, half and quarter dose application of tebuconazole or HGCA2 may significantly reduce disease and toxin levels, in some instances, when applied up to 3 days either side of mid-anthesis.

8. Lodging of the crop can lead to large increases in the toxin content of grain, overwhelming any effects of fungicide application to the ear.

9. Double fan nozzles combined with reduced traverse speed of fungicide application can improve disease control.

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**APPENDIX 1: Tables of results from field trials over three years.**

**Table A.1. Disease scores at GS 75 and GS 85 and thousand grain weight - CSL 1999**

| Fungicide | Disease GS75 | Disease GS85 | TGW |
|---|---|---|---|
| **Untreated** | 4.20 | 34.18 | 44.59 |
| **HGCA1** | 3.71 | 28.10 | 44.81 |
| **HGCA2** | 2.63 | 10.82* | 46.35 |
| **HGCA3** | 3.60 | 25.31* | 47.35 |
| **Difenoconazole** | 5.12 | 28.52 | 44.67 |
| **Epoxiconazole + Kresoxim-methyl** | 2.89 | 17.76* | 47.79 |
| **Metconazole** | 2.84 | 13.07* | 45.28 |
| **MBC (bavistim)** | 3.39 | 10.98* | 47.63 |
| **Azoxystrobin** | 4.81 | 18.51* | 47.77 |
| **Tebuconazole** | 2.33 | 14.76* | 46.74 |
| **Prochloraz** | 3.60 | 20.31* | 46.60 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.2. Trichothecene mycotoxin content of grain - CSL 1999**

| Fungicide | DON | 15AcDON | 3AcDON | NIV | FUS X[1] | DAS[1] | HT2 | T2 |
|---|---|---|---|---|---|---|---|---|
| **Untreated** | 12.12 | 0.14 | 0.11 | 1.29 | 0.00 | 0.00 | 0.04 | 0.01 |
| **HGCA1** | 10.58 | 0.09 | 0.10 | 0.71 | 0.00 | 0.00 | 0.00 | 0.00 |
| **HGCA2** | 1.05* | 0.07 | 0.00 | 0.26 | 0.00 | 0.00 | 0.00 | 0.04 |
| **HGCA3** | 9.56 | 0.07 | 0.11 | 0.79 | 0.00 | 0.03 | 0.02 | 0.00 |
| **Difenoconazole** | 11.19 | 0.11 | 0.11 | 0.91 | 0.00 | 0.00 | 0.02 | 0.01 |
| **Epoxiconazole + Kresoxim-methyl** | 5.69* | 0.07 | 0.07 | 0.36 | 0.00 | 0.00 | 0.00 | 0.04 |
| **Metconazole** | 2.71* | 0.04 | 0.07 | 0.37 | 0.00 | 0.00 | 0.01 | 0.01 |
| **MBC (derosal)** | 1.85* | 0.05 | 0.01 | 0.24 | 0.00 | 0.00 | 0.03 | 0.00 |
| **Azoxystrobin** | 10.41 | 0.12 | 0.17 | 0.90 | 0.00 | 0.03 | 0.02 | 0.02 |
| **Tebuconazole** | 2.76* | 0.02 | 0.03 | 0.31 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Prochloraz** | 3.96* | 0.02 | 0.05 | 0.35 | 0.00 | 0.00 | 0.00 | 0.01 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

[1] Below the level of detection

**Table A.3. Amount of *Fusarium* species and *Microdochium nivale* varieties in grain - CSL 1999**

| Fungicide | *F. culmorum* | *F. graminearum* | *F. avenaceum* | *F. poae* | *M.n.* var *majus* | *M.n.* var *nivale* |
|---|---|---|---|---|---|---|
| **Untreated** | 3.38 | 0.56 | 0.19 | 0.41 | 0.02 | 0.04 |
| **HGCA1** | 4.82 | 0.45 | 0.11 | 0.33 | 0.02 | 0.04 |
| **HGCA2** | 0.24* | 0.04* | 0.03* | 0.29 | 0.01 | 0.04 |
| **HGCA3** | 4.69 | 0.61 | 0.22 | 0.42 | 0.01 | 0.05 |
| **Difenoconazole** | 2.87 | 0.47 | 0.10 | 0.25 | 0.02 | 0.04 |
| **Epoxiconazole + Kresoxim-methyl** | 1.89 | 0.44 | 0.16 | 0.36 | 0.01 | 0.05 |
| **Metconazole** | 0.96 | 0.19 | 0.15 | 0.42 | 0.02 | 0.05 |
| **MBC (derosal)** | 0.29* | 0.14 | 0.04 | 0.26 | 0.02 | 0.05 |
| **Azoxystrobin** | 3.88 | 0.69 | 0.12 | 0.65 | 0.01 | 0.04 |
| **Tebuconazole** | 0.49 | 0.12 | 0.03* | 0.22 | 0.01 | 0.05 |
| **Prochloraz** | 1.09 | 0.12 | 0.05 | 0.23 | 0.01 | 0.04 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.4. Disease scores at GS 75 and GS 85, yield, thousand grain weight (TGW) and specific weight (SpWt) - HAUC 1999**

| Fungicide | Disease 75 | Disease 85 | Yield | TGW | SpWt |
|---|---|---|---|---|---|
| **Untreated** | 14.15 | 49.38 | 6.80 | 44.04 | 55.25 |
| **HGCA1** | 12.05 | 42.53 | 6.92 | 47.38 | 57.43 |
| **HGCA2** | 6.83 | 39.40 | 8.15+ | 52.52+ | 63.4+ |
| **HGCA3** | 11.85 | 45.13 | 7.15 | 49.32 | 59.05 |
| **Difenoconazole** | 15.00 | 46.03 | 7.22 | 47.55 | 56.90 |
| **Epoxiconazole + Kresoxim-methyl** | 9.98 | 42.60 | 7.91 | 50.45+ | 58.75 |
| **Metconazole** | 8.53 | 48.15 | 8.27+ | 53.00+ | 62.88+ |
| **MBC (bavistim)** | 12.43 | 44.98 | 6.67 | 45.82 | 55.65 |
| **Azoxystrobin** | 16.25 | 43.20 | 6.96 | 46.63 | 55.93 |
| **Tebuconazole** | 7.18 | 39.65 | 7.96 | 52.72+ | 61.85+ |
| **Prochloraz** | 9.88 | 48.55 | 7.48 | 48.65 | 58.88 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.5. Trichothecene mycotoxin content of grain -HAUC 1999**

| Fungicide | DON | 15AcDON | 3AcDON | NIV | FUS X[1] | DAS | HT2 | T2 |
|---|---|---|---|---|---|---|---|---|
| **Untreated** | 9.29 | 0.09 | 0.09 | 1.39 | 0.00 | 0.00 | 0.04 | 0.01 |
| **HGCA1** | 10.53 | 0.01 | 0.24 | 1.85 | 0.00 | 0.00 | 0.06 | 0.00 |
| **HGCA2** | 2.63* | 0.03 | 0.02 | 1.02 | 0.00 | 0.00 | 0.03 | 0.00 |
| **HGCA3** | 7.85 | 0.06 | 0.22 | 1.38 | 0.00 | 0.04 | 0.04 | 0.03 |
| **Difenoconazole** | 7.82 | 0.13 | 0.15 | 1.11 | 0.00 | 0.04 | 0.06 | 0.00 |
| **Epoxiconazole + Kresoxim-methyl** | 8.53 | 0.08 | 0.17 | 1.36 | 0.00 | 0.03 | 0.03 | 0.02 |
| **Metconazole** | 2.66* | 0.04 | 0.05 | 0.88 | 0.00 | 0.00 | 0.05 | 0.00 |
| **MBC (bavistim)** | 8.24 | 0.09 | 0.24 | 1.46 | 0.00 | 0.00 | 0.03 | 0.01 |
| **Azoxystrobin** | 12.13 | 0.08 | 0.23 | 2.16 | 0.00 | 0.00 | 0.03 | 0.00 |
| **Tebuconazole** | 3.43* | 0.01 | 0.05 | 0.51 | 0.00 | 0.00 | 0.02 | 0.00 |
| **Prochloraz** | 6.04 | 0.07 | 0.18 | 1.14 | 0.00 | 0.00 | 0.03 | 0.11 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

[1] Below the level of detection

**Table A.6. Amount of *Fusarium* species and *Microdochium nivale* varieties in grain - HAUC 1999**

| Fungicide | *F. culmorum* | *F. graminearum* | *F. avenaceum* | *F. poae* | *M.n.* var *majus* | *M.n.* var *nivale* |
|---|---|---|---|---|---|---|
| **Untreated** | 6.70 | 0.10 | 0.10 | 0.02 | 0.10 | 0.05 |
| **HGCA1** | 6.42 | 0.13 | 0.13 | 0.06 | 0.08 | 0.06 |
| **HGCA2** | 3.36 | 0.02* | 0.06 | 0.06 | 0.14 | 0.08 |
| **HGCA3** | 4.07 | 0.16 | 0.09 | 0.04 | 0.03 | 0.04 |
| **Difenoconazole** | 5.72 | 0.16 | 0.12 | 0.08 | 0.11 | 0.08 |
| **Epoxiconazole + Kresoxim-methyl** | 6.29 | 0.14 | 0.05 | 0.19+ | 0.04 | 0.04 |
| **Metconazole** | 2.04* | 0.09 | 0.04 | 0.02 | 0.21 | 0.11 |
| **MBC (bavistim)** | 5.11 | 0.08 | 0.10 | 0.10 | 0.12 | 0.07 |
| **Azoxystrobin** | 5.32 | 0.08 | 0.05 | 0.07 | 0.08 | 0.06 |
| **Tebuconazole** | 2.81 | 0.08 | 0.23 | 0.02 | 0.36 | 0.14+ |
| **Prochloraz** | 6.05 | 0.14 | 0.03 | 0.07 | 0.07 | 0.08 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.7. Disease scores at GS 85, yield and thousand grain weight - MRC 1999**

| Fungicide | Disease GS85 | Yield | TGW |
|---|---|---|---|
| **Untreated** | 19.88 | 9.54 | 44.71 |
| **HGCA1** | 17.17 | 10.10+ | 46.24 |
| **HGCA2** | 14.5* | 10.72+ | 47.06 |
| **HGCA3** | 17.25* | 10.04 | 44.11 |
| **Difenoconazole** | 18.38 | 9.88 | 44.71 |
| **Epoxiconazole + Kresoxim-methyl** | 14.13* | 10.60+ | 46.86 |
| **Metconazole** | 13.13* | 10.72+ | 46.36 |
| **MBC (derosal)** | 17.88 | 10.37+ | 46.43 |
| **Azoxystrobin** | 18.00 | 10.18+ | 44.61 |
| **Tebuconazole** | 13.88* | 10.78+ | 46.40 |
| **Prochloraz** | 15.5* | 10.36+ | 44.37 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.8. Trichothecene mycotoxin content of grain -MRC 1999**

| Fungicide | DON | 15AcDON | 3AcDON | NIV | FUS X[1] | DAS[1] | HT2 | T2 |
|---|---|---|---|---|---|---|---|---|
| **Untreated** | 0.44 | 0.03 | 0.04 | 2.53 | 0.00 | 0.04 | 0.06 | 0.00 |
| **HGCA1** | 0.53 | 0.03 | 0.04 | 2.35 | 0.00 | 0.00 | 0.10 | 0.09 |
| **HGCA2** | 0.51 | 0.05 | 0.00 | 1.33* | 0.00 | 0.00 | 0.13 | 0.03 |
| **HGCA3** | 0.57 | 0.08 | 0.06 | 2.44 | 0.00 | 0.00 | 0.10 | 0.05 |
| **Difenoconazole** | 0.50 | 0.06 | 0.00 | 2.34 | 0.00 | 0.00 | 0.08 | 0.06 |
| **Epoxiconazole + Kresoxim-methyl** | 0.29 | 0.00 | 0.00 | 1.08* | 0.00 | 0.00 | 0.05 | 0.05 |
| **Metconazole** | 0.46 | 0.03 | 0.00 | 2.21 | 0.00 | 0.00 | 0.11 | 0.05 |
| **MBC (derosal)** | 0.30 | 0.03 | 0.00 | 0.89* | 0.00 | 0.04 | 0.06 | 0.00 |
| **Azoxystrobin** | 0.46 | 0.03 | 0.00 | 1.62 | 0.00 | 0.00 | 0.13 | 0.02 |
| **Tebuconazole** | 0.47 | 0.06 | 0.00 | 1.60 | 0.00 | 0.00 | 0.09 | 0.03 |
| **Prochloraz** | 0.30 | 0.03 | 0.00 | 1.31* | 0.00 | 0.00 | 0.08 | 0.00 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

[1] Below the level of detection

**Table A.9. Amount of *Fusarium* species and *Microdochium nivale* varieties in grain - MRC 1999**

| Fungicide | *F. culmorum* | *F. graminearum*[1] | *F. avenaceum* | *F. poae* | *M.n.* var *majus*[1] | *M.n.* var *nivale*[1] |
|---|---|---|---|---|---|---|
| **Untreated** | 8.17 | 0.01 | 1.28 | 0.02 | 0.01 | 0.04 |
| **HGCA1** | 5.77 | 0.00 | 1.11 | 0.02 | 0.01 | 0.03 |
| **HGCA2** | 2.47* | 0.00 | 0.35* | 0.02 | 0.01 | 0.04 |
| **HGCA3** | 4.32 | 0.00 | 1.09 | 0.04 | 0.01 | 0.04 |
| **Difenoconazole** | 5.67 | 0.00 | 1.30 | 0.02 | 0.01 | 0.04 |
| **Epoxiconazole + Kresoxim-methyl** | 2.75* | 0.01 | 0.95 | 0.04 | 0.01 | 0.03 |
| **Metconazole** | 2.32* | 0.00 | 0.88 | 0.02 | 0.01 | 0.04 |
| **MBC (derosal)** | 2.99* | 0.00 | 0.93 | 0.05 | 0.01 | 0.03 |
| **Azoxystrobin** | 7.22 | 0.00 | 0.99 | 0.05 | 0.01 | 0.04 |
| **Tebuconazole** | 2.38* | 0.00 | 0.63* | 0.06 | 0.01 | 0.04 |
| **Prochloraz** | 3.44 | 0.01 | 0.79 | 0.04 | 0.01 | 0.04 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

[1] Below the level of detection

**Table A.10. Amount of *Fusarium* species and *Microdochium nivale* varieties in grain - HAUC 2000**

| Fungicide | *F. culmorum* | *F. graminearum* | *F. poae* | *F. avenaceum* | *M.n.* var *majus* | *M.n.* var *nivale* |
|---|---|---|---|---|---|---|
| **Untreated** | 0.12 | 2.38 | 0.00 | 5.90 | 0.29 | 0.15 |
| **Tebuconazole (x1)** | 0.06 | 1.46 | 0.00 | 2.07 | 0.29 | 0.23 |
| **Tebuconazole (x0.5)** | 0.05 | 2.39 | 0.00 | 4.06 | 0.32 | 0.16 |
| **Metconazole (x1)** | 0.03* | 0.58 | 0.00 | 4.04 | 0.37 | 0.16 |
| **Metconazole (x0.5)** | 0.08 | 1.23 | 0.00 | 6.90 | 0.36 | 0.22 |
| **Azoxystrobin (x1)** | 0.16 | 2.56 | 0.01 | 7.43 | 0.19 | 0.05* |
| **Azoxystrobin (x0.5)** | 0.16 | 3.43 | 0.00 | 5.84 | 0.15* | 0.04* |
| **HGCA2 (x1)** | 0.04 | 1.99 | 0.00 | 2.35 | 0.20 | 0.20 |
| **HGCA2 (x0.5)** | 0.07 | 2.05 | 0.00 | 3.78 | 0.25 | 0.20 |
| **Azoxy (x0.5) + Teb (x0.5)** | 0.10 | 1.94 | 0.00 | 6.20 | 0.16* | 0.06* |
| **Azoxy (x0.5) + Met (x0.5)** | 0.08 | 3.71 | 0.00 | 7.71 | 0.16* | 0.06 |
| **HGCA4 (x1)** | 0.07 | 1.27 | 0.01 | 4.66 | 0.14* | 0.07 |
| **Epoxiconazole (x1)** | 0.13 | 1.43 | 0.00 | 4.94 | 0.33 | 0.19 |
| **Epoxiconazole (x0.5)** | 0.14 | 1.59 | 0.01 | 4.92 | 0.33 | 0.21 |
| **Azoxy (x0.5) + Epoxi (x0.5)** | 0.15 | 2.42 | 0.00 | 6.92 | 0.16* | 0.06* |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.11. Disease scores at GS 75 and GS 85 yield, thousand grain weight, specific weight and DON - HAUC 2000**

| Fungicide | Disease GS75 | Disease GS85 | Yield | TGW | SpWt | DON |
|---|---|---|---|---|---|---|
| **Untreated** | 47.5 | 62.03 | 6.83 | 48.84 | 63.85 | 7.33 |
| **Tebuconazole (x1)** | 44.6 | 61.27 | 7.10 | 52.99 | 66.78 | 4.61 |
| **Tebuconazole (x0.5)** | 42.55 | 58.41 | 7.48 | 50.31 | 65.93 | 4.19 |
| **Metconazole (x1)** | 47.36 | 58.28 | 7.47 | 53.60 | 66.95 | 2.66 |
| **Metconazole (x0.5)** | 47.47 | 57.32* | 6.83 | 51.39 | 65.48 | 4.26 |
| **Azoxystrobin (x1)** | 46.54 | 53.83* | 7.35 | 53.02 | 65.33 | 7.58 |
| **Azoxystrobin (x0.5)** | 50.07 | 55.39* | 7.50 | 51.76 | 64.15 | 14.91+ |
| **HGCA2 (x1)** | 46.5 | 57.32* | 7.66 | 53.12 | 66.75 | 4.30 |
| **HGCA2 (x0.5)** | 46.41 | 58.59 | 7.14 | 51.44 | 66.25 | 5.33 |
| **Azoxy (x0.5) + Teb (0.5)** | 44.08 | 54.06* | 7.64 | 56.01+ | 68.3+ | 7.50 |
| **Azoxy (x0.5) + Met (0.5)** | 45.5 | 56.87* | 7.62 | 53.26 | 66.43 | 6.90 |
| **HGCA4 (x1)** | 46.36 | 55.26* | 7.49 | 55.63+ | 68+ | 3.95 |
| **Epoxiconazole (x1)** | 46.87 | 57.68 | 7.76 | 52.90 | 66.50 | 4.73 |
| **Epoxiconazole (x0.5)** | 50.03 | 60.19 | 7.07 | 50.57 | 64.58 | 6.93 |
| **Azoxy (0.5) + Epoxi (0.5)** | 42.99 | 54.38* | 7.67 | 53.63 | 66.05 | 5.64 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.12. Amount of *Fusarium* species and *Microdochium nivale* varieties in grain - CSL 2000**

| Fungicide | *F. culmorum* | *F. graminearum* | *F. avenaceum* | *F. poae* | *M.n.* var *nivale* | *M.n.* var *majus* |
|---|---|---|---|---|---|---|
| **Untreated** | 0.57 | 2.85 | 1.51 | 0.03 | 0.01 | 0.07 |
| **Tebuconazole (x1)** | 0.10* | 0.64* | 0.59 | 0.02 | 0.01 | 0.08 |
| **Tebuconazole (x0.5)** | 0.2* | 0.95* | 0.49 | 0.02 | 0.01 | 0.08 |
| **Metconazole (x1)** | 0.15* | 0.58* | 0.05* | 0.02 | 0.01 | 0.06 |
| **Metconazole (x0.5)** | 0.25 | 1.55 | 0.66 | 0.03 | 0.01 | 0.05 |
| **Azoxystrobin (x1)** | 0.51 | 1.22* | 0.58 | 0.04 | 0.00 | 0.01* |
| **Azoxystrobin (x0.5)** | 0.57 | 1.85 | 0.58 | 0.04 | 0.00* | 0.02* |
| **Epoxiconazole + Kresoxim-methyl** | 0.31 | 1.86 | 1.15 | 0.03 | 0.00 | 0.02 |
| **Epoxiconazole (x1)** | 0.26 | 1.27* | 0.85 | 0.02 | 0.00 | 0.03 |
| **Epoxiconazole (x0.5)** | 0.25 | 1.49 | 1.72 | 0.02 | 0.00 | 0.03 |
| **Azoxy (x0.5) + Teb (x0.5)** | 0.27 | 1.29* | 0.81 | 0.03 | 0.00 | 0.01* |
| **Azoxy (x0.5) + Met (x0.5)** | 0.21 | 0.94* | 0.43 | 0.05 | 0.00 | 0.01* |
| **Azoxy (x0.5) + Epoxi (x0.5)** | 0.39 | 1.67 | 1.12 | 0.03 | 0.00* | 0.01* |
| **HGCA2 (x1)** | 0.05* | 0.45* | 0.06 | 0.02 | 0.00 | 0.03 |
| **HGCA2 (x0.5)** | 0.13* | 0.46* | 0.11 | 0.02 | 0.00 | 0.03 |
| **HGCA4 (x1)** | 0.15* | 0.34* | 0.15 | 0.03 | 0.00 | 0.01* |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.13. Disease scores at GS 75 and GS 85, thousand grain weight and DON - CSL 2000**

| Fungicide | Disease 75 | Disease 85 | TGW | DON |
|---|---|---|---|---|
| **Untreated** | 1.58 | 30.38 | 43.78 | 13.63 |
| **Tebuconazole (x1)** | 0.46* | 5.58* | 47.90+ | 4.26* |
| **Tebuconazole (x0.5)** | 0.72* | 10.87* | 47.88+ | 7.62* |
| **Metconazole (x1)** | 0.35* | 6.02* | 49.22+ | 5.79* |
| **Metconazole (x0.5)** | 0.71* | 12.28* | 48.38+ | 7.39* |
| **Azoxystrobin (x1)** | 0.62* | 18.77* | 49.48+ | 10.28 |
| **Azoxystrobin (x0.5)** | 0.84* | 24.36 | 48.73+ | 10.38 |
| **Epoxiconazole + Kresoxim-methyl** | 0.52* | 9.78* | 48.11+ | 7.39* |
| **Epoxiconazole (x1)** | 0.7* | 10.95* | 48.35+ | 3.74* |
| **Epoxiconazole (x0.5)** | 0.77* | 15.16* | 47.82+ | 5.61* |
| **Azoxy (x0.5) + Teb (x0.5)** | 0.42* | 12.22* | 47.96+ | 7.74* |
| **Azoxy (x0.5) + Met (x0.5)** | 0.36* | 10.14* | 50.11+ | 5.7* |
| **Azoxy (x0.5) + Epoxi (x0.5)** | 0.82* | 11.15* | 48.58+ | 8.35* |
| **HGCA2 (x1)** | 0.33* | 7.08* | 49.16+ | 1.91* |
| **HGCA2 (x0.5)** | 0.56* | 3.92* | 50.26+ | 3.65* |
| **HGCA4 (x1)** | 0.32* | 5.14* | 49.97+ | 3.58* |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.14. Amount of *Fusarium* species and *Microdochium nivale* varieties in grain - MRC 2000**

| Fungicide | *F. culmorum* | *F. graminearum* | *F. avenaceum* | *F. poae* | *M.n.* var *majus* | *M.n.* var *nivale* |
|---|---|---|---|---|---|---|
| **Untreated** | 0.18 | 0.06 | 1.57 | 0.00 | 0.33 | 0.05 |
| **Tebuconazole (x1)** | 0.05 | 0.03 | 1.86 | 0.00 | 0.43 | 0.10 |
| **Tebuconazole (x0.5)** | 0.08 | 0.01 | 1.44 | 0.00 | 0.41 | 0.07 |
| **Metconazole (x1)** | 0.09 | 0.04 | 1.44 | 0.00 | 0.40 | 0.09 |
| **Metconazole (x0.5)** | 0.10 | 0.04 | 1.40 | 0.01 | 0.38 | 0.11 |
| **Azoxystrobin (x1)** | 0.18 | 0.12 | 2.27 | 0.00 | 0.11* | 0.02* |
| **Azoxystrobin (x0.5)** | 0.20 | 0.08 | 3.26 | 0.00 | 0.13* | 0.02* |
| **Epoxiconazole + Kresoxim-methyl** | 0.11 | 0.05 | 1.87 | 0.00 | 0.22 | 0.04 |
| **Epoxiconazole (x1)** | 0.11 | 0.04 | 2.27 | 0.01 | 0.33 | 0.07 |
| **Epoxiconazole (x0.5)** | 0.13 | 0.03 | 1.67 | 0.01 | 0.32 | 0.08 |
| **Azoxy (x0.5) + Teb (x0.5)** | 0.17 | 0.10 | 1.75 | 0.01 | 0.12* | 0.02 |
| **Azoxy (x0.5) + Met (x0.5)** | 0.16 | 0.10 | 2.26 | 0.00 | 0.15* | 0.01* |
| **Azoxy (x0.5) + Epoxi (x0.5)** | 0.13 | 0.08 | 1.73 | 0.00 | 0.15* | 0.02 |
| **HGCA2 (x1)** | 0.08 | 0.06 | 0.90 | 0.00 | 0.20 | 0.09 |
| **HGCA2 (x0.5)** | 0.11 | 0.05 | 1.17 | 0.00 | 0.20 | 0.06 |
| **HGCA4 (x1)** | 0.11 | 0.19 | 1.25 | 0.00 | 0.11* | 0.02 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.15. Disease score at GS 85 yield, thousand grain weight, specific weight and DON -MRC 2000**

| Fungicide | Disease GS 85 | Yield | TGW | SpWt | DON |
|---|---|---|---|---|---|
| **Untreated** | 45.00 | 8.20 | 37.52 | 71.94 | 2.59 |
| **Tebuconazole (x1)** | 29.75* | 8.85+ | 39.53 | 72.98 | 1.73 |
| **Tebuconazole (x0.5)** | 30.5* | 8.68 | 37.49 | 72.41 | 2.80 |
| **Metconazole (x1)** | 30.75* | 8.61 | 38.41 | 71.96 | 1.85 |
| **Metconazole (x0.5)** | 34.25* | 8.48 | 38.61 | 72.09 | 2.75 |
| **Azoxystrobin (x1)** | 24.25* | 9.09+ | 40.74+ | 73.45 | 5.11+ |
| **Azoxystrobin (x0.5)** | 26.75* | 9.03+ | 40.23+ | 72.98 | 6.33+ |
| **Epoxiconazole + Kresoxim-methyl** | 20* | 9.56+ | 41.9+ | 73.97 | 2.53 |
| **Epoxiconazole (x1)** | 25.25* | 9.09+ | 40.25+ | 73.04 | 2.64 |
| **Epoxiconazole (x0.5)** | 27.5* | 8.94+ | 38.54 | 71.36 | 2.74 |
| **Azoxy (x0.5) + Teb (x0.5)** | 20.75* | 9.48+ | 41.66+ | 74.07+ | 3.95 |
| **Azoxy (x0.5) + Met (x0.5)** | 23.5* | 9.08+ | 39.95+ | 73.46 | 4.03 |
| **Azoxy (x0.5) + Epoxi (x0.5)** | 19* | 9.34+ | 40.69+ | 74.21+ | 4.21 |
| **HGCA2 (x1)** | 23.25* | 9.03+ | 40.92+ | 73.57 | 2.44 |
| **HGCA2 (x0.5)** | 26.75* | 8.95+ | 38.02 | 73.21 | 2.61 |
| **HGCA4 (x1)** | 20.75* | 9.67+ | 40.68+ | 74.31+ | 3.63 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.16. Amount of *Fusarium* species and *Microdochium nivale* varieties in grain - CSL 2001**

| Fungicide | *F. culmorum* | *F. graminearum* | *F. avenaceum* | *F. poae* | *M.n.* var *majus*[1] | *M.n.* var *nivale*[1] |
|---|---|---|---|---|---|---|
| **Untreated** | 1.03 | 0.03 | 0.04 | 0.01 | 0.00 | 0.00 |
| **Azoxystrobin (x0.5)- pre** | 0.33 | 0.01 | 0.03 | 0.01 | 0.00 | 0.00 |
| **Azoxystrobin (x0.25)- pre** | 0.67 | 0.02 | 0.02 | 0.01 | 0.00 | 0.00 |
| **Tebuconazole (x0.5)- pre** | 0.17* | 0.01 | 0.02 | 0.01 | 0.00 | 0.00 |
| **Tebuconazole (x0.25)- pre** | 0.23 | 0.02 | 0.02 | 0.01 | 0.00 | 0.00 |
| **Azoxy (x0.5) + Teb (x0.5)- pre** | 0.47 | 0.01 | 0.19 | 0.01 | 0.00 | 0.00 |
| **Azoxy (x0.25) + Teb (x0.25)- pre** | 0.56 | 0.01 | 0.13 | 0.01 | 0.00 | 0.00 |
| **HGCA2 (x0.5)- pre** | 0.23 | 0.01 | 0.02 | 0.01 | 0.00 | 0.00 |
| **HGCA2 (x0.25)- pre** | 0.19 | 0.01 | 0.03 | 0.01 | 0.00 | 0.00 |
| | | | | | | |
| **Azoxystrobin (x0.5)- post** | 0.46 | 0.02 | 0.08 | 0.01 | 0.00 | 0.00 |
| **Azoxystrobin (x0.25)- post** | 0.86 | 0.03 | 0.06 | 0.01 | 0.00 | 0.00 |
| **Tebuconazole (x0.5)- post** | 0.21 | 0.01 | 0.03 | 0.01 | 0.00 | 0.00 |
| **Tebuconazole (x0.25)- post** | 0.30 | 0.02 | 0.03 | 0.01 | 0.00 | 0.00 |
| **Azoxy (x0.5) + Teb (x0.5)- post** | 0.33 | 0.01 | 0.03 | 0.01 | 0.00 | 0.00 |
| **Azoxy (x0.25) + Teb (x0.25)- post** | 0.54 | 0.02 | 0.02 | 0.01 | 0.00 | 0.00 |
| **HGCA2 (x0.5)- post** | 0.15* | 0.01 | 0.02 | 0.01 | 0.00 | 0.00 |
| **HGCA2 (x0.25)- post** | 0.47 | 0.01 | 0.02 | 0.01 | 0.00 | 0.00 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

[1] Below the level of detection

**Table A.17. Disease scores at GS 75 and GS 85, thousand grain weight and DON -CSL 2001**

| Fungicide | Disease GS75 | Disease GS85 | TGW | DON |
|---|---|---|---|---|
| **Untreated** | 0.34 | 5.49 | 48.68 | 2.66 |
| **Azoxystrobin (x0.5)- pre** | 0.32 | 2.65 | 48.96 | 1.21* |
| **Azoxystrobin (x0.25)- pre** | 0.28 | 1.55* | 49.45 | 1.08* |
| **Tebuconazole (x0.5)- pre** | 0.19 | 0.92* | 49.34 | 0.88* |
| **Tebuconazole (x0.25)- pre** | 0.15 | 1.66* | 48.87 | 1.19* |
| **Azoxy (x0.5) + Teb (x0.5)- pre** | 0.30 | 0.93* | 50.13 | 1.18* |
| **Azoxy (x0.25) + Teb (x0.25)- pre** | 0.17 | 3.03 | 50.27 | 1.83 |
| **HGCA2 (x0.5)- pre** | 0.16 | 1.14* | 49.22 | 0.76* |
| **HGCA2 (x0.25)- pre** | 0.25 | 2.24 | 48.30 | 0.92* |
| | | | | |
| **Azoxystrobin (x0.5)- post** | 0.33 | 1.95* | 47.95 | 1.05* |
| **Azoxystrobin (x0.25)- post** | 0.35 | 5.06 | 47.99 | 1.56 |
| **Tebuconazole (x0.5)- post** | 0.29 | 0.67* | 48.68 | 1.00* |
| **Tebuconazole (x0.25)- post** | 0.44 | 2.00* | 49.43 | 1.00* |
| **Azoxy (x0.25) + Teb (x0.25)- post** | 0.53 | 1.04* | 49.38 | 0.96* |
| **Azoxy (x0.5) + Teb (x0.5)- post** | 0.23 | 1.41* | 49.58 | 0.8* |
| **HGCA2 (x0.5)- post** | 0.15 | 1.15* | 48.74 | 1.53 |
| **HGCA2 (x0.25)- post** | 0.34 | 2.67 | 49.51 | 1.25* |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.18. Amount of *Fusarium* species and *Microdochium nivale* varieties in grain - HAUC 2001**

| Fungicide | *F. culmorum* | *F. graminearum* | *F. avenaceum* | *F. poae* | *M.n.* var *majus*[1] | *M.n.* var *nivale*[1] |
|---|---|---|---|---|---|---|
| **Untreated** | 5.80 | 0.04 | 1.68 | 0.00 | 0.00 | 0.00 |
| **Azoxystrobin (x0.5)- pre** | 4.47 | 0.06 | 3.17 | 0.01 | 0.00 | 0.00 |
| **Azoxystrobin (x0.25)- pre** | 6.74 | 0.08 | 2.90 | 0.00 | 0.00 | 0.00 |
| **Tebuconazole (x0.5)- pre** | 3.33 | 0.08 | 2.09 | 0.01 | 0.00 | 0.00 |
| **Tebuconazole (x0.25)- pre** | 2.61 | 0.06 | 1.77 | 0.00 | 0.00 | 0.00 |
| **Azoxy (x0.5) + Teb (x0.5)- pre** | 4.94 | 0.02 | 2.11 | 0.00 | 0.00 | 0.00 |
| **Azoxy (x0.25) + Teb (x0.25)- pre** | 3.93 | 0.15 | 2.63 | 0.00 | 0.00 | 0.00 |
| | | | | | | |
| **Azoxystrobin (x0.5)- post** | 3.02 | 0.08 | 1.31 | 0.00 | 0.00 | 0.00 |
| **Azoxystrobin (x0.25)- post** | 6.81 | 0.11 | 2.16 | 0.00 | 0.00 | 0.00 |
| **Tebuconazole (x0.5)- post** | 1.31* | 0.02 | 1.40 | 0.00 | 0.00 | 0.00 |
| **Tebuconazole (x0.25)- post** | 2.49 | 0.04 | 0.89 | 0.00 | 0.00 | 0.00 |
| **Azoxy (x0.5) + Teb (x0.5)- post** | 4.81 | 0.07 | 0.85 | 0.00 | 0.00 | 0.00 |
| **Azoxy (x0.25) + Teb (x0.25)- post** | 1.69* | 0.01 | 0.87 | 0.00 | 0.00 | 0.00 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

[1] Below the level of detection

**Table A.19. Disease scores at GS 75 and GS 85 yield, thousand grain weight, specific weight and DON -HAUC 2001**

| Fungicide | Disease GS75 | Disease GS85 | Yield | TGW | SpWt | DON |
|---|---|---|---|---|---|---|
| **Untreated** | 7.06 | 7.00 | 8.92 | 52.85 | 74.35 | 2.13 |
| **Azoxystrobin (x0.5)- pre** | 4.94 | 7.45 | 9.64 | 52.50 | 73.53 | 2.37 |
| **Azoxystrobin (x0.25)- pre** | 7.71 | 6.88 | 9.10 | 52.05 | 73.48 | 1.93 |
| **Tebuconazole (x0.5)- pre** | 1.82* | 3.70 | 9.59 | 52.05 | 74.55 | 1.43 |
| **Tebuconazole (x0.25)- pre** | 3.35 | 4.60 | 9.45 | 52.10 | 75.15 | 1.85 |
| **Azoxy (x0.5) + Teb (x0.5)- pre** | 2.47* | 1.93 | 9.33 | 52.30 | 74.00 | 2.22 |
| **Azoxy (x0.25) + Teb (x0.25)- pre** | 2.65 | 2.07 | 9.30 | 51.80 | 74.30 | 1.84 |
| | | | | | | |
| **Azoxystrobin (x0.5)- post** | 2.88 | 5.13 | 9.34 | 54.70 | 74.40 | 2.53 |
| **Azoxystrobin (x0.25)- post** | 5.00 | 8.60 | 9.30 | 51.90 | 74.10 | 2.34 |
| **Tebuconazole (x0.5)- post** | 4.41 | 1.88 | 9.49 | 52.70 | 74.50 | 1.13 |
| **Tebuconazole (x0.25)- post** | 3.24 | 3.40 | 9.67 | 53.05 | 74.95 | 1.31 |
| **Azoxy (x0.5) + Teb (x0.5)- post** | 3.35 | 1.88 | 9.54 | 52.15 | 75.25 | 1.24 |
| **Azoxy (x0.25) + Teb (x0.25)- post** | 3.06 | 3.03 | 9.77 | 53.85 | 75.40 | 1.58 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

**Table A.20. Amount of *Fusarium* species and *Microdochium nivale* varieties in grain - MRC 2001**

| Fungicide | *F. culmorum* | *F. graminearum* | *F. avenaceum* | *F. poae* | *M.n.* var *majus*[1] | *M.n.* var *nivale*[1] |
|---|---|---|---|---|---|---|
| **Untreated** | 12.95 | 0.43 | 14.70 | 0.02 | 0.00 | 0.00 |
| **Azoxystrobin (x0.5)- pre** | 32.31 | 0.44 | 15.53 | 0.02 | 0.00 | 0.00 |
| **Azoxystrobin (x0.25)- pre** | 18.85 | 0.62 | 19.76 | 0.02 | 0.00 | 0.00 |
| **Tebuconazole (x0.5)- pre** | 11.26 | 0.32 | 7.18 | 0.01 | 0.00 | 0.00 |
| **Tebuconazole (x0.25)- pre** | 15.32 | 0.37 | 14.65 | 0.03 | 0.00 | 0.00 |
| **Azoxy (x0.5) + Teb (x0.5)- pre** | 11.60 | 0.51 | 20.29 | 0.02 | 0.00 | 0.00 |
| **Azoxy (x0.25) + Teb (x0.25)- pre** | 22.82 | 0.27 | 21.64 | 0.02 | 0.00 | 0.00 |
| **HGCA2 (x0.5)- pre** | 12.59 | 0.43 | 12.74 | 0.01 | 0.00 | 0.00 |
| **HGCA2 (x0.25)- pre** | 12.26 | 0.44 | 8.90 | 0.02 | 0.00 | 0.00 |
| **Azoxystrobin (x0.5)- post** | 19.48 | 0.68 | 13.87 | 0.03 | 0.00 | 0.00 |
| **Azoxystrobin (x0.25)- post** | 18.94 | 0.61 | 14.30 | 0.02 | 0.00 | 0.00 |
| **Tebuconazole (x0.5)- post** | 5.75 | 0.34 | 12.83 | 0.01 | 0.00 | 0.00 |
| **Tebuconazole (x0.25)- post** | 13.01 | 0.46 | 18.21 | 0.02 | 0.00 | 0.00 |
| **Azoxy (x0.5) + Teb (x0.5)- post** | 17.22 | 0.79 | 24.62 | 0.03 | 0.00 | 0.00 |
| **Azoxy (x0.25) + Teb (x0.25)- post** | 12.19 | 0.78 | 19.54 | 0.02 | 0.00 | 0.00 |
| **HGCA2 (x0.5)- post** | 6.85 | 0.45 | 8.17 | 0.01 | 0.00 | 0.00 |
| **HGCA2 (x0.25)- post** | 5.52 | 0.26 | 5.48 | 0.01 | 0.00 | 0.00 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)

[1] Below the level of detection

**Table A.21. Disease scores at GS 75 and GS 85 yield, thousand grain weight, specific weight and DON -MRC 2001**

| Fungicide | Disease GS75 | Disease GS85 | Yield | TGW | SpWt | DON |
|---|---|---|---|---|---|---|
| **Untreated** | 45.50 | 62.50 | 9.10 | 41.53 | 64.20 | 15.13 |
| **Azoxystrobin (x0.5)- pre** | 35.5* | 33.75* | 9.69 | 43.27 | 63.27 | 20.86 |
| **Azoxystrobin (x0.25)- pre** | 33.5* | 41.25* | 9.21 | 42.39 | 63.44 | 22.77 |
| **Tebuconazole (x0.5)- pre** | 35.5* | 47.50 | 9.38 | 44.07 | 66.25 | 11.96 |
| **Tebuconazole (x0.25)- pre** | 35.25* | 55.00 | 9.18 | 43.21 | 65.27 | 14.86 |
| **Azoxy (x0.5) + Teb (x0.5)- pre** | 34.5* | 32.5* | 9.78 | 44.44 | 65.41 | 14.10 |
| **Azoxy (x0.25) + Teb (x0.25)- pre** | 30.25* | 40* | 9.26 | 44.62 | 65.54 | 17.21 |
| **HGCA2 (x0.5)- pre** | 36.25* | 62.50 | 9.71 | 44.93 | 66.85 | 12.00 |
| **HGCA2 (x0.25)- pre** | 36.25* | 68.75 | 9.14 | 45.17 | 65.27 | 12.63 |
| **Azoxystrobin (x0.5)- post** | 34.25* | 36.75* | 9.77 | 42.45 | 65.45 | 13.72 |
| **Azoxystrobin (x0.25)- post** | 32.75* | 37.5* | 9.54 | 43.24 | 64.94 | 18.97 |
| **Tebuconazole (x0.5)- post** | 35.5* | 43.75* | 9.48 | 45.59 | 67.24 | 9.91 |
| **Tebuconazole (x0.25)- post** | 33* | 46.25 | 9.29 | 43.13 | 66.86 | 11.32 |
| **Azoxy (x0.5) + Teb (x0.5)- post** | 24* | 20* | 9.53 | 45.15 | 67.49 | 12.16 |
| **Azoxy (x0.25) + Teb (x0.25)- post** | 27.5* | 31.25* | 9.80 | 45.58 | 65.93 | 14.84 |
| **HGCA2 (x0.5)- post** | 29.5* | 50.00 | 10.13 | 45.22 | 67.99+ | 9.72 |
| **HGCA2 (x0.25)- post** | 30* | 52.50 | 10.15 | 43.84 | 68.31+ | 6.96 |

* significantly lower than untreated (P,0.05)

+ significantly higher than untreated (P<0.05)