Project Report No. 383

February 2006

Price: £4.50

Improving the deposition and coverage of fungicides on ears to control Fusarium ear blight and reduce mycotoxin contamination of grain

by

C S Parkin[1], P C H Miller[1], E S Powell[2], J H Orson[2], J Gill[2]
N Magan[3], and D Aldred[3]

[1]Silsoe Research Institute, Wrest Park, Silsoe, Bedfordshire, MK45 5HS
[2]The Arable Group, Wymondham, Norfolk, NR18 9DB, UK
[3]Cranfield University, Silsoe, Bedford, MK45 4DT, UK

This is the final report of a thirty-six month project that commenced in October 2002. The work was funded by a contract for £166,168 from the Home-Grown Cereals Authority (Project No. 2743).

CONTENTS

Page No.

ABSTRACT

Fusarium ear blight (FEB) infections in wheat can significantly reduce grain yield and produce mycotoxins that are potentially harmful when consumed. Limits on the levels of mycotoxins found in grain are being introduced. Fungicidal sprays need to not only control FEB but also reduce mycotoxin levels.

A three-year study aimed at developing techniques to both control FEB and reduce toxin levels was carried out. Spray deposition from a range of application techniques was evaluated in the laboratory using a wind tunnel and. Field experiments were carried out using established and emerging fungicides and commercial field plots were inoculated with mycotoxin producing *Fusarium* species. Samples of ears were assessed for FEB and analysed by HPLC techniques for trichothecene mycotoxins.

Deposition onto sensitive sites such as the spikelets was increased by angling nozzles and selecting an appropriate spray quality. With some fungicides this could improve disease control. Recently-introduced fungicides performed better than established fungicides. Application technique had no clear effect on mycotoxin production.

Despite inoculation with mycotoxin-producing *Fusarium* sp., levels of disease established in the experimental plots varied from year to year. Factors such as the naturally occurring *Fusarium* – *Microdochium* complex and its variable response to fungicides appeared to mask any differences due to changes in fungicide deposit. All grain samples analysed had mycotoxin levels below the forthcoming EU standard of 1250 $\mu g\ kg^{-1}$ for unprocessed wheat.

More reliable disease control will result from angling nozzles backwards. Medium spray quality or air-included sprays may provide better control than fine sprays.

SUMMARY

A three-year study was carried out to investigate the settings and nozzle designs that increase deposition on the ear and more specifically deposition onto the sites of FEB infection. The study aimed to identify spray application techniques suited to UK conditions that could increase deposition onto FEB sensitive sites on the ear and to select fungicides to provide both effective control of *Fusarium* sp. and reduced mycotoxin levels.

Because FEB control and its subsequent effect on mycotoxin levels is complex, only certain parameters were considered in this research. Issues such as formulation chemistry, spray timing and volume application rate were not considered. Particular attention was paid to the mycotoxins deoxynivalenol (DON) and nivaelenol (NIV). A standardised volume rate of 150 l/ha was used throughout because it is representative of current UK practice.

A series of wind tunnel experiments using a tray-grown crop were carried out in 2003 and 2004. These experiments were used to explore the effect of different nozzle designs and configurations on spray deposition to the ear. Deposition was measured by tracing dyes, fluorescent pigments and active ingredient. The emphasis was on using angled nozzles and those that provided a range of spray qualities. Nozzles used in tests during the project included conventional and air-induction flat-fan nozzles orientated vertically and 45° forwards and backwards. Conventional and air-induction hollow-cone nozzles were also used. An air induction flat-fan nozzle angled at 10° (Amistar Az) was also tested.

Field experiments were carried out in 2003, 2004 and 2005 using different fungicides and application systems selected using the results from the wind tunnel experiments. Treatments were applied to a crop of winter wheat at mid-anthesis using a factorial design. All spray treatments were applied with adjustments to speed and nozzle arrangements on the boom made when necessary to accommodate nozzle characteristics. To help ensure the presence of ear diseases, the site was inoculated with known DON-producing *Fusarium sp.* before fungicide application. Establishment of the fungi was encouraged by regular misting following inoculation prior to fungicide application. Spray deposits on ears were measured in 2003 and 2004. FEB control, mycotoxin production and yield were monitored throughout.

In 2003, field and wind tunnel data suggested a trend for a higher deposit on the back of the ear compared to the front for all treatments, with deposits slightly higher on the front or the back of the ear from the Amistar nozzle. This application system tended to give the highest yields in the field experiment for the three fungicide treatments evaluated. Wind tunnel experiments using the fungicide tebuconazole as a tracer carried out in 2004 showed good penetration of fungicide onto the rachis with the air-included hollow-cone and the angled conventional nozzle showing the highest deposits. Similar measurements with samples from the field experiment showed lower levels of tebuconazole penetrating the rachis. This could be due to differences

between the varieties or crop densities used in the field and the wind tunnel or to environmental conditions. It was not possible to discern significant differences in tebuconazole deposition in the field caused by varying application method, although the angled air-included spray had the highest deposit levels.

High levels of NIV were detected in 2003 and were thought to be due to the presence of *F. poae* affecting individual plots for some treatments. In 2005, the results were similarly affected despite the increased misting in that year and the more humid ambient conditions. However, lower temperatures and humidity were encountered in 2004 and this appears to have reduced the levels of FEB infection and as a result detectable levels of DON were not found. Detectable levels of DON were found in 2005 but with so few samples showing contamination there was no clear relationship between application method and DON levels. All samples were below 1250 μg kg^{-1}.

Although fungicide deposits on ears can be manipulated by nozzle design and orientation, and under certain circumstances deposit levels can be linked to disease control, the influence of application system on mycotoxin production is far from clear. Factors such as the level of natural disease pressure, the *Fusarium* sp. complex and its reaction to fungicides and crop meteorology appear to strongly influence mycotoxin production even when experiments are carried out with irrigated crops inoculated by known DON-producing strains.

Of the fungicide treatments evaluated in 2003, the full dose of Prosaro resulted in the lowest infection of FEB and highest resulting grain yield. This was irrespective of application system used suggesting that for this dose of fungicide, application system was less important. The Amistar + Folicur treatment appeared to be more dependent on application system and, in particular, the Amistar nozzle tended to result in lower infection of FEB and higher grain yield for this fungicide treatment.

The work has shown that more reliable disease control will result from angling nozzles backwards, and that medium or air-included sprays may provide better control than fine sprays.

1 INTRODUCTION

In the UK *Fusarium* ear blight (FEB) on wheat is predominantly caused by *F. culmorum* but in some cases by complexes of *F. poae*, *F. avenaceum* and *F. graminearum* and one *Microdochium* species *M. nivale* vars. *nivale* and *majus* (formerly *Fusarium nivale*). *Fusarium* infection is of concern owing to the reduction in crop yield that can result, with the extent of the loss being related to the species involved and the humidity around the ear which affects the intensity of infection. Infection can also produce mycotoxins in grain that are potentially harmful to humans and animals when consumed. In particular, *F. culmorum* is important as it readily produces the mycotoxins deoxynivalenol (DON) and nivalenol (NIV) on ripening ears of cereals. Because of concerns for human health, the EU has set maximum limits for mycotoxins found in cereal products intended for human consumption. These limits will come into force in July 2006. For DON the limit is 1250 $\mu g\ kg^{-1}$ for unprocessed wheat.

The link between FEB control and mycotoxin levels is not simple. With naturally occurring infections, Ioos *et al.* (2005) reported that treatments with tebuconazole, metconazole and azoxystrobin produced samples that had high levels of the known DON and NIV producing *F. graminearum* and *F. culmorum* with low mycotoxin levels and highly contaminated samples that were only lightly infected by those species. They concluded that this was likely to be due to the several biotypes of *Fusarium* sp. reacting differently to different fungicides. Inoculated field trials have also shown that the different members of the FEB complex react differently to different fungicides. In a review of *Fusarium* mycotoxins Magan *et al.* (2002) reported that field studies with tebuconazole and metconazole showed good control of FEB and reduced production of DON, but azoxystrobin and related fungicides sometimes increased DON production. There is evidence to suggest that because azoxystrobin controls *Microdochium nivale,* which does not produce mycotoxins, more sites are available for the growth of *Fusarium* species that can produce mycotoxins. Also, low doses of fungicide and environmental factors such as water stress are implicated in increased DON production.

It is clear that to suppress FEB using fungicides, sprays should be targeted onto the site of infection on the ear. It is well known that angling spray nozzles can increase spray deposition onto cereal crops (Bryant *et al.* (1984); Miller *et al.* (2002); Wolf *et al.* (2002)). What is not known are the settings and nozzle designs that specifically increase deposition on the ear and more specifically deposition onto the sites of FEB infection. Work by ARVALIS (Debroize, 2002) demonstrated increased control of FEB using two sets of fine spray quality nozzles, one pointing forward, one back. However, recent work in Canada (Pilsner, 2000) contradicted these findings with a single coarser spray providing better control.

In 2003 this three-year study was initiated to investigate the settings and nozzle designs that increase deposition on the ear and more specifically deposition onto the sites of FEB infection. The project consortium included Silsoe Research Institute, The Arable Group and Cranfield University, Silsoe. The study aimed to identify spray application techniques suited to UK conditions that could increase deposition onto FEB sensitive sites on the ear and to select fungicides that will provide both effective control of *Fusarium* sp. and reduce mycotoxin levels.

Because FEB control and its subsequent effect on mycotoxin levels is complex, only certain parameters were considered in this research. The interaction of the main parameters involved in FEB control and mycotoxin production are shown in diagrammatic form in Fig. 1. Issues such as formulation chemistry, spray timing and volume application rate were not considered. A standardised volume rate of 150 l/ha was used throughout because it represents current UK practice.

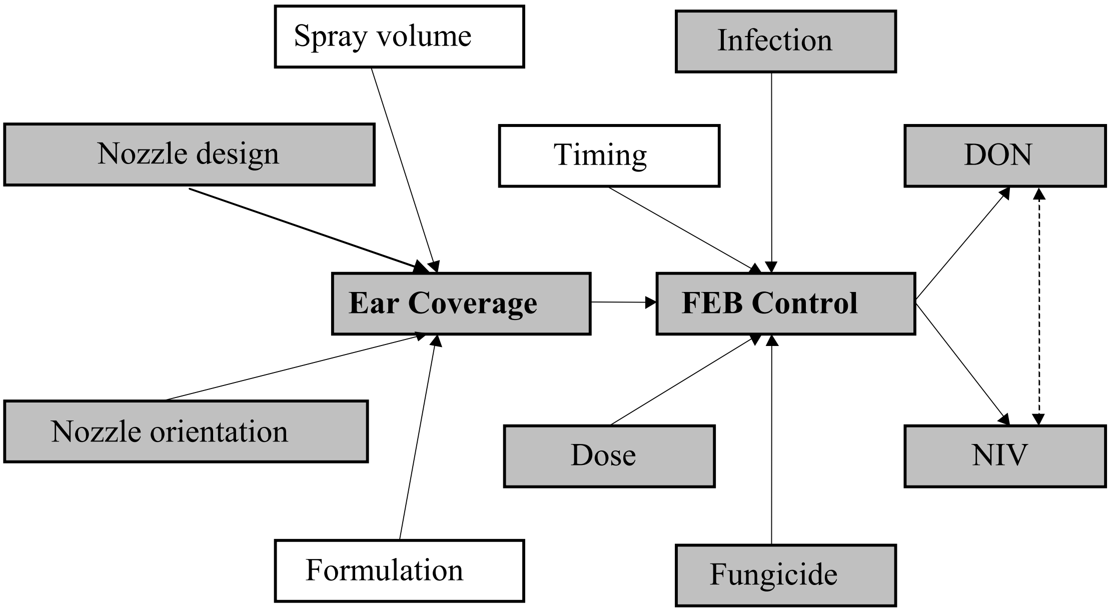


Fig. 1. Interactions between the inputs and outputs involved in the control of FEB and mycotoxins on wheat; shaded boxes indicate parameters under investigation in this project.

The project objectives were:

1. To examine the total spray deposits on wheat ears achieved with a range of different application systems so as to identify those systems that give relatively high total deposit on crop ears.

2. For systems that give a high level of deposit on crop ears, examine the distribution of this deposit so as to identify those systems giving a higher level of uniformity of deposit particularly in the front/back directions with reference to the direction of travel and the level of penetration of spray into the ear.

3. To conduct a series of plot trials over two cropping seasons with at least three different nozzle systems identified from initial experimental work (to include a conventional reference system) and three different fungicide mixtures in which the level of fusarium control was to be assessed. Assessments of spray deposits would be made and plots taken through to yield.

4. To identify combinations of application method and fungicide mixture to maximise the control of *Fusarium sp*., mycotoxins (as measured by DON and NIV).

5. To validate the performance of the recommended system in a series of field trials.

Some aspects of this work have been published (Powell *et al.,* 2004; Parkin *et al.,* 2006).

2 METHODS & MATERIALS

2.1 Selection of test nozzles

Nozzles were selected on the basis that they were likely to provide a range of sprays that could be differentiated by measurement of deposit pattern, and/or biological effect. Some nozzles had a history of use for fungicide ear washes, (e.g. Amistar Az nozzle) and others characterised a particular form of spray (e.g. air-induction). Because deposition of spray onto ears is strongly effected by the angle of projection (Bryant *et al.* 1984), the selection of nozzles included those mounted in twin-cap holders at 45° and designs such as the Amistar AZ nozzle and hollow-cone nozzles where angled spray projection built-in to the nozzle design. It was a requirement that the nozzles selected could be readily available to growers and used on current sprayers without major modifications. The decision on each year's test selection was based on the prior experience gained from the previous year's research and information on spray deposition available from published and private sources. The nozzles chosen each year are presented in Sections 2.6 to 2.10.

To provide benchmark data on the performance of the nozzles selected, the drop spectra of the nozzles selected were measured using a Malvern SprayTec laser drop size analyser. The SprayTec is capable of measuring sprays with air-inclusions (Kippax *et al.,* 2002) and is therefore suitable for evaluating air-induction nozzles. The tests were carried out in the Silsoe Research Institute nozzle laboratory using standard protocols. Measurements were made using a standard liquid (0.1% aqueous solution of Agral non-ionic surfactant) and an x-y transporter to ensure adequate sampling of the complete spray pattern.

2.2 Spray deposit analysis

A key aspect of the project was the assessment of spray deposition onto ears or parts of ears. Spray deposition was assessed using a variety of techniques; the technique chosen being based on its ability to provide the level of resolution required. In this section we review the methods adopted. Further details of the methods adopted are provided in the sections describing individual experiments.

Spray volume collected onto whole ears was assessed using a dye tracer technique. This is a technique that has been used for some time by Silsoe Research Institute to establish spray deposition on natural surfaces. The dye used, Green S (Merck Chemicals) is an approved food dye. It is easily recovered from biological material and when used on cereals it does not suffer from background interference. Although the technique has the advantage of providing a simple and relatively rapid method of assessment, it has the disadvantage of not behaving as a fungicide particularly after the spray has impacted onto the ear. As a result it cannot be used reliably to determine partitioning of the spray within the ear.

Although the front and rear spikelets were assessed separately using the dye tracer technique, most measurements of spray partitioning within the ear utilised HPLC. Tebuconazole, a constituent of many fungicide ear sprays, was used as the tracer with extraction made using a mixture of solvents. Using this technique it was possible to determine the total volume deposited on the ear and the quantity deposited on the rachis and spikelets. The technique was not rapid but it was able to provide more detail than the dye tracer technique and it had the added advantage of using a commercially important fungicide.

Fluorescent pigment tracers were utilised as visual assessment technique in wind tunnel tests. This technique provided images of spray deposits and assisted with the interpretation of analytical data from other techniques. The technique was not used as a primary assessment tool.

2.3 Fungicide selection

The selection of fungicides was based on the requirement to include both existing and emerging products. In 2003 and 2004 field experiments included the then standard treatment of Amistar (azoxystrobin) and Folicur (tebuconazole). In 2003 it was clear that several potentially important products were in development and that they were likely to reach the market before the completion of the project. To ensure the results of this project remained relevant following completion of this work, these compounds were incorporated into the field experiment programme. It should be emphasised that this project did not aim to field test fungicides and the results should not be taken as being indicative of the performance of individual fungicides.

2.4 Crop establishment, inoculation and spraying

It was necessary to grow wheat plants that could be used to assemble an artificial crop in the wind tunnel and establish a commercial crop suitable for field experiments. For the wind tunnel experiments wheat (cv. Claire) was grown outdoors in trays at Silsoe Research Institute within a commercial crop of wheat. The trays then received the standard treatments applied to the commercial crop. However, because their roots were restricted by the trays their head count was lower than a commercial crop (200 – 250 per m^2) and they required added irrigation to maintain vigour. Nevertheless, with careful selection the trays could be arranged to provide a realistic spray target. An example of the tray grown crop is shown in Fig. 2.

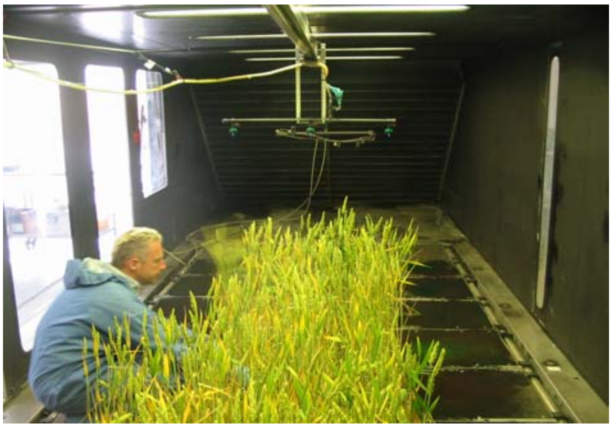


Fig. 2. Tray grown wheat crop and spray transporter in Silsoe wind tunnel

For field experiments crops of winter wheat (cv. Napier) were grown at The Arable Group, Morley, Norfolk. They were established using conventional approaches and treated with epoxiconazole plus chlorothalonil (0.3 l Opus + 1.0 l/ha Bravo) at the second node detectable stage and trifloxystrobin plus epoxiconazole (0.8 l Twist + 0.4 l/ha Opus) at full flag leaf emergence. Plot size was 6 m wide x 24 m long to ensure that reliable yields were obtained from the centre of the plot.

To help ensure the presence of ear diseases, the site was inoculated with *Fusarium culmorum* prior to the experimental fungicide applications using inoculum produced by Cranfield University and applied by sprayer in 2003 and 2004. In 2005 a mixture of *F. culmorum* and *F. graminearum* were used. The former species is a well characterised DON and NIV producer (Hope *et al.*, 2003)
and the latter was a know DON producer kindly provided by Dr. P. Jennings (CSL, York, U.K.). The spore inoculum was grown on sterile moist wheat grain at 25°C for approx. 3 weeks prior to suspensions being prepared and checked with a particle counter. The final concentration of spores used was 10^5 spores ml^{-1} of *F. culmorum* in 2004 and 2005, and a mixture of 10^4 and 10^4 spores ml^{-1} of *F. culmorum* and *F. graminearum* respectively. After application the control plots were analysed and the initial level of contamination of the ears at anthesis was about 10^3 spores $gram^{-1}$. This was analysed by homogenising 10 g sub-samples in 90 ml of sterile water in a Colworth stomacher for 10 min. and serially diluting on malt extract agar. The establishment of the fungi was encouraged by regular ‘misting’ using hollow cone nozzles applying 800 l/ha water, each evening, for three days following inoculation prior to fungicide application. Fig. 2 shows a typical misting application.

Fig. 3. Misting a wheat crop to promote *Fusarium* Ear Blight

The experimental fungicide treatments were applied at mid-anthesis and the experiments comprised a factorial design. All field treatments were applied using a commercial scale farm sprayer (Fig. 3).

Fig. 4. Farm-scale application to experimental plots at Morley, Norfolk

2.5 Mycotoxin extraction and analysis

Analysis of DON and NIV was carried out using a modified method of that reported in Cooney *et al.* (2001). Each sample was finely ground and mixed well. The samples were extracted by mixing with acetonitrile + methanol (14:1; 40 ml), shaking for 2 hours and then filtering through Whatman No 1 filter paper. For mycotoxin analysis a 2 ml aliquot was passed through a cleanup cartridge consisting of a 2 ml syringe packed with a disc of Whatman No 1 filter paper, a 5 ml plug of glass wool and 300 mg of alumina/activated carbon (20:1, 500 mg). The sample was allowed to gravity feed through the cartridge and the eluent collected. The column was washed with a mixture of acetonitrile + methanol + water (80:5:15; 500 µl), and the combined eluate was evaporated with compressed air, at 50°C, to dryness and then re-suspended in methanol + water (5:95; 500 µl).

Quantification of DON and NIV was accomplished via HPLC, using a Luna C18 reverse phase column (100 mm x 4.6 mm, 5 mm particle size; Phenomenex) connected to a 4 x 3 mm guard column filled with the same mobile phase. Separation was achieved using an isocratic mobile phase of methanol + water (12:88, v/v) at 1.5 ml min^{-1}. Eluates (50 µl) were detected using a UV detector set at 220 nm with an attenuation of 0.01 AUFS. The retention time for DON was 13.3 minutes. Quantification was relative to external standards of 1 to 4 µg ml^{-1} in methanol + water (5:95) and the quantification limit was 5ng g^{-1}. This method has been described by Ramirez et al. (2004). Two replicates of all treatments were also subjected to full trichothecene analysis using GC-MS in 2003 and 2004. In both years only DON and NIV were detected in the harvested grain samples.

The relative amount of ear blight present in treatments was recorded about two weeks after fungicide application and samples taken for mycotoxin analyses. The harvested grain samples were also examined for the level of visible contamination with pink grains and contamination checked by plating on malt extract agar and determining levels of *Fusarium* contamination. In 2003 and 2004 Taqman PCR was also employed to examine the relative amounts of DNA of Fusarium species in the final grain samples using a method developed by Waalwijk (2002).

2.6 Wind tunnel experiments 2003

An initial set of measurements assessing total ear deposits and deposits to the front and back of the ear was carried out with nine different application systems (Table 1). The front of the ear was considered to be the side that the spray liquid made contact with first when the spray boom was travelling forward.

Table 1. Details of application systems evaluated in 2003 wind tunnel experiments

Nozzle	Description	Spray quality	Volume
Lurmark F110-03	Conventional flat fan nozzle	Medium	150 l/ha
Amistar	10° angled back air induction nozzle[1]	-	150 l/ha
2 x Lurmark F110-015	2 conventional flat fan nozzles angled at 45°, one forward & one back	Fine	150 l/ha
Delevan WRW2	Wide angle hollow cone nozzle	Coarse	150 l/ha
Lurmark F110-015	Conventional flat fan nozzle angled 45° back	Fine	150 l/ha
Lurmark F110-04	Conventional flat fan nozzle angled 45° back	Medium	150 l/ha
D5-23	Hollow cone nozzle	Fine	150 l/ha
Lurmark F110-04LD	Pre-orifice flat fan nozzle	Coarse	150 l/ha
Amistar	10° angled back air-induction nozzle	-	100l/ha

The tray grown crop was arranged in the tunnel to simulate a realistic canopy with guard trays surrounding the tray to be treated. To determine deposition on the ear a spray liquid comprising 0.4% Green S tracer dye and 0.1% Agral (surfactant) was used. For each treatment 10 ears were sampled, seven for whole ear analysis and three to determine the amounts deposited on the front and back facing surfaces of the ears. The concentration of recovered dye was measured using spectrophotometry. Visual assessments of deposit patterns were carried out on four of the application systems that were selected for assessment in the field (Table 2). The spray liquid for these experiments was a 2% suspension of Saturn Yellow fluorescent pigment with 0.1% Agral.

2.7 Field trial 2003

A field experiment was established evaluating a range of fungicides and application systems. One of the fungicide treatments evaluated was a development fungicide which was not yet commercially available and was included in the project as a code only and evaluated at two doses. The application systems were selected following visual assessments of deposit patterns from the initial wind tunnel experiments and selection was such that the application systems were examples of current commercial practice or were likely to give a range of spray deposition. Details of the fungicides, doses and application systems used are shown in Table

---

[1] To date there is no agreed classification for air-induction sprays. A measure of spray quality can be estimated from drop size (see Table 6) but this ignores differences in amount of air included.

2. All spray treatments were applied at a spray volume of 150 l/ha with adjustments to speed and nozzle arrangements on the boom made when necessary to accommodate nozzle characteristics.

Table 2. Application systems and fungicides evaluated in the 2003 field experiment

Nozzles	**Fungicides**
Lurmark/Hypro flat-fan F110-03	0.3 l ha ^{1} Amistar (azoxystrobin 250 g l^{-1}) 0.3 l ha^{-1} Folicur (tebuconazole 250 g l^{-1})
Amistar Az angled air-induction nozzle	0.75 l ha^{-1} Prosaro[2] (125 g l^{-1} prothioconazole; 125 g l^{-1} tebuconazole)
Lurmark/Hypro F110-04 flat-fan angled nozzle	1.5 l ha^{-1} Prosaro (125 g l^{-1} prothioconazole; 125 g l^{-1} tebuconazole)
Lurmark/Hypro Hollow-cone D5-23	

One hundred ears per plot were collected two weeks after spraying for visual assessment of FEB and analysis of DON and NIV. Assessment of FEB was carried out on 10 randomly selected ears from the 100 ear sample. For each sub sample, the area affected by FEB was assessed and expressed as a percentage of the total area. Plots were harvested by replicate using a plot combine which used electronic weighing, moisture and specific weight determination (Harvest Master HM-400 with Grain Gauge).

For each application system evaluated in the field trial a single plot was also treated with a Green S tracer dye. These plots were located alongside the field trial. Total deposition on whole ears was measured using a similar technique to the wind tunnel experiments.

2.8 Wind tunnel measurements 2004

A second series of experiments was conducted in the wind tunnel at Silsoe Research Institute laboratory in 2004 using a winter wheat crop (cultivar Claire) grown outdoors in trays. Measurements investigating deposition onto spikelets and rachis were made using the fungicide tebuconazole (as Folicur applied at 1.0 l ha^{-1}) and HPLC. Each treatment was replicated three times and five ears were selected from each replicate. Tebuconazole was recovered from the spikelets and rachis of each ear. Because the work carried out in 2003 had indicated that the form and location of the spray deposit on the ear may be important, angled spray treatments with varying spray quality were tested. Details of the application techniques are given in Table 3.

---

[2] When this product was applied it had not yet received approval and was not named. When reported (Powell *et al.,* 2004) it was coded as HGCA 1.

Table 3. Details of application systems evaluated in 2004 wind tunnel experiment

Application	Nozzles	Manufacturer	Spray quality	Volume
Conventional vertical flat-fan nozzle	03F110VB	Lurmark Hypro	medium	150 l ha^{-1}
Flat-fan nozzle angled 45° back	03F110VB	Lurmark Hypro	medium	150 l ha^{-1}
Flat-fan nozzles 45° twin cap	015F110VB	Lurmark Hypro	fine	150 l ha^{-1}
Flat-fan nozzles 45° twin cap	03F110VB	Lurmark Hypro	medium	300 l ha^{-1}
Air-induction nozzle 45° back	PJ-03	Sprays International	air-included	150 l ha^{-1}
Hollow-cone air-induction nozzle	80-025 HC	Bfs/ Agrotop	air-included	150 l ha^{-1}

2.9 Field experiments 2004

In 2004 a field experiment was established at The Arable Group, Morley, Norfolk evaluating the application systems selected following the wind tunnel and field experiments in 2003 and using different fungicides. Application systems were selected to test the hypothesis that coarse spray directed to the ears would give improved results[3]. This was based on the work carried out in 2003 and the work of Wolf *et al.* (2002). Treatments were applied to a crop of winter wheat (cultivar Napier) at mid-anthesis and the trial comprised a factorial design. Details of the fungicides, application systems used are shown in Table 4. All spray treatments were applied at a volume rate of 150 l ha^{-1} using farm-scale sprayers to 6 m wide by 24 m long plots. Adjustments were made to speed and nozzle arrangements to accommodate nozzle characteristics.

Two fungicidal treatments were used, azoxystrobin plus tebuconazole applied as Amistar (Syngenta) at 0.3 l ha^{-1} plus Folicur (Bayer CropScience) at 0.3 l ha^{-1} and fluoxastrobin plus prothioconazole applied at 0.7 l ha^{-1}. This was known as the coded product Bayer (UK 187) but it is now sold as Fandango (Bayer CropScience). The crop was established using conventional approaches and inoculated with a mixture of *Fusarium culmorum* and *F. graminearum* five days before fungicide application. In 2003 the inoculum had consisted solely of *F. culmorum*. The *Fusarium* mixture was adopted because it was considered to be more representative of the infections currently found in Southern England.

As in 2003 the establishment of the fungi was encouraged by regular misting. The experimental plots were monitored, sampled for yield and analysed for mycotoxins using the methods reported by Powell *et al.*

---

[3] The air-included hollow-cone nozzle was not available for use in the field in 2004.

(2004).To determine deposition onto ears four samples, each of 10 ears, were taken from the plots where tebuconazole was applied and subjected to a similar analysis to the wind tunnel samples.

Table 4. Details of application systems evaluated in 2004 field experiment

Description	Nozzles	Manufacturer	Spray quality	Spray direction
Conventional flat-fan nozzle	03F110	Lurmark Hypro	medium	vertical
Flat-fan nozzle 45^o back	03F110	Lurmark Hypro	medium	back
Flat-fan nozzles 45^o twin cap	015F110	Lurmark Hypro	fine	fore & back
Air-induction nozzle 10° back	Amistar	Syngenta	air-included	back
Air-induction nozzle vertical	PJ-03	Sprays International	air-included	vertical
Air-induction nozzle 45^o back	PJ-03	Sprays International	air-included	back
Hollow-cone nozzle	D5-23	Lurmark Hypro	fine	multi

2.10 Field experiments 2005

In 2005 a further field experiment using the same methodology was carried out using the applications set out in Table 5. Because the emphasis for the experiments was to investigate application effects, a single fungicide was selected. The fungicide chosen was prothioconazole/ tebuconazole applied at 1.5 and 0.7 l ha^{-1} as Prosaro (Bayer CropScience).

The crop was established using similar methods as in 2004 and inoculated with a mixture of *Fusarium culmorum* and *F. graminearum*. The establishment of the fungi was again encouraged by misting but with an increased frequency of application to maximise infection. The methods reported by Powell *et al.* (2004) were used to assess FEB, yield and mycotoxin content.

Table 5. Details of application systems evaluated in 2005 field experiment

Description	Nozzles	Manufacturer	Spray quality	Spray direction
Conventional flat-fan nozzle	03F110	Lurmark Hypro	medium	vertical
Flat-fan nozzle 45° back	03F110	Lurmark Hypro	medium	back
Flat-fan nozzles 45° twin cap	015F110	Lurmark Hypro	fine	fore & back
Air-induction nozzle vertical	AI11003	Tee Jet	air-included	vertical
Air induction nozzle 45° back	AI11003	Tee Jet	air-included	back
Hollow-cone nozzle	D5-23	Lurmark Hypro	fine	multi
Flat-fan nozzles - two booms	015F110	Lurmark Hypro	fine	fine
Hollow-cone air-induction	80-025HL	Bfs/ Agrotop	air-included	multi

3 RESULTS

3.1 Wind tunnel experiment 2003

The total ear deposits from each of the application systems evaluated are illustrated in Figure 5. The differences in deposit between application systems were relatively small although there was a suggestion that the highest deposits were achieved using the wide angle hollow-cone nozzle (WRW2) and the F110-015 and F110-04 nozzles angled backwards. Many of the application systems resulted in a similar distribution of deposit on the front and the back of the ear (Fig. 6) apart from the two F110-015 nozzles angled forward and backwards and the single F110-015 nozzle angled backwards which resulted in higher deposits on the front of the ear.

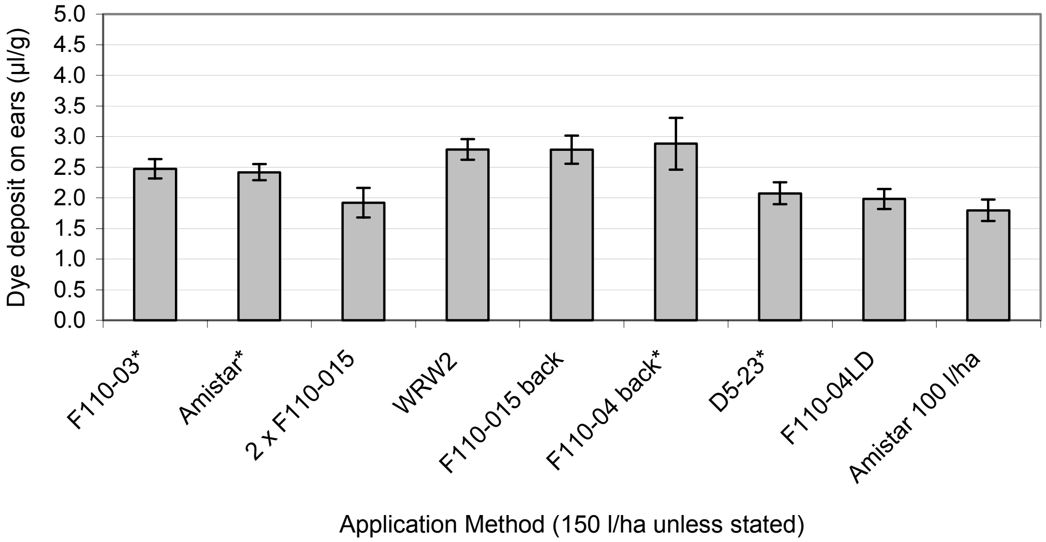


Figure 5. Mean total deposits on wheat ears (µl g^{-1}) from all application systems in the wind tunnel experiments (* = application systems further evaluated in the field trial)

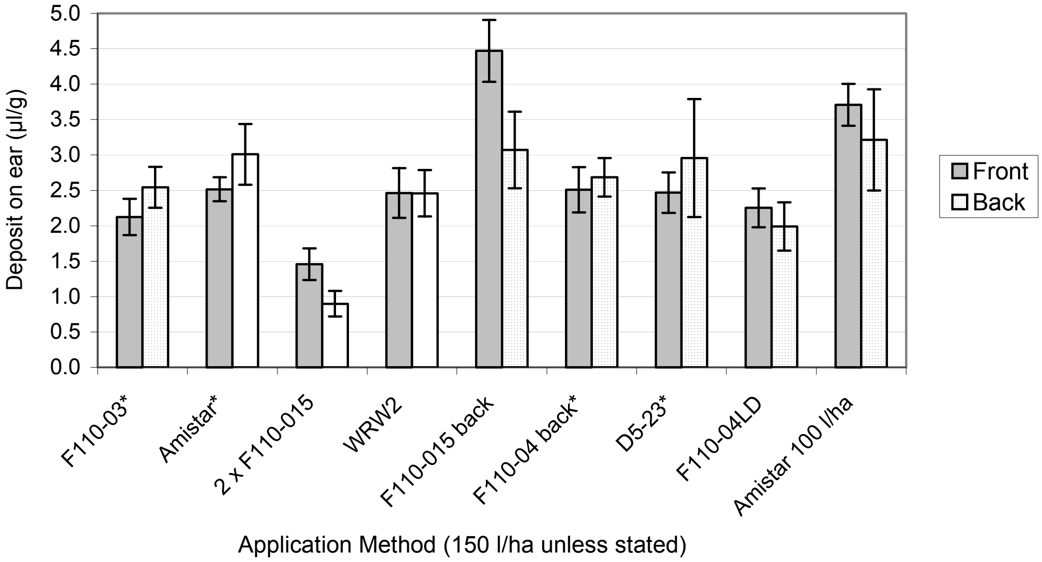


Fig. 6. Mean deposit distribution on the front and back of wheat ears (µl g^{-1}) from all application systems in the wind tunnel experiments (* = application systems further evaluated in the field trial)

3.2 Field experiment 2003

Fig. 7 shows the grain yield achieved from the range of application systems and fungicides tested. A full dose of Prosaro (1.5 l ha^{-1}) achieved the highest yield of grain with all of the application systems evaluated and a clear dose response between the half and full dose of this fungicide was apparent. The grain yield achieved by Prosaro was higher than the current standard treatment for FEB control, Amistar + Folicur, particularly at the full dose. There was a trend for the three other application systems to result in higher grain yield than the conventional flat fan-nozzle with a trend for the Amistar nozzle to give the highest yield particularly when applying Amistar + Folicur or the full dose of Prosaro.

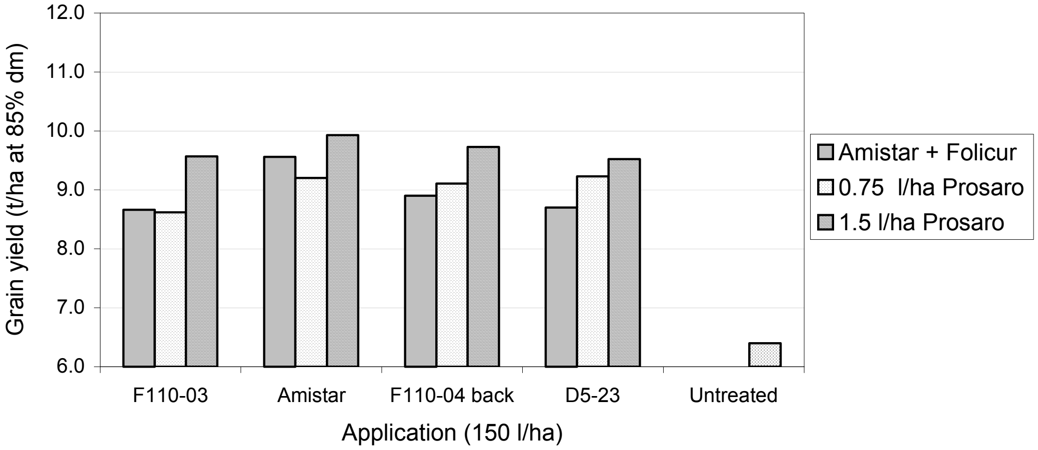


Fig. 7. Grain yield (t/ha at 85% dm) with three fungicide treatments and four application systems in the field trial

Fig. 8 shows the percentage disease infection of FEB on wheat ears, two weeks after fungicide application. A full dose of Prosaro significantly reduced the visible infection of FEB on ears with all application systems compared to the untreated ears. The percentage disease infection was unexpectedly low on the untreated ears and hence there were no significant differences between the reduced dose of Prosaro or Amistar + Folicur with any of the application systems when compared to the untreated.

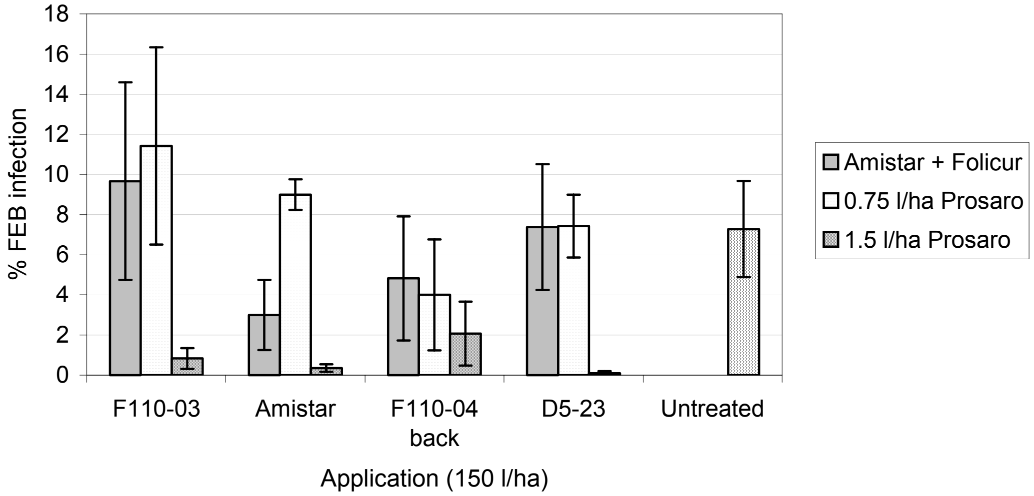


Fig. 8. Percentage infection of FEB on ears two weeks after spraying fungicides (error bars show SEM)

Figure 9 shows the effect of treatments on relative amounts of biomass of *F. culmorum* present in the harvested grain. The highest amount using quantitative PCR was present in the control. The least was present in the nozzle treatments which were most effective and again confirms the effectiveness of the Prosaro treatment at the higher (recommended) application rate.

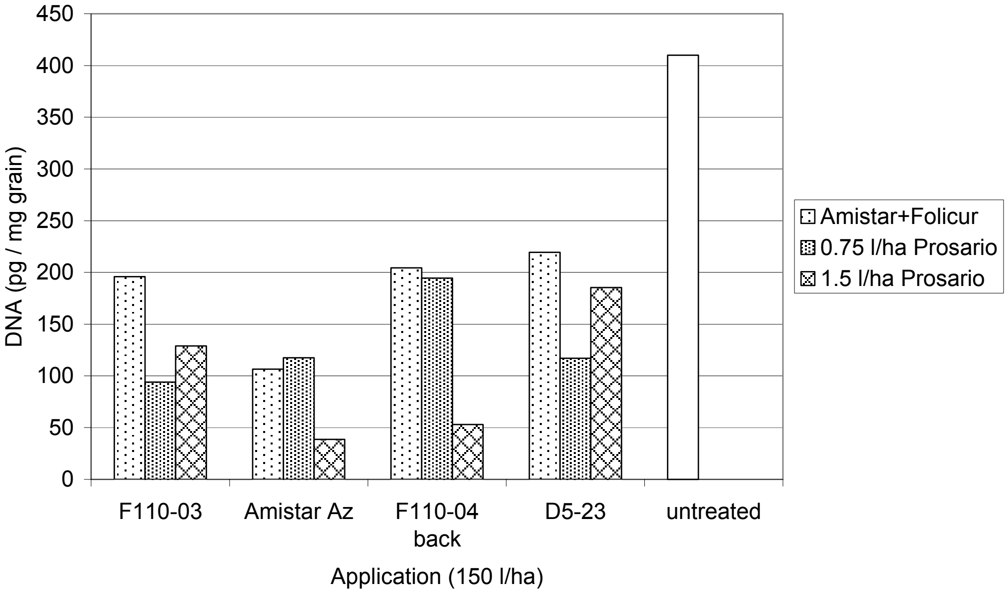


Fig. 9. The mean concentration biomass of *F. culmorum* in grain treated with different spray systems and different fungicides in 2003

Fig. 10 shows DON and NIV levels for wheat ears two weeks after spraying. There was a trend for levels of NIV to be generally higher than those of DON for most treatments but particularly for the backward angled F110-04 nozzle and the hollow-cone nozzle applying Amistar + Folicur or 0.75 l ha^{-1} Prosaro. The levels of DON observed were generally low for all treatments and unexpectedly there were very low levels (0.15 µg kg^{-1}) detected on the untreated ears.

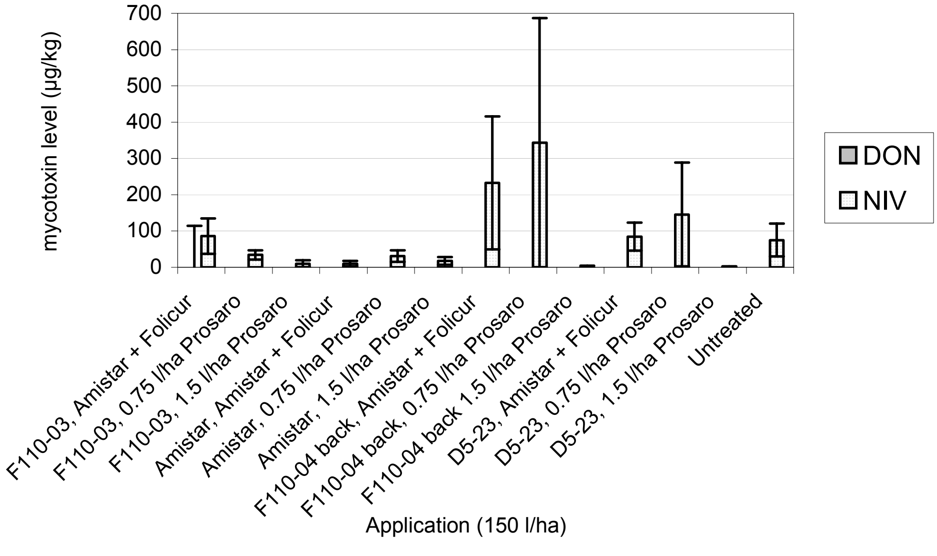
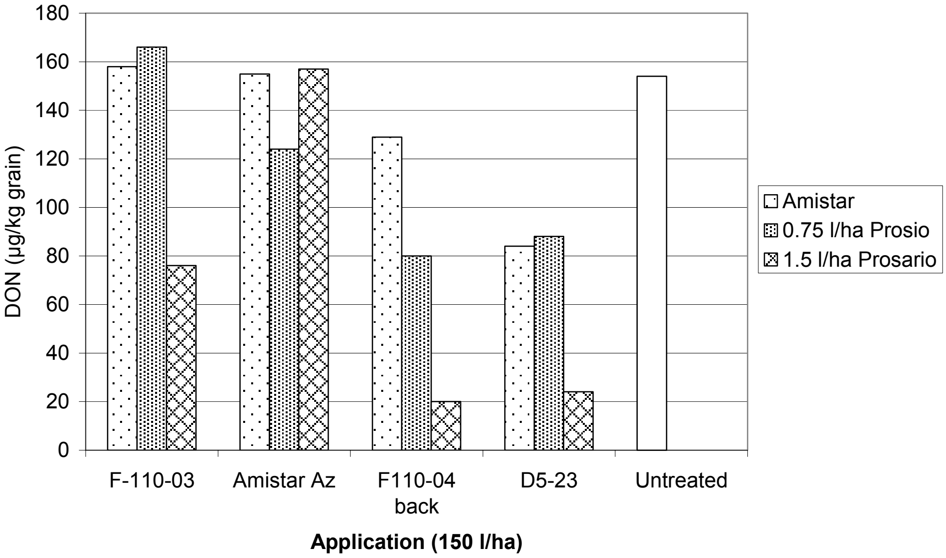


Figure 10. DON and NIV levels (µg/kg) on ears two weeks after spraying (error bars show SEM)

The effect of treatments on DON and NIV in 2003 is shown in Figure 11. In both 2003 and 2004 GC-MS showed that these were the only trichothecenes present. The levels of DON were lower than those for NIV. Generally, based on these results there was very little effect of nozzle targeting on relative amounts of NIV present in the harvested grain. There was some effect of the Prosario fungicide at the normal rate of application with some nozzle treatments but there was no particular trend.



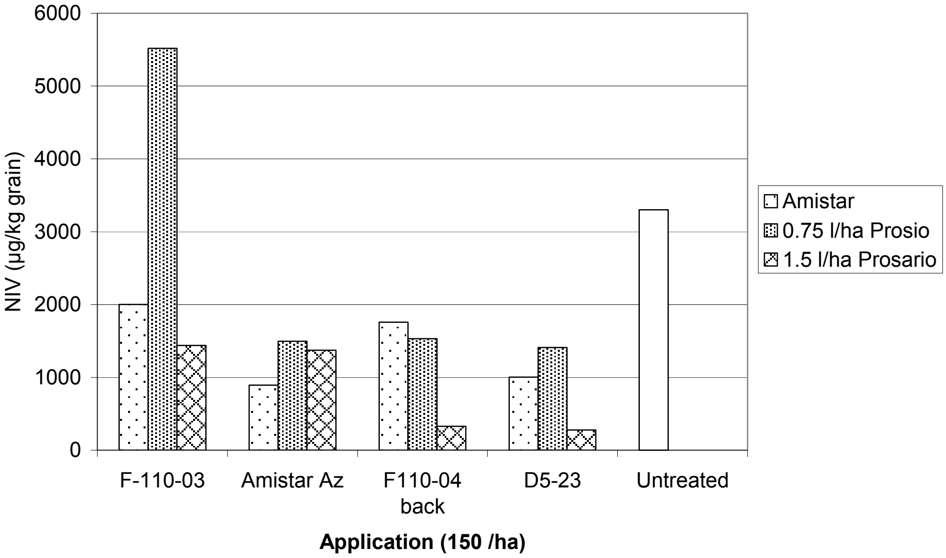


Figure 11. Effect of spray treatments and fungicide applications on content of DON and NIV in harvested wheat grain in 2003; data is mean of GC-MS analyses.

3.3 Wind tunnel experiment 2004

The ear deposits of tebuconazole from each of the application systems evaluated in the wind tunnel in 2004 are illustrated in Fig. 12. Although the variability of deposits was high it appeared that significant amounts of tebuconazole were detected on the rachis indicating that there was penetration into the ear. The data also suggested that an angled medium quality spray and the air-included hollow-cone spray deposited more fungicide on the ear.

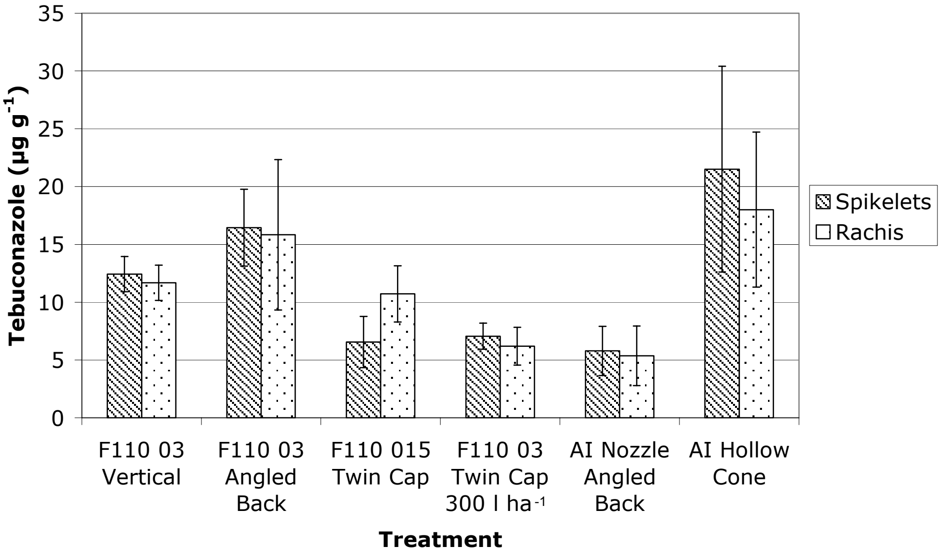


Fig. 12. Mean total deposits on wheat ears from application systems in the wind tunnel experiments in 2004 normalised to 150 l ha^{-1}

3.4 Field experiment 2004

Deposits of tebuconazole from the field samples (Fig. 13) were lower than those from the wind tunnel but this reflected the lower dose used. However, the proportion of chemical penetrating into the rachis was also lower. Although deposits with each application system were broadly similar, unlike the wind tunnel data the air-included angled spray produced the highest deposit.

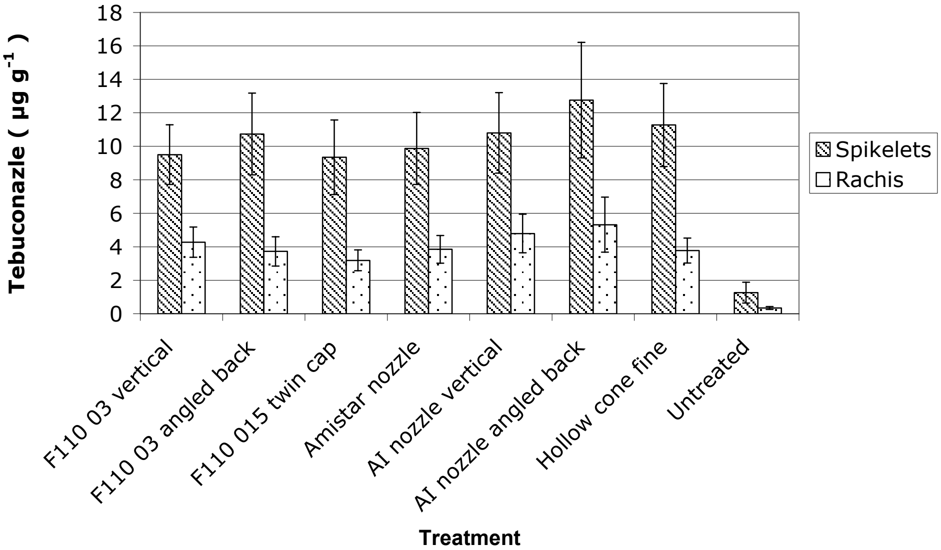


Fig. 13. Mean total deposits on wheat ears from application systems in the field experiments in 2004 normalised to 150 l ha^{-1}

Disease assessments following spraying showed that, unlike the 2003 season there was little FEB established in the crop. Extraction of mycotoxins from ears showed that only NIV was present. With mean values 50-100 µg kg^{-1} this was much less than the 400-600 µg kg^{-1} found in 2003. In harvested grain again only NIV was present and at a maximum of 36 µg kg^{-1}. Differences in yield could not be detected with any application method.

3.5 Field experiment 2005

The field treatments in 2005 were scored for ear colour using a 1-5 scale. The results are shown in Fig. 14. Although few significant differences were observed between treatments, fungicide dose appeared to have an effect and the angled conventional nozzle (F110-03) at the higher dose rate produced a significantly better score. Differences in yield could not be detected with either application method or fungicide dose. Samples taken from the plots were found to be infected by ear blight at similar levels to 2003 (Fig. 13), although the variability of the infection was greater making if difficult to observe any trends. However, plots where the fungicide was applied with the angled air-induction nozzle and the air-induction hollow-cone nozzle had low levels of infection. Despite *Fusarium* infection being established in the crop; only six of the 51 samples showed detectable levels of DON. Where DON was present it was in the range 500 – 550 µg kg^{-1}. As a result, it was not possible to discern any relationship between DON levels and treatment. Levels of NIV were higher (Fig. 14) but the high variability between replicate samples made it difficult to determine trends.

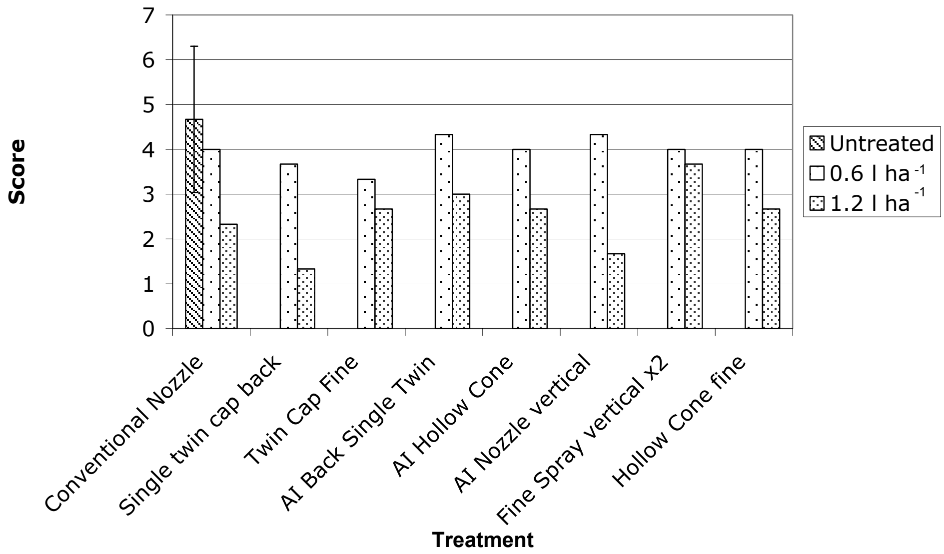


Fig. 12. Ear colour score for fungicide treatments in 2005; 1 = bright, 5 = dark; error bar on the untreated plot represents the LSD

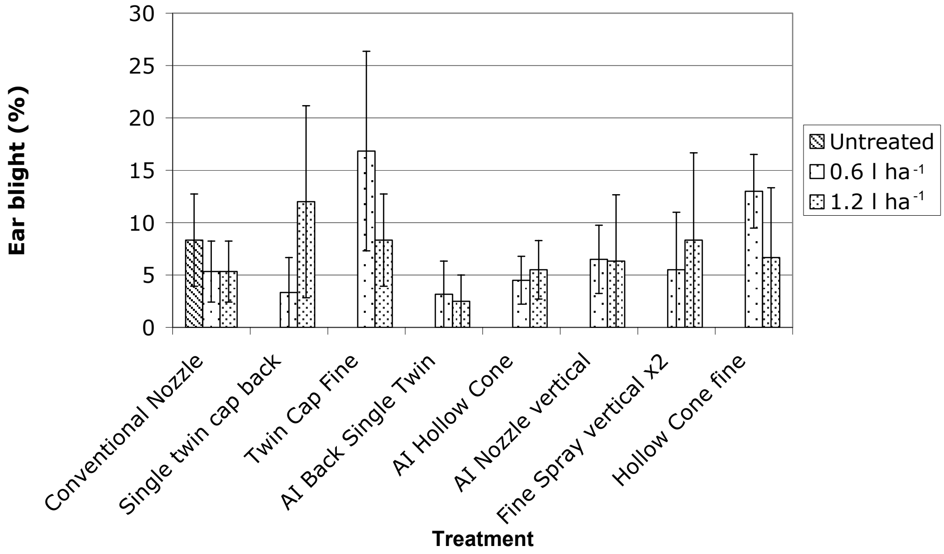


Fig. 14. Ear blight infection two weeks after fungicide treatments in 2005; error bars represent the SEM

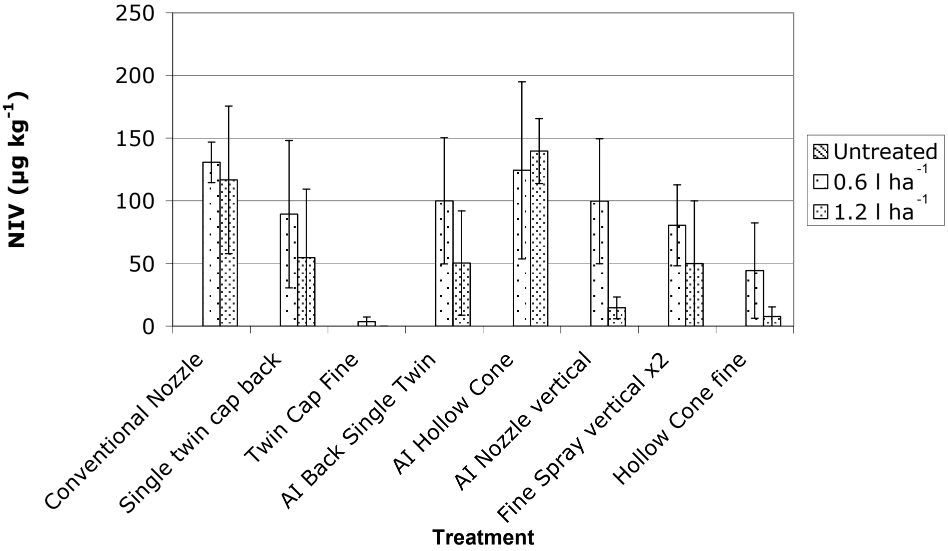


Fig. 15. Nivalenol (NIV) levels in grain samples after fungicide treatments in 2005; error bars represent the SEM

3.6 Drop spectra measurements

The drop spectra of the nozzles selected for use in the project are presented in Table 6. The hollow-cone WRW nozzle could not be measured using the existing sampling arrangement in the nozzle laboratory because of inherent sampling problems caused by the wide angle of the cone. The results show the wide variation in drop spectra used for the experiments and confirm the spray quality descriptions used in Tables 1,3 4, and 5. The results also show the wide variation in drop size spectra that are obtained from air-induction nozzles of a similar size emphasising the need for a classification of spray for air-included sprays.

Table 6. Drop spectra of nozzles used in wind tunnel and field experiments

Manufacturer	Code	Description	Pressure bar	Flowrate l/min	VMD[4] µm
Lurmark/Hypro	03F110	Reference nozzle	3	1.20	192.1
Lurmark/Hypro	015F110	Fine nozzle	3	0.91	177.1
Lurmark/Hypro	04F110	Coarse spray	2	1.30	226.2
Lurmark/Hypro	04F110LD	Pre-orifice flat-fan	3	1.32	284.5
Lechler	ID120-03	Air-induction flat-fan	3	1.32	589.5
bfs/agrotop	80-025 HC	Air-induction hollow-cone	3	0.97	447.7
Lechler	TR 90-03	Fine hollow-cone	3	1.15	195.4
Syngenta	Az	Air-induction angled (150 l/ha)	4	1.17	294.8
Syngenta	Az	Air-induction angled (100 l/ha)	3	1.02	281.7
Sprays International	PJ-03	Air-induction flat-fan	3	1.09	502.9
Spraying Systems	D5-23	Fine hollow-cone	3	0.83	202.9
Delavan	WRW	Hollow-cone	3	0.80	n/a

---

[4] Volume Median Diameter – 50% of the volume of the spray is in drops with diameter greater than this value.

4 DISCUSSION

4.1 Results

The results in 2003 indicated that variations in total ear deposits were relatively small for all the application systems evaluated in the wind tunnel and many of the application systems also resulted in similar deposit distribution between the front and the back of the ear. Of the four application systems that were further evaluated in the field trial (as shown by * on Figs. 5 and 6), the deposit distribution on the front and the back of the ear suggested a slight trend for a higher deposit on the back of the ear compared to the front for all these treatments. The deposit patterns also suggested that for these four application systems, there was a slightly higher deposit on both the front and the back of the ear from the Amistar nozzle, and this was the application system that tended to give the highest yields in the field trial for the three fungicide treatments evaluated.

Of the fungicide treatments evaluated in 2003, the full dose of Prosaro resulted in the lowest infection of FEB and highest resulting grain yield. This was irrespective of application system used suggesting that for this dose of fungicide, application system was less important. The Amistar + Folicur treatment appeared to be more dependent on the application system and in particular the Amistar nozzle tended to result in lower infection of FEB and higher grain yield for this fungicide treatment.

The high levels of NIV detected in 2003 are thought to be due to the presence of *F. poae* and *F.avenaceum* which was also detected in the ears and is capable of producing relatively high levels of NIV. Similar increases in NIV have been observed in trials at Harper Adams University College (Dr. S. Edwards, Personal Communication). The presence of this fungus complicated the results by affecting individual plots for some treatments (e.g. the backward angled F110-04 nozzle applying Amistar + Folicur and the hollow cone nozzle applying the reduced dose of Prosaro) which resulted in high variation in the results.

The DON levels in 2003 were low for all treatments and the untreated ears and this is likely to be owing to the lower than expected levels of infection of FEB caused by *F.culmorum*. DON levels for all treatments were well below the proposed legislative limit.

It was concluded from the 2003 results that variations between the spraying systems evaluated appeared to be relatively small, although these small differences in spray deposit could be more important when applying some fungicides than others. This may be influenced by the mode of action of the fungicide and the importance of targeting the spray deposit to the target and ability to reach the site of *Fusarium* infection may vary between different fungicides. It may be that for some fungicides the target may not be the whole ear but a specific part of the ear.

Wind tunnel experiments with tebuconazole carried out in 2004 showed good penetration of fungicide onto the rachis with the air-included hollow-cone and the angled conventional nozzle showing high deposits. Similar measurements with samples from the field experiment showed lower levels of tebuconazole penetrating the rachis. This could be due to differences between the varieties or crop densities used in the field and the wind tunnel or to environmental conditions. It was not possible to discern significant differences in tebuconazole deposition in the field caused by varying application method although the angled air-included spray had the highest deposit levels.

The high levels of NIV detected in 2003 were thought to be due to the presence of *F. poae* and *F.avenaceum* producing relatively high levels of NIV and effecting individual plots for some treatments. It is likely that the 2005 results were similarly affected despite the increased misting and the more humid conditions. The lower temperatures may also have had an influence on infection. Unlike 2004, detectable levels of DON were found in 2005 but with so few samples showing contamination there was no clear relationship between application method and DON levels. All samples were below 1250 µg kg^{-1}.

It was clear from the results of the field experiments over the three years that, despite inoculation with DON producing *Fusarium sp*., disease pressure and DON production were variable and this made determining differences due to application method difficult. In 2003 it appeared that inoculation produced reasonable disease pressure, but in 2004 using the same techniques produced little disease and no significant difference between treatments. Because of these difficulties, the 2005 inoculation was carefully monitored and misting increased in frequency. However, the natural variability of FEB between plots appears to have influenced the results. Because the weather between inoculation and treatment is crucial to the establishment of disease, this influence of weather was investigated. A summary of the Morley weather data is presented in Table 7.

Table 7. Summary weather data from Morley, Norfolk for the period between *Fusarium* inoculation and fungicide spraying

	Year		
	2003	2004	2005
Mean maximum temperature (°C)	20.3	23.0	18.0
Mean % Relative humidity at 09:00hrs	80.8	74.0	86.6
Total rainfall (mm)	1.48	0.03	1.03

The data shows that during the crucial period between inoculation and spraying it was hotter and drier in 2004 than in 2003 and that in 2005 it was cooler and wetter than 2003. This may help explain why FEB levels were lower in 2004 than in 2003 and why in 2005 there was significant natural variability between plots. To illustrate this further the trends in Morley weather data are plotted in Figs 16, 17 & 18.

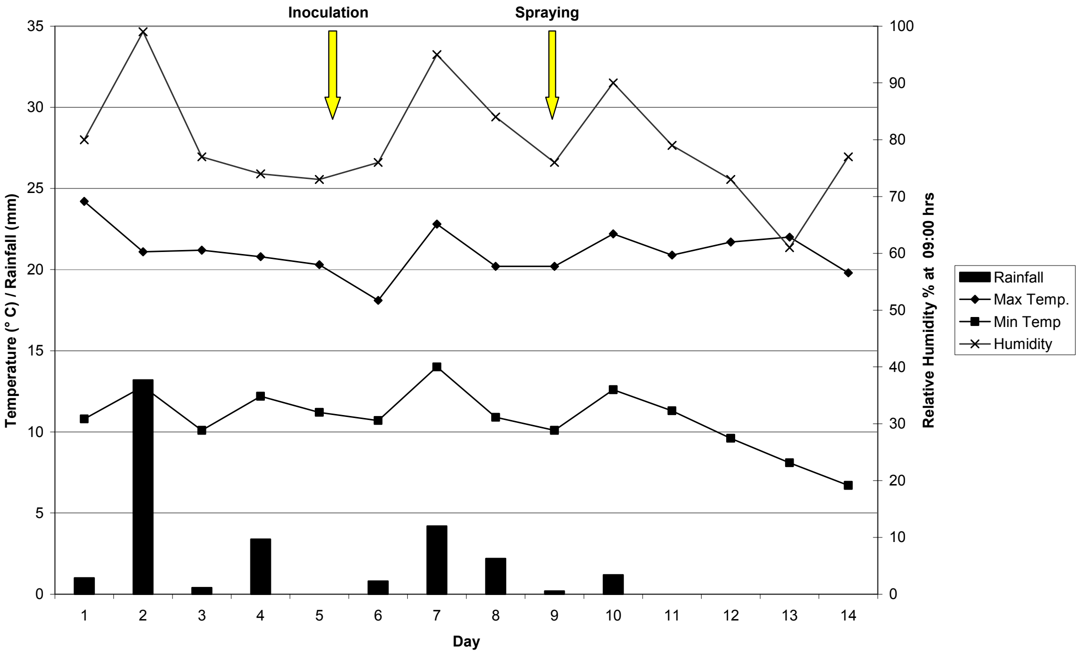


Fig. 17. Meteorological data from Morley Research Centre during the 2003 field experiment.

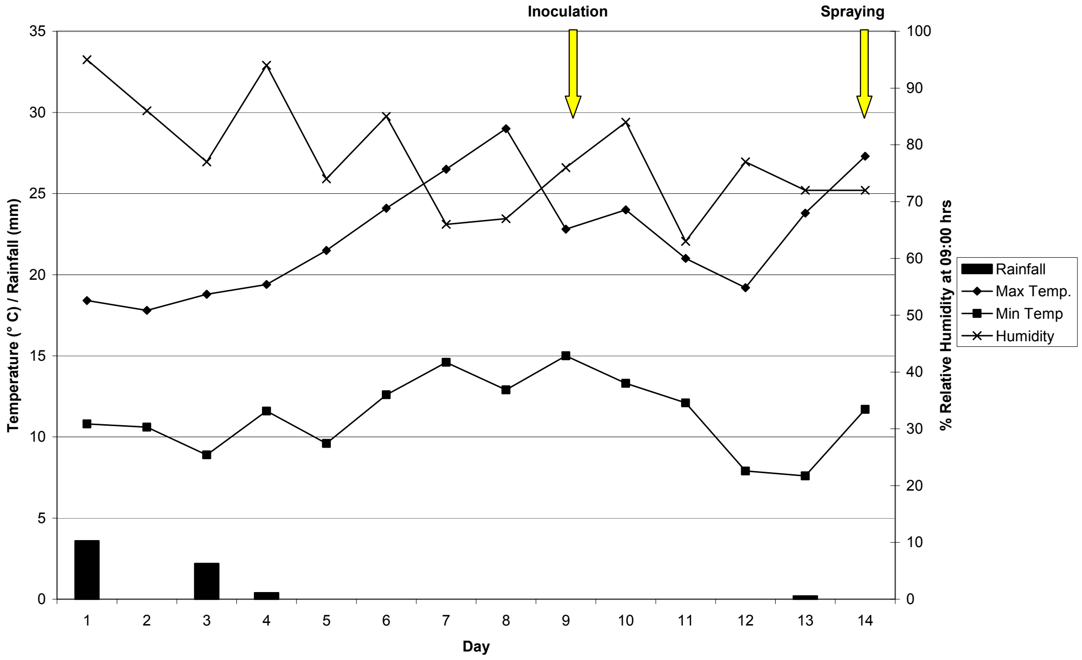


Fig. 18. Meteorological data from Morley Research Centre during the 2004 field experiment

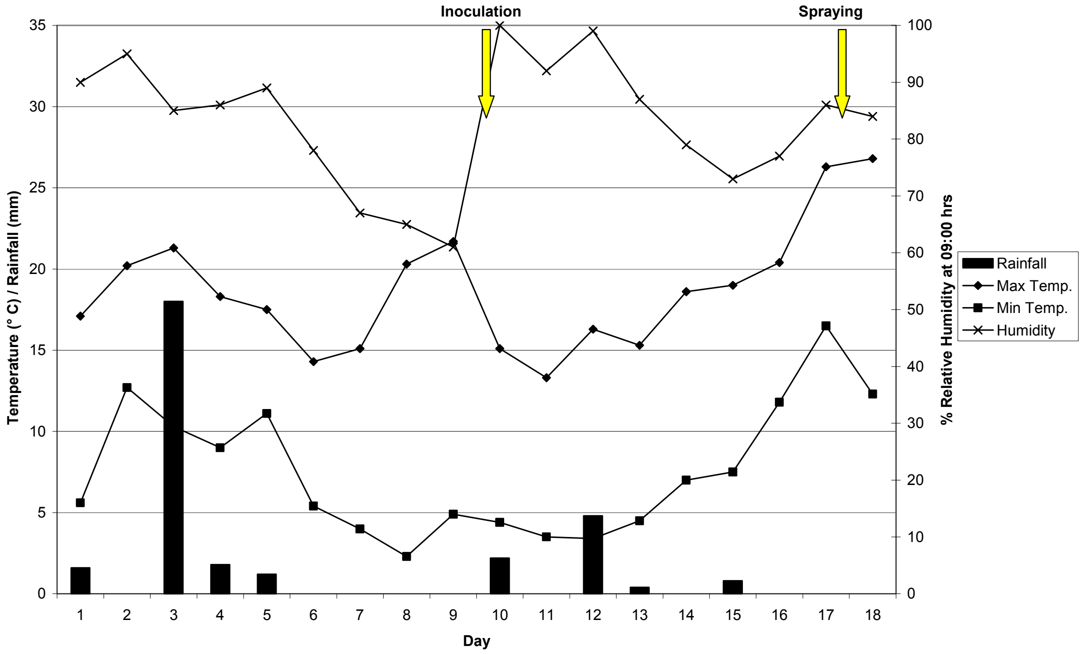


Fig. 17. Meteorological data from Morley Research Centre during the 2005 field experiment

The rainfall that occurred in between inoculation and spraying in 2003 and 2005 appears to have been crucial in producing FEB. However, the cooler temperatures, particularly the overnight minimum, appear may be linked to the increased variability of disease in 2005.

It therefore appears that although fungicide deposits on ears can be manipulated by nozzle design and orientation, and that under certain circumstances deposit levels can be linked to disease control, the influence of application system on mycotoxin production is far from clear. Factors such as the level of natural disease pressure, the *Fusarium* sp. complex and its reaction to fungicides and crop meteorology appear to strongly influence mycotoxin production even when experiments are carried out with irrigated crops inoculated by known DON producing strains. Inoculation was done with levels of spores significantly higher than that occurring naturally (10^6 spores/ml) with actual levels of at least 10^3 spores/gram of ear tissue present after inoculation. The wetness periods at anthesis are critical to determining whether establishment and infection occurs. This was conducive in 2003, but not so in 2004 when the weather after inoculation was dry. In 2005, dry weather slightly delayed the application of the inoculum and fungicides although additional misting was carried out. Establishment of ear blight was as good as in 2003. However, the infection was more variable than in 2005 within plots which was shown by the larger standard errors. This is consistent with the sometimes patchy nature of ear blight infection.

In terms of making recommendations for application systems, the work has shown it is likely that more reliable disease control will result from angling nozzles backwards, and that medium or air-included sprays may provide better control than fine sprays.

4.2 Implication for levy payers

The HGCA Nozzle Selection Chart (published in March 2002) recommends that in terms of efficacy, ear sprays are applied by fine flat-fan spray nozzles. This advice is based on the conventional approach; vertically projected sprays. Whilst the evidence from this project is not clear-cut it appears that a better alternative would be to recommend the use of angled air-induction nozzles or angled medium quality sprays. This approach would offer some advantages in terms of ear deposition and FEB control and it would also have the advantage of improved drift control. Although air-included hollow-cone nozzles are relatively new to the market, they also appear to offer advantages in terms of ears sprays.

Ear blight does not directly correlate with mycotoxin contamination. A range of variables, biological and non-biological, interact to influence the levels of ear blight and the amount of mycotoxin contamination. The disease pressure was high in 2003 and 2005. The levels of DON were low, but that of NIV were very high in harvested grain in 2003 because of natural contamination with other Fusarium species in that year. The important outcome of the project is that provided fungicides are applied at the recommended rate using the best possible spraying systems then the risk from DON contamination can be minimised. Although throughout the project the disease pressure in the treatment plots was low, even with inoculation by known DON producing strains of *Fusarium* the 1250 µg kg^{-1} limit for DON was not exceeded by any of the samples taken throughout 2003-2005. Best control was achieved at the recommended rates.

4.3 Opportunities for further research

It is clear that producing high and uniform levels of FEB in the field such that differences in control caused by application can be determined remains a challenge. Even inoculating a crop with known DON producing strains of *Fusarium* produced sufficient disease in 2003 and 2005 but not enough DON/NIV to be able to distinguish effectively between treatments. In 2004 the adverse weather conditions resulted in poor establishment in the field experiment. In the wetter but colder season in 2005 there was a lack of disease uniformity and this caused difficulties. Further work on inoculation and the maintenance of FEB by irrigation would assist future work on application techniques and also assist with the development of techniques for fungicide selection. This could include work on assessing the influence of soil moisture on FEB.

More detailed studies could be carried out in the wind tunnel with marked *Fusarium* strains (e.g. Green Fluorescent Protein, mutants) in controlled studies to optimise application type directly on ripening ears during the critical anthesis phase. This could further elucidate the complex interactions between spray deposition, fungicides and control of different combinations of *Fusarium* species (e.g. *F.culmorum*, *F.graminearum*, *F.poae*, *F.avenaceum* and mixtures of these) in different humidity regimes and lead to further optimisation of spray application techniques. The influence of *Microdochium – Fusarium* interactions on trichothecene production also requires further work.

5 CONCLUSIONS & RECOMMENDATIONS

Although fungicide deposits on ears can be manipulated by nozzle design and orientation, and that under certain circumstances deposit levels can be linked to disease control, the influence of application system on mycotoxin production is far from clear. Factors such as the level of natural disease pressure, the *Fusarium sp*. complex and its reaction to fungicides and crop meteorology appear to strongly influence mycotoxin production even when experiments are carried out with irrigated crops inoculated by known DON producing strains.

In terms of making recommendations for application systems, this work has shown that it is likely that more reliable disease control will result from angling nozzles backwards, and that medium or air-included sprays may provide better control than fine sprays.

6 ACKNOWLEDGEMENTS

The authors of the report wish to recognise the contribution of many people involved in this work and thank them for their input. Particular thanks are due to;

`

- the HGCA for funding the study and to the staff and members of the R&D Committee for their helpful comments during the course of this project;
- staff at The Arable Group, particularly Marion Self who carried the statistical analysis of the cropping data.
- staff at Applied Mycology Group, Cranfield University, particularly Ester Baxter and Carlos Pires;
- Dave Baker, John Power, and Andy Lane of the Chemical Application Group at Silsoe Research Institute for their assistance with wind tunnel and field experiments;
- Colin Mitchell, Christine O'Sullivan and Paul Rooney of the Analytical Laboratory at Silsoe Research Institute for their analysis of spray deposits.

7 REFERENCES

Bryant J E, Parkin C S, Wyatt J C. (1984). Partitioning of pesticide spray on and under a cereal canopy. *Proceedings British Crop Protection Council Conference - Pests & Diseases*, 1007 -1012

Cooney JM, Lauren DR, di Menna ME, (2001). Impact of competitive fungi on trichothecene production by *Fusarium graminearum*. *Journal of Agricultural food and chemistry 49, 522-526*

Debroize D (2002). Personal communication

Hope R, Magan N (2003). Two dimensional environmental profiles of growth, deoxynivalenol and nivalenol production by *Fusarium culmorum* on a wheat-based substrate. *Letters in Applied Microbiology,* **37**, 70-74

Ioos R, Belhadj A, Menez M, Faure A. (2005). The effects of *Fusarium* spp. and *Microdochium nivale* and their associated trichothecene mycotoxins in French naturally-infected grains. *Crop Protection* 24: 894-902

Kippax, P, Parkin CS, Tuck CR (2002) Particle size characterisation of agricultural sprays using laser diffraction, *Proceedings of ILASS –Europe 2002*, Zaragoza, Spain 9 –11 September

Magan N, Hope R, Colleate A, Baxter E S. (2002). Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment. *European Journal of Plant Pathology*, 108:685-690

Miller P C H, Lane A G, Wheeler H C. (2002). Optimising fungicide application according to crop canopy characteristics in wheat. *Project Report No. 277, HGCA*, London

Parkin C S, Miller P C H , Magan N, Aldred D, Gill J, Orson J H. (2006). The deposition of fungicides on ears to control *Fusarium* ear blight and the mycotoxin contamination of grain. *Aspects of Applied Biology*, to be published

Pilsner RE, (2000) *Research Project 494.6*, Agriculture Canada

Powell E S, Orson J H, Parkin C S, Miller P C H, Aldred, D, Magan N. (2004). Improving the deposition and coverage of fungicides on ears to control *Fusarium* ear blight and reduce mycotoxin contamination of grain. International advances in pesticide application, *Aspects of Applied Biology* 71:215-222

Ramirez ML, Chulze S, Magan N (2004). Impact of environmental factors on growth and deoxynivalenol production by *Fusarium graminearum* isolates from Argentinean wheat. *Crop Protection,* **23**, 117-125.

Waalwijk C (2002). *Fusarium* species on wheat in the Netherlands: inventory and molecular identification. *Journal of Applied Genetics,* **43A**, 125-130

Wolf T, Kutcher R, Gilbert J, Fernandez M (2002). Optimising the application of foliar sprays for *Fusarium* Head Blight control in wheat, unpublished report, Agriculture Canada

Proceedings papers and posters originating from this work

Aldred D, Magan N, Parkin C S, Miller P C H, Gill J, & Orson J G. (2005). Fungicide targeting on ripening ears for improved control of Fusarium ear blight and the mycotoxins deoxynivalenol and nivalenol. BCPC *Crop Science and Technology* 5B, 417-422.

Aldred D, Magan N, Orson J, Parkin, C S, & Miller P C H (2004). Ear spray targeting for improved ear blight and mycotoxin control. In: Canty, S.M., Boring T, Wardwell J & Ward R W (Eds). *Proceedings of the 2nd International Symposium on Fusarium Head Blight; incorporating the 8th European Fusarium Seminar, 2004*, 11-15 December, Orlando, FL, USA. Michigan State University, East Lansing. MI., 285-286.

Powell E S, Orson J H, Parkin C S, Miller P C H, Aldred, D, Magan N. (2004). Improving the deposition and coverage of fungicides on ears to control *Fusarium* ear blight and reduce mycotoxin contamination of grain. International advances in pesticide application, *Aspects of Applied Biology* 71:215-222

Parkin C S, Miller P C H , Magan N, Aldred D, Gill J, Orson J H. (2006). The deposition of fungicides on ears to control *Fusarium* ear blight and the mycotoxin contamination of grain. *Aspects of Applied Biology*, In Press