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EPIDEMIOLOGY AND CONTROL OF FUSARIUM EAR BLIGHT

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EPIDEMIOLOGY AND CONTROL OF FUSARIUM EAR BLIGHT

by

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ABSTRACT

A series of related investigations was undertaken during the three year period 1993 - 1995 to investigate various aspects of the epidemiology of *Fusarium* ear blight on wheat in the UK, the effect of the disease on yield and control of the disease using fungicides.

The effects of ear blight caused by *Fusarium culmorum* on yield and yield components was investigated for three years in small plots inoculated at GS 65 and irrigated subsequently. There was a strong linear association between ear blight severity and thousand grain weight, assessed either on whole plots or on single tillers. There were no significant differences between data obtained by the two methods, nor were differences between the three years significant. Amalgamating all data for the three years, a statistically significant yield loss relationship was determined, which indicated that $y = 47.72 - 0.22x$, where y = thousand grain weight and x = percentage of spikelets infected.

The possibility that ears may become infected systemically from stem-base lesions caused by *F. culmorum*, *F. graminearum* and *Microdochium nivale* was studied in a glasshouse under conditions designed to avoid the splash dispersal of conidia from infected compost. Each species was recovered from stem tissues above soil level in some, apparently symptomless, plants. There was an inverse relationship between recovery of the pathogen and the height above stem base from which the stem tissue was excised. *F. culmorum* was the most frequently isolated fungus and it was also recovered from the highest position in plants. Only 3% of plants were colonised above the second node and none of the fungal species were recovered from either the fifth node or the ear. This suggests that systemic colonisation of winter wheat from *Fusarium* infected stem bases is unlikely to contribute to the development of ear blight symptoms in winter wheat.

Small-scale field experiments were carried out to determine the effect of increasing relative humidity on the development of the five major species of *Fusarium* known to cause ear blight in the UK. A mist irrigation system, controlled by an in-plot relative humidity sensor, was used to manipulate relative humidity levels in plots artificially inoculated with single species of either *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* or *Microdochium nivale*. The degree of ear infection by all five inoculated species was related to relative humidity, with evidence of differing optima. Yield data indicated that losses could occur even under conditions of low relative humidity, and that *F. culmorum* and *F. graminearum* were the most aggressive pathogens, capable of reducing yield by up to 25%. The mycotoxin content of grain increased with increasing humidity, with highest levels of mycotoxin contamination being associated with infection by either *F. culmorum* or *F. graminearum*. Mean levels of mycotoxins were low, and not significant in terms of risk to human or animal health, but one sample from grain infected with *F. culmorum* did have mycotoxin levels above the guidelines on mycotoxin content used in Canada.

The relationship between *Fusarium* ear blight and meteorological factors was studied using data from the ADAS/CSL winter wheat disease surveys for the period 1988-1995 and from Monthly Weather Reports from the Meteorological Office. Seasons with high levels of *Fusarium* ear blight were those with wetter than average conditions in June and July, following a dry November and warmer than average January.

In an evaluation of fungicides for control of *Fusarium* ear blight, and also black point (*Alternaria alternata*), few showed activity against either disease. Products containing tebuconazole gave some control of ear blight, and tebuconazole plus triadimenol plus

anilazine reduced black point incidence when applied at GS 75. Irrigation in early July markedly increased the incidence of black point. In experiments on its protectant and eradicant activity, tebuconazole was applied on a series of dates before or after inoculation at early anthesis with *F. culmorum*, and irrigation was provided after inoculation. Ear blight was reduced by up to 90% by tebuconazole applied up to seven days after inoculation, but there was protectant activity only when applied two days before inoculation. At non-irrigated sites, *Fusarium* ear blight incidence was very low in each of the years 1994-1996, and there was no effect on yield of tebuconazole applied at or after GS 57.

INTRODUCTION

Fusarium species are common and important pathogens of cereals, causing seedling blight, stem-base lesions and a variety of ear symptoms, ranging from ear blight which kills part or all of an ear to smaller and often superficial lesions on glumes. The incidence of *Fusarium* ear blight in the UK is erratic. ADAS/CSL surveys of disease in winter wheat have shown that the incidence of *Fusarium* ear blight is high in some seasons, particularly 1982 and 1991, but low in many others. There is an association with rain during or shortly after anthesis, and infection of grain before harvest may also be favoured by wet weather and lodging, but the precise conditions which favour ear blight are poorly understood. Recent work at Harper Adams Agricultural College on the epidemiology of *Fusarium* diseases of cereals identified rain splash of spores as an important mechanism for *Fusarium* dispersal from infected stem bases to ears (Jenkinson & Parry, 1994). Dispersal by arthropod vectors and systemic infection have also been suggested as mechanisms of ear infection (Parry *et al.*, 1995; Hutcheon & Jordan, 1992). In addition, preliminary investigations into the development of a forecasting system for *Fusarium* ear blight have shown that the effects of temperature, moisture and growth stage of wheat are critical for disease development. Contaminated grain resulting from *Fusarium* ear blight can provide a primary source of inoculum for the development of seedling blight and foot rot. Some of the common *Fusarium* spp. on cereals are also capable of producing mycotoxins (Snijders, 1990c), which could have implications for human and animal health. The current status of *Fusarium* ear blight was reviewed by Parry *et al.* (1995).

PROJECT OBJECTIVES

There were five main objectives within the project, each of which is covered in a separate section within this report:

(i) To determine the effect of *Fusarium* ear blight on yield of wheat

(ii) To investigate the role of systemic infection of ears in the epidemiology of ear blight.

(iii) To investigate the environmental conditions favouring *Fusarium* infection of ears of wheat and mycotoxin production under field conditions.

(iv) To establish relationships between ear blight and factors which may influence its occurrence and severity by analysis of historical disease, meteorological and geographical data.

(v) To evaluate fungicides for activity against *Fusarium* ear blight and black point, and to determine the optimum timings for fungicide application to reduce the severity of *Fusarium* ear blight

SECTION 1: EFFECT OF *FUSARIUM* EAR BLIGHT ON THE YIELD OF WINTER WHEAT

P Jenkinson & D W Parry, Harper Adams Agricultural College

Objective

To determine the effect of ear blight caused by *F. culmorum* on yield and yield components of winter wheat.

Materials and methods

In each of three successive growing seasons (1994-96 harvest years), six plots (45 m x 2 m) of the winter wheat cultivar Avalon were sown during the first two weeks of October. All plots received standard crop husbandry throughout the growing season. Each plot was sub-divided into 16 'mini-plots' (2m x 2m). At GS 65 (Tottman 1987), ears within 24 mini-plots (4 in each main plot) were artificially inoculated with a conidial spore suspension ($5x10^5$ spores ml^{-1} water) of *F. culmorum* at a rate of 50 ml m^{-2}. Immediately after inoculation, all plots were mist irrigated for 10 seconds approximately every 15 minutes for 21 days (between the hours of 09.00 and 17.00). The exact frequency of irrigation was determined by a leaf wetness sensor placed at ear height within the crop.

At 14, 21 and 28 days post-inoculation, the incidence (% of ears infected) and the severity (% of spikelets infected) of ear blight was assessed for 25 ears selected at random in each of the 96 mini-plots.

In order to compare the validity of the mini-plot design with the single tiller approach used by Scott & Hollins (1974) and Richardson *et al.* (1975), 1200 ears (randomly distributed throughout the experiment) were tagged 10 days post-inoculation. Tagged tillers were selected so as to provide ears with a wide range of disease severities. All tagged ears were inspected 14, 21 and 28 days post-inoculation and the percentage of spikelets infected recorded.

When mature, all tagged ears were harvested and each placed into a sulphide bag. On the same day, all ears found within a quadrat area of 1.0 m^2 were harvested from each mini-plot and counted. All samples were threshed using a Hege single ear thresher and the grain collected dried to 15% moisture content. For each of the 1200 single ears, the number and weight of grains yielded was recorded, from which a thousand grain weight was calculated. For each mini-plot, total grain was recorded along with four replicate thousand grain weight counts. Given the total weight of grain yielded, the mean thousand grain weight and the number of ears harvested, the average number of grains per ear was estimated for each mini-plot.

Each year, in order to determine if symptoms of ear blight observed were a direct result of the artificial inoculation procedure, 500 spikelets were plated on to potato dextrose agar supplemented with streptomycin sulphate (100 mg l^{-1}) and chloramphenicol (50 mg l^{-1}). After incubating at 15°C for 14 days all emerging *Fusarium* colonies were identified.

Linear regression analysis was carried out on all single ear and mini-plot data in order to determine if ear blight had a significant effect on the number of grains yielded per ear or

thousand grain weight. Similar analysis was also carried out on the incidence and severity of ear blight in mini-plots in order to establish if a relationship existed between the two. In addition, for each assessment date, linear relationships between ear blight severity and yield components derived from the two approaches (i.e. mini-plot and single tiller) were compared using analysis of parallelism. Analysis of parallelism was also used to determine if linear relationships, derived for ear blight severity and yield components, differed between 1994, 1995 and 1996.

Results

Isolation of *Fusarium* spp. from the 500 spikelets revealed that all symptoms observed were due to infection by *F. culmorum*.

For all three years (1994-96), the relationship between the incidence of blighted ears and the severity of ear blight 14, 21 and 28 days post-inoculation can be seen in Figures 1.1, 1.2 and 1.3 respectively. For each assessment, increasing the incidence of blighted ears resulted in a concurrent increase in the severity of ear blight. Linear regression analysis revealed that all linear relationships were significant ($P < 0.001$), with up to 97% of variance accounted for in some cases. Analysis of parallelism also revealed that, for each assessment date, linear relationships did not significantly differ between the three years.

The effect of ear blight severity 14 , 21 and 28 days post-inoculation on thousand grain weight of plants harvested from the mini-plots during the three years (1994-96) can be seen in Figures 1.4, 1.5 and 1.6 respectively. Linear regression analysis revealed that increasing the severity of ear blight significantly reduced individual grain weight in all three years at $P < 0.001$. Analysis of parallelism also revealed that, for the disease assessment 28 days post-inoculation, linear relationships derived from mini-plots did not significantly differ between the three seasons.

The effect of ear blight severity 14, 21 and 28 days post-inoculation on thousand grain weight of single tillers harvested during 1994-96 can be seen in Figures 1.7, 1.8 and 1.9 respectively. As observed with the mini-plots, increasing the severity of ear blight significantly reduced individual grain weight in all three years ($P < 0.001$). Analysis of parallelism also revealed that for the assessment 28 days post-inoculation, linear relationships derived single tillers did not differ significantly between the three years.

Analysis of parallelism revealed no significant differences in linear relationships between ear blight 28 days post-inoculation and thousand grain weight derived from both mini-plot and single tiller approaches during each of the three years. As a result, all data for this disease assessment date were collated and a common linear relationship determined (Figure 1.10).

Although linear regression analysis revealed significant relationships between ear blight and grain number per ear, the strength of these relationships were poor with less than 30% of variance accounted for and, as such, were not considered reliable.

Discussion

Results from the study suggest that incidence and severity of *Fusarium* ear blight are closely related. Although linear relationships between incidence and severity of ear blight at each of the assessment dates were not significantly different between years, the strength of the

relationship was lower for data obtained in 1994. In contrast with 1995 and 1996, where over 80% of variance was accounted for, less than 76% of variance was acounted for during 1994. This difference may be explained by the absence of naturally infected ears in 1995 and 1996. During 1994, the isolation of pathogens from spikelets revealed that not all ears exhibiting symptoms of blight were infected with *F. culmorum* and that some ears were infected with *Microdochium nivale* and *F. avenaceum*. During 1995 and 1996, isolation of pathogens revealed that all diseased spikelets were infected with *F. culmorum*. This would suggest that relationships between the incidence and severity of ear blight may be determined by the species responsible for disease.

In all three years, *Fusarium* ear blight was shown to reduce significantly individual grain weight of winter wheat. Using either the single tiller (Scott & Hollins, 1974, Richardson *et al.*, 1975) or the mini-plot approach, produced similar linear relationships between ear blight and individual grain weight. The fact that analysis of parallelism failed to identify any significant difference between relationships at 28 days post-inoculation derived from either the single tiller or mini-plot approach, suggests that either method is equally valid for determining yield loss relationships. As such, all data could be collated and a common yield loss relationship of $y = 47.72 - 0.22x$ (where y is thousand grain weight and x is the % of spikelets infected) determined (Figure 1.10).

After inoculating 32 wheat genotypes in 1987 and 54 genotypes in 1989 with *F. culmorum*, Snijders (1990b) demonstrated a significant reduction in grain yield due to ear blight. For the 1987 data, a significant regression relationship of $y = 6x$ (where y is the yield of grain and x is the severity of ear blight) was obtained with 49% of variance accounted for). For the 1989 data, a relationship of $y = 7.2x$ was determined with 62% of variance accounted for. Regression relationships between ear blight and thousand grain weight for mini-plots in this study compare well with those of Snijders (1990b). Field studies carried out by Parry (1991) between 1983 and 1990, in which plots of the winter wheat, cv. Avalon, were artificially inoculated with *F. culmorum* and *F. avenaceum*, revealed that ear blight (expressed as % area of ear affected) significantly reduced individual grain weight of single ears. Linear regression relationships of $y = 31.93 - 0.37x$, $y = 30.70 - 0.65x$ and $y = 55.25 - 0.43x$ (where y is thousand grain weight an x is the % of ears infected) were determined for the years 1983, 1987 and 1990, respectively. Regression relationships obtained from single tillers in this study compare well with those determined by Parry (1991) with slopes ranging between 0.20 and 0.61 depending on assessment date and season.

Although increasing the severity of ear blight was shown to reduce significantly the number of grains yielded per ear in 1994, no such effect was observed in 1995. No assessment of grains per ear was made during 1996 on the evidence of data obtained in the previous two years. It is suggested that ear blight has a very weak influence on the number of grains per ear.

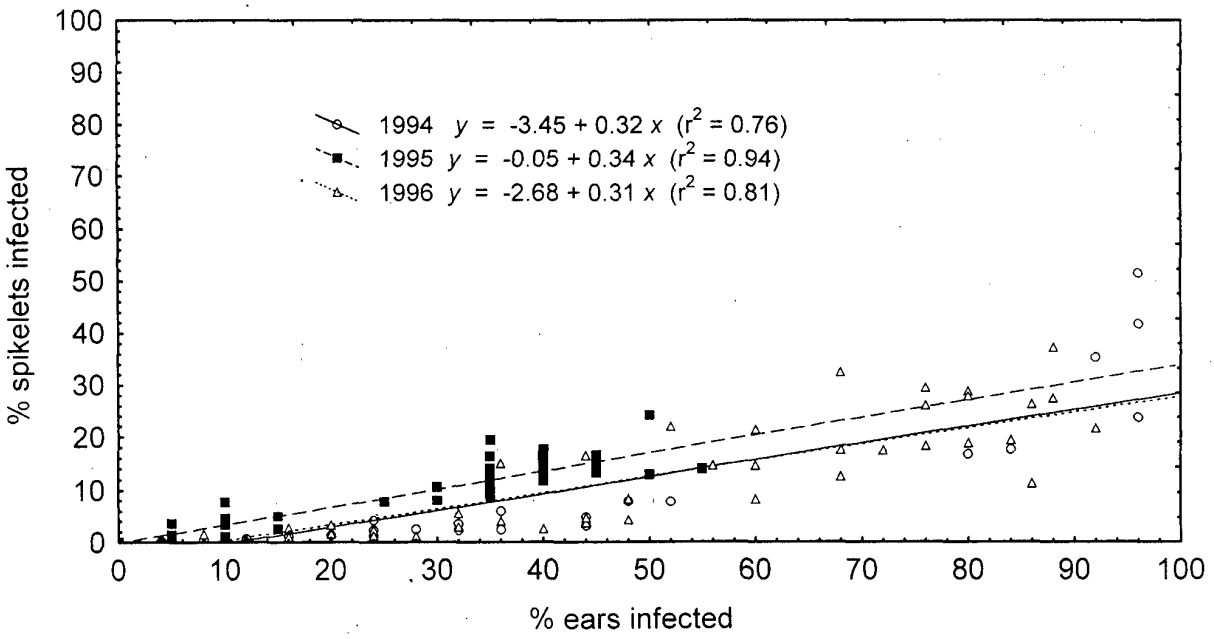


Figure 1.1. Relationship between incidence and severity of *Fusarium* ear blight 14 days post-inoculation, 1994-1996.

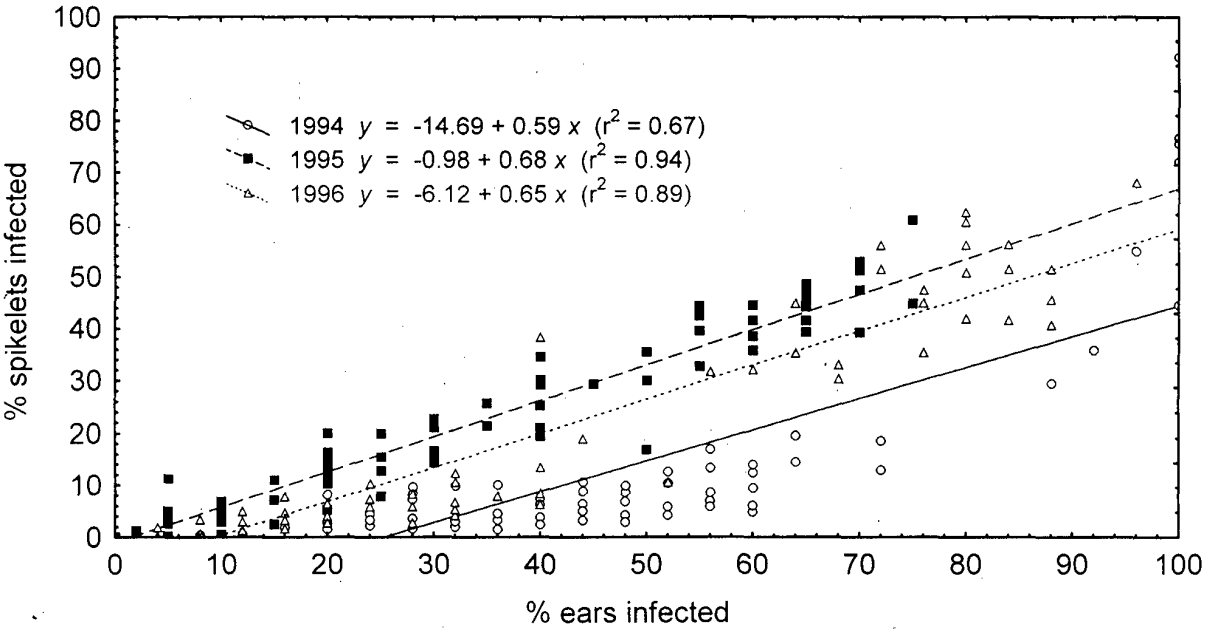


Figure 1.2. Relationship between incidence and severity of *Fusarium* ear blight 21 days post-inoculation, 1994-1996.

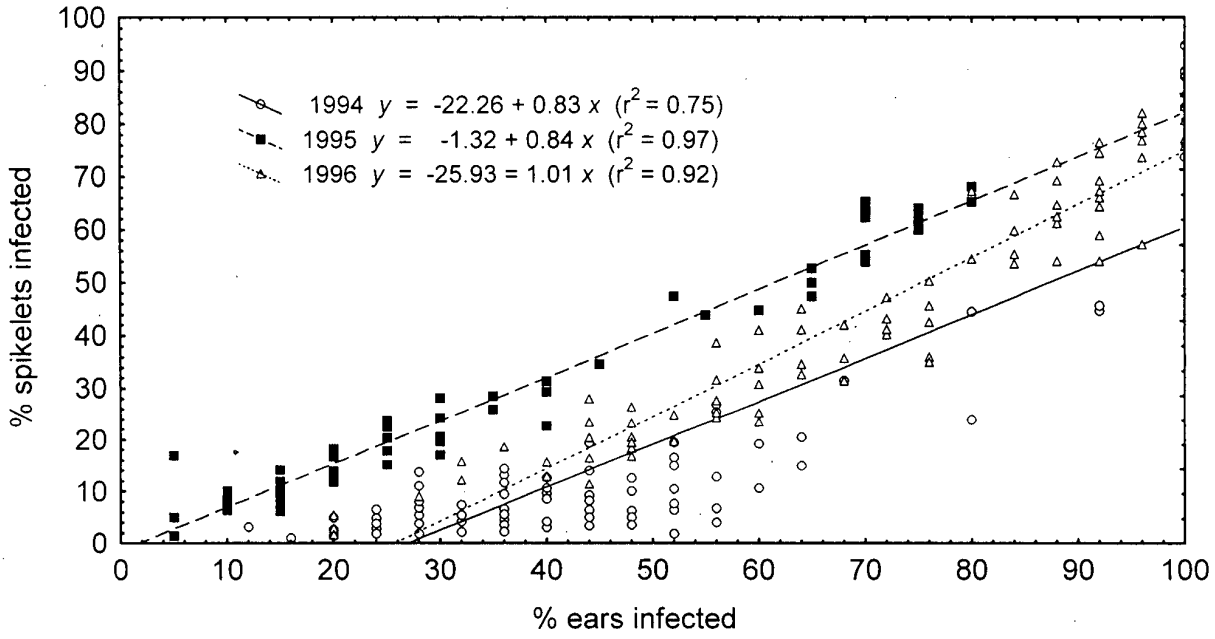


Figure 1.3. Relationship between incidence and severity of *Fusarium* ear blight 28 days post-inoculation, 1994-1996.

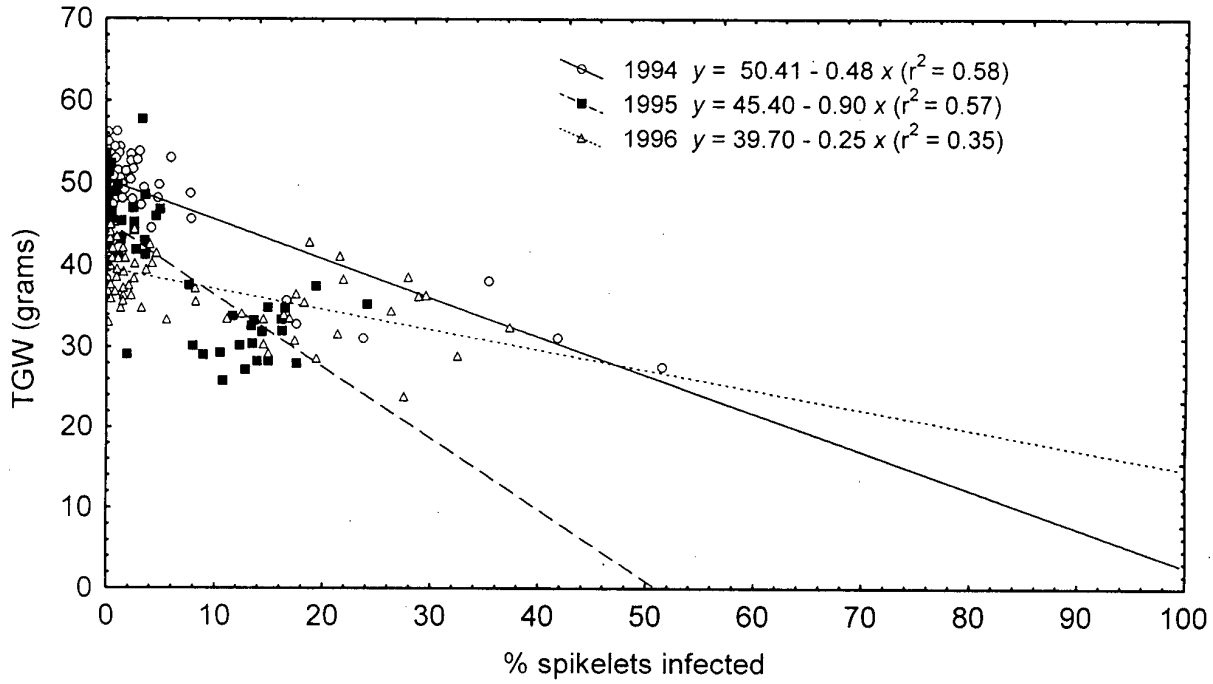


Figure 1.4. Effect of *Fusarium* ear blight 14 days post-inoculation on thousand grain weight of wheat., 1994-1996

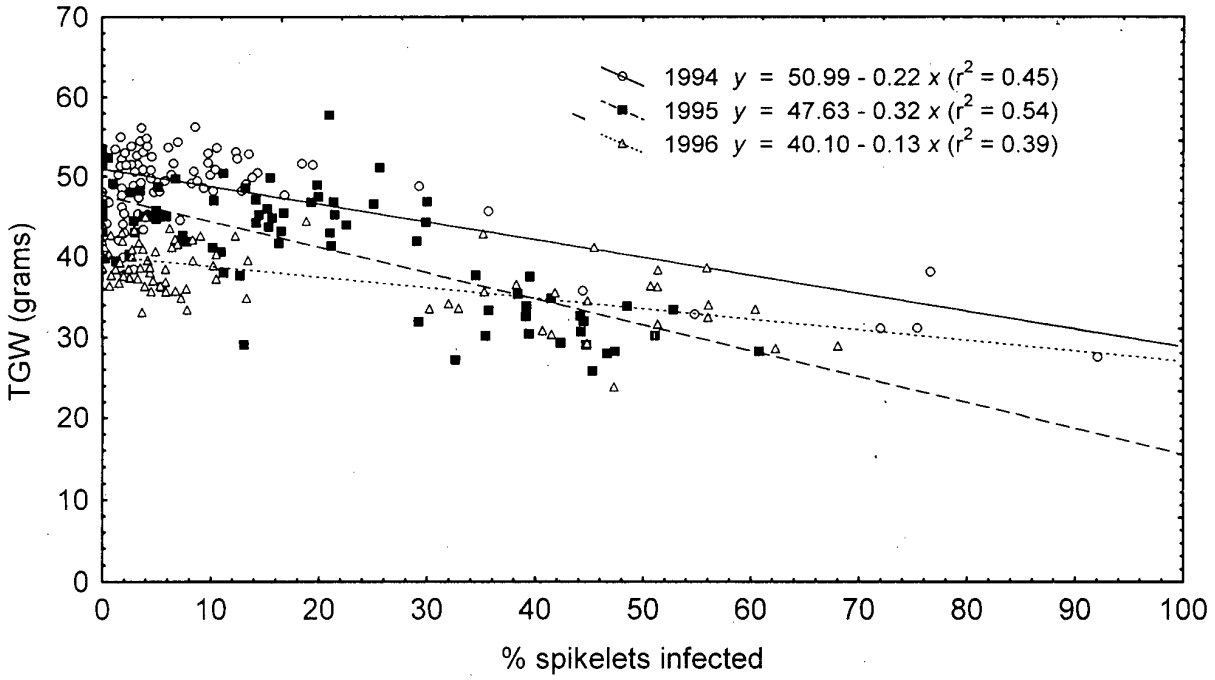


Figure 1.5. Effect of *Fusarium* ear blight 21 days post-inoculation on thousand grain weight of wheat., 1994-1996

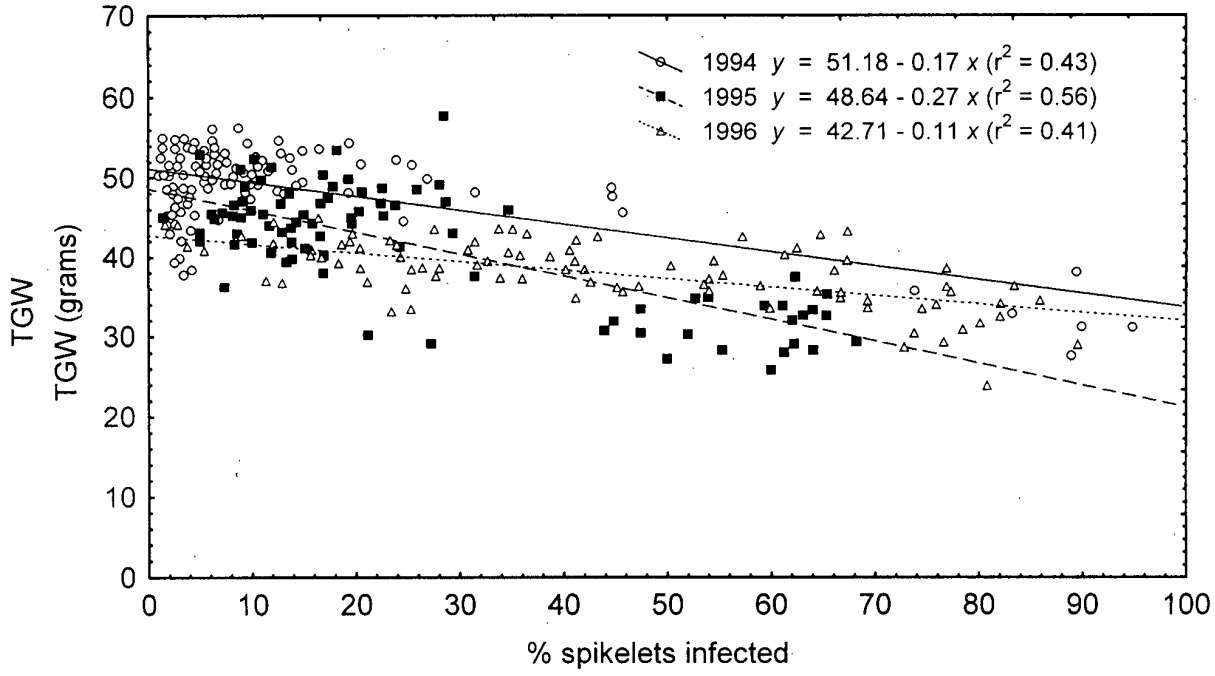


Figure 1.6. Effect of *Fusarium* ear blight 28 days post-inoculation on thousand grain weight of wheat., 1994-1996

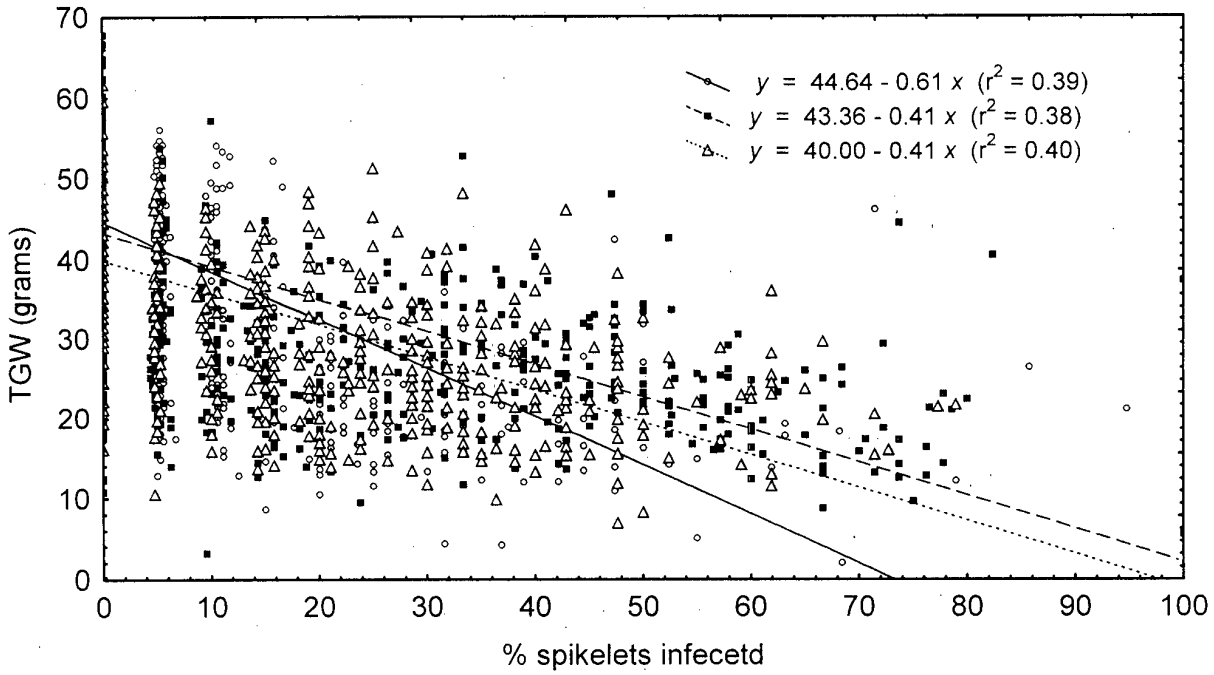


Figure 1.7. Effect of *Fusarium* ear blight 14 days post-inoculation on thousand grain weight of single tillers of wheat, 1994-1996.

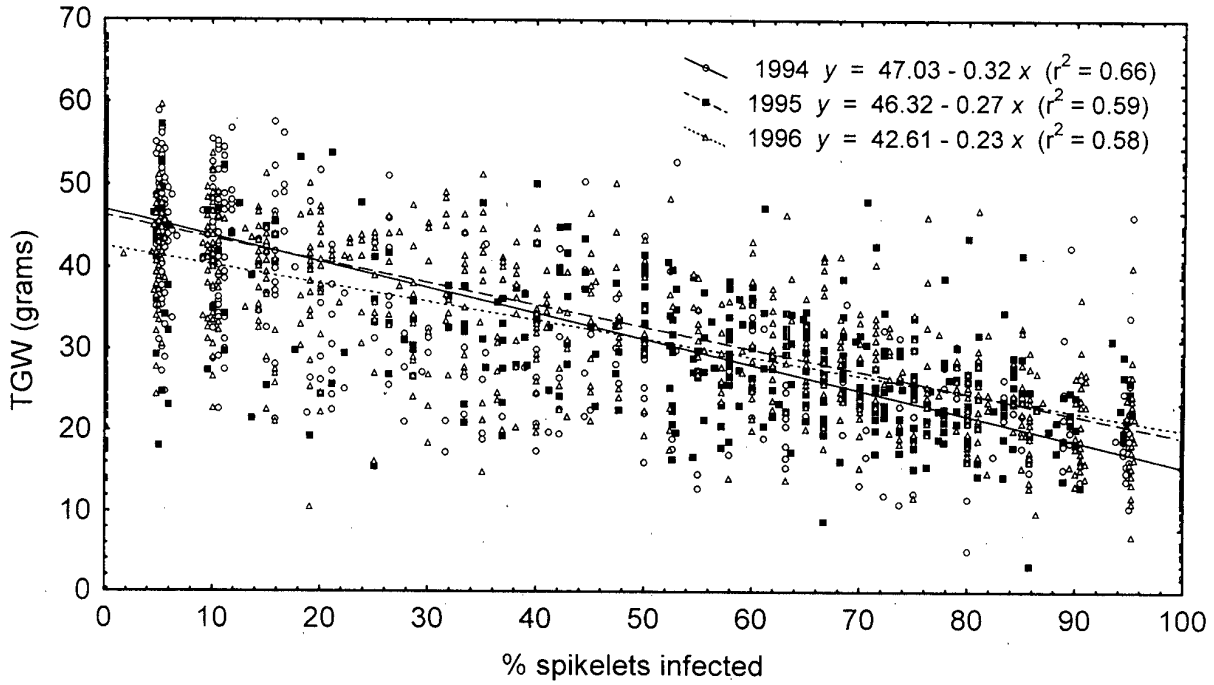


Figure 1.8. Effect of *Fusarium* ear blight 21 days post-inoculation on thousand grain weight of single tillers of wheat, 1994-1996.

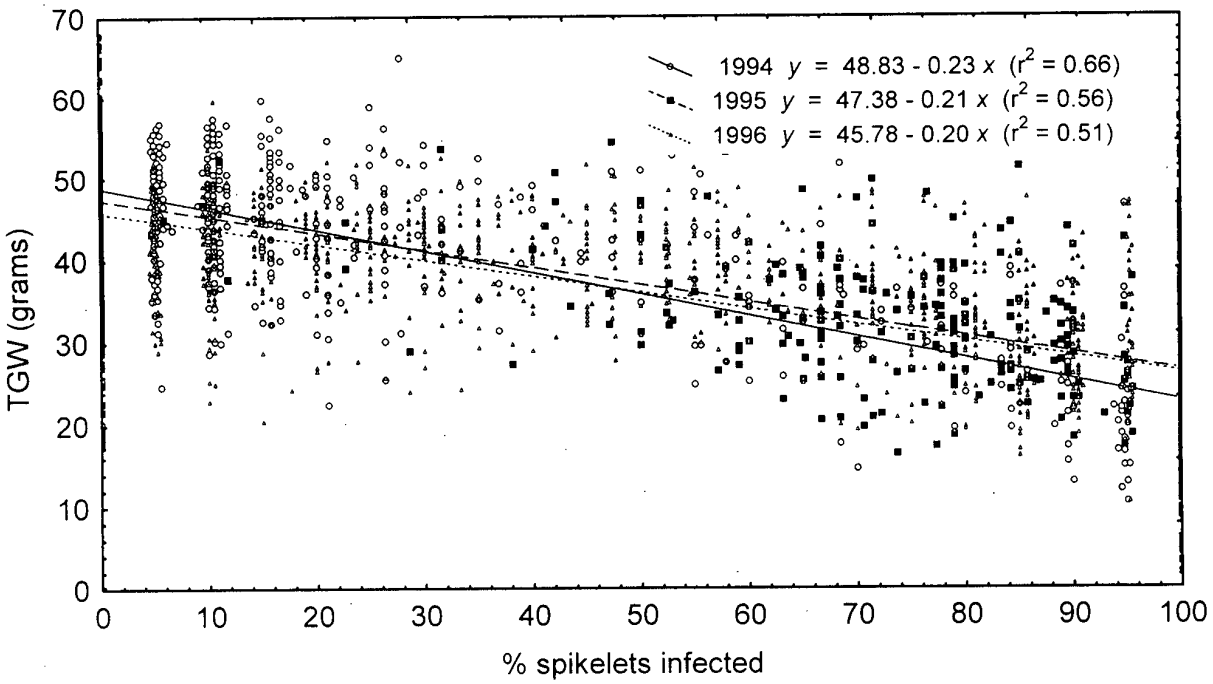


Figure 1.9. Effect of *Fusarium* ear blight 28 days post-inoculation on thousand grain weight of single tillers of wheat, 1994-1996.

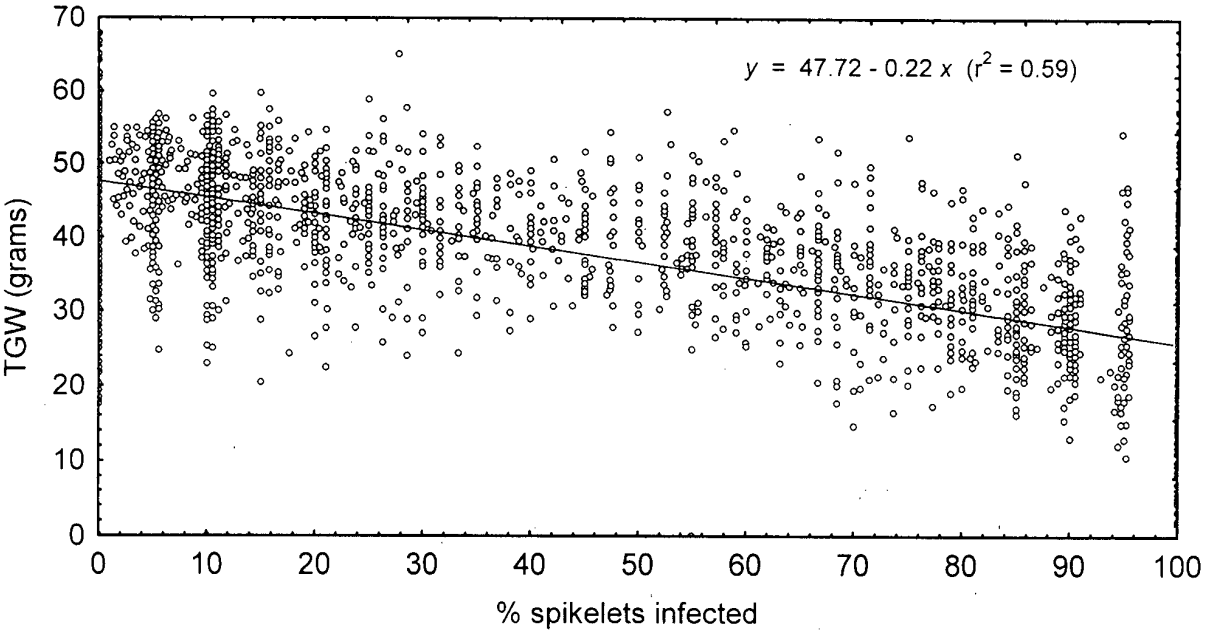


Figure 1.10. Effect of *Fusarium* ear blight 28 days post-inoculation on thousand grain weight of winter wheat using combined data from mini-plots and single tiller assessments, 1994-1996.

SECTION 2: THE ROLE OF SYSTEMIC INFECTION IN EAR BLIGHT EPIDEMIOLOGY

J A Clement & D W Parry, Harper Adams Agricultural College

Objective

To determine the degree to which systemic growth of *Fusarium* species and *Microdochium nivale* contributes to the development of ear blight of winter wheat.

Materials and Methods

Untreated seed of winter wheat cv. Cadenza was surface sterilised in 4% sodium hypochlorite (0.5% available chlorine) for 3 minutes, rinsed in three changes of sterile distilled water, and dried at ambient temperature on sterile filter paper. Seed was sown in trays of autoclaved John Innes No. 2 compost. The compost had previously been autoclaved in small volumes (approx.12.5 l) for 3 × 55 minutes at 126°C and the trays had been sterilised by immersion in 4% sodium hypochlorite for 30 minutes. Planted trays were incubated in plant growth cabinets (Fitotron or Conviron) operating at 70% r.h. with 16h day at 15°C. The temperature was reduced to 10°C during each dark period. These conditions were maintained throughout the experiment.

Seedlings were transplanted at GS 12 into 72 × 15cm pots containing autoclaved J.I No. 2 compost, 5 plants per pot. Spore suspensions of *Fusarium graminearum*, *F. culmorum* and *Microdochium nivale* were prepared from single spore cultures grown on potato dextrose agar. The fungal spores were applied as a soil drench (2×10^4 spores g^{-1} compost) when the plants were at GS 13. One third of the pots (24) were inoculated with *F. graminearum,* a further third were treated with *F. culmorum* and the final third were exposed to *M.nivale*. From this stage each inoculated pot stood on an individual saucer and was watered only from the base. The treatments were incubated as three randomised blocks. At GS 33 half the pots in each treatment were sprayed with prochloraz (405 g in 220 l) using a track-mounted precision pot sprayer.

At GS33 (prior to fungicide treatment), GS 59, GS 77-87 and at harvest, one plant was uprooted from each pot and a 3 cm segment of tissue was removed from the stem base, from each node and from the ear (post emergence). The height, relative to the stem base, from which each tissue segment was removed was measured. Segments were surface sterilised (4% sodium hypochlorite for 3 minutes), rinsed in sterile distilled water and blotted on sterile filter paper. The segments were bisected longitudinally. One half was fixed in 3% glutaraldehyde (0.05M phosphate buffer, pH 7) for scanning electron microscopy and the other was plated on potato dextrose agar amended with antibiotics (streptomycin sulphate 10 $\mu g\ ml^{-1}$, neomycin 5 g μml^{-1} and chloramphenicol 5 $\mu g\ ml^{-1}$). The incubation temperature for the agar plates was varied to reflect the optimum for growth of the species which had been originally used to inoculate the compost (*F. graminearum*, 25°C; *F. culmorum*, 20°C; *M. nivale*, 15°C). After 3-7 days, recovery of each species was assessed by colony morphology, pigmentation and spore morphology.

Glutaraldehyde fixed samples were dehydrated through an ethanol series to dry acetone and then critical point dried. Dried specimens were mounted, sputter coated with gold and examined in a Cambridge S200 Stereoscan electron microscope operating at 10 kV. To assess the relationship between symptoms visible on outside of the culm and internal fungal

colonisation, some culms collected at GS 95 were bisected longitudinally and examined under epi-illumination using a Zeiss Jenalab light microscope.

Results

At GS 33, depending on the species used for inoculation, recovery from stem base segments varied from 88 to 100% (Figure 2.1). At this growth stage 14% of plants were colonised above the stem base but there was no isolation from above the second node. With all three fungal species there was a inverse relationship between recovery and the height above stem base from which the tissue was excised. *F. culmorum* was the most frequently isolated fungus (Figure 2.1) and it was also recovered from the highest position in plants (Figure 2.2).

In plants sampled at GS 59 stem base recovery was almost ubiquitous but less than 6% showed colonisation above this height (Figure 2.1). In this latter group 75% of the plants had been treated with fungicide at GS 33. Of those plants in which there was no vertical colonisation above the stem base, 46% had been treated with fungicide. *F. culmorum* was again recovered most frequently and in one plant it was isolated from the tissue segment which contained the fourth node (Figures 2.1 & 2.2). No species were recovered from tissue segments taken from either the highest node on each plant or the ear.

The occurrence of stem base infestation remained high at GS 77-87 (Figure 2.1). The three treatment species were recovered above the stem base in 44% of plants and, of these, 39% had previously been treated with fungicide. Where no fungal colonisation occurred above the stem base 66% of the plants had received a fungicide spray. More than 60% of plants from pots inoculated with *F. culmorum* were infected at the first node and this species was again isolated from one plant at the fourth node (Figures 2.1 & 2.2). Again there was no recovery from the highest stem segment sampled or from the ear.

In the final sampling at harvest maturity 53% of plants showed clear symptoms of *Fusarium* foot rot and 97% of stem bases were infested (Figure 2.1). In the group showing clear symptoms 47% had received fungicide treatment. Vertical colonisation above the stem base occurred in 61% of plants. Of the remainder, in which there was no recovery of fungi above the stem base, 44% had been sprayed with fungicide. Seventeen per cent and 13% of plants respectively, from pots inoculated with *F. culmorum* or *F. graminearum,* were infected up to the third node and *F. culmorum* was recovered from one plant at the fourth node (Figures 2.1 & 2.2). Out of a total of 286 plants examined, only nine were infected above the second node. Six of these had received fungicide treatment.

Scanning electron microscopy of samples taken at early growth stages (GS 33 & GS 59) confirmed that colonisation of the lower culm was usually limited to a sparse surface mycelium. In stem base specimens all three fungal species colonised the spaces between successive leaf sheaths. Infestation was typically greatest between the outer sheaths at approximately soil level. Fungal growth occurred within tissues of the outermost leaf sheath and hyphae often penetrated and/or emerged along the line of the anticlinal walls between epidermal cells or entered directly through the epidermal cell wall. Above the stem base mycelial development between leaf sheaths was localised and very sparse. Some hyphae appeared flattened and they were often closely adpressed in the anticlinal groove between epidermal cells. In one of the plants from which *F. culmorum* was recovered from the second node at GS 33, hyphae were present within the pith cavity below the node and intercellular hyphae were present within the second node.

At the later growth stages (GS 77-87 & GS 95) each of the fungal species colonised the pith cavity below the first node. There were no obvious differences between species in the morphological features of this colonisation. Typically hyphae were present on the walls of parenchyma cells lining the cavity (Figures 2.3 & 2.4) and from this mycelium aerial hyphae grew into the cavity space. Often a denser mycelium formed below each node against the lower face of the diaphragm (Figure 2.3). There were also intercellular and intracellular hyphae growing within the parenchyma tissue of both the culm and the nodes (Figures 2.4-2.6). At GS 95 intercellular hyphae were sometimes observed growing within vascular bundles of the culm, typically associated with xylem vessels (Figures 2.7-2.9). At these later growth stages an increasing proportion of plants showed discolouration towards the base of the culm. Colonisation of the pith cavity appeared to be associated with plants showing these macroscopic symptoms. At GS 95 the mean distance to which mycelium within the culm or pith cavity extended above discolouration was similar for each fungal species (Table 2.1).

Table 2.1. Extent of fungal growth above discolouration of the culm.

Fungal species	Mean distance (mm)	Standard error	Number of culms
Fusarium culmorum	33.7	5.8	28
Fusarium graminearum	25.2	5.1	31
Microdochium nivale	27.9	5.5	19

Discussion

All three pathogens used in this study have been implicated in ear blight disease of winter wheat (Jenkins *et al.*, 1988). The mode of transmission of the infection from soil level to the ear has not yet been conclusively demonstrated (Parry *et al.*, 1995). Early work (Bennett, 1928) indicated that diseased ears and grain were due solely to conidia distributed from external sources and a mechanism involving splash dispersed inocula of *F. culmorum* and *F. avenaceum* has been demonstrated *in vitro* (Jenkinson & Parry, 1994). Systemic growth of *F. culmorum* has been suggested as a method by which sporodochia may be formed some distance above the stem base but no evidence was found of systemic growth leading to infected ears (Snijders, 1990a). In contrast, in a study by Hutcheon & Jordan (1992), there was a claim that ears may become infected through internal systemic colonisation by any one of three *Fusarium* species and *M. nivale*. If transmission of ear blight disease is non-systemic then improved chemical control may be achieved by better disease forecasting and accurate timing of late-season sprays (Hutcheon & Jordan, 1992; Parry *et al.*, 1995). However, if systemic growth provides a significant alternative route for ear colonisation then effective seed treatment may be expected to minimise yield losses. Consequently, when devising chemical control strategies, it is important to determine the degree to which systemic fungal colonisation contributes to ear blight in winter wheat.

Results from the present study suggest that each of the species is capable of upward growth from the stem base of some, apparently symptomless, plants. In most cases, during the

vegetative phase of plant development, this growth does not appear to be systemic but it is rather a colonisation of protected exterior plant surfaces. In addition the lack of symptoms suggests that this limited fungal development may not be strictly, pathogenic. In young, fast growing, healthy plants the hydration and permeability of epidermal cells in these locations would be relatively high. This may create a niche in which sufficient water and other compounds are available to support a restricted latent "endophytic" infestation. It seems probable that such infestations may develop more rapidly or become pathogenic in those plants which are subject to stress. Certainly it is likely that colonisation of the pith cavity, which was rare in young plants in our experiments, might be more common among plants grown in sub-optimal field conditions.

The lack of any fungicide control was unexpected since procloraz has activity against all three fungi (Parry *et al.*, 1994). Successful application does however rely on foliar wetting and run-off which transfers the active compound to the stem-base. Because plants received no foliar watering it is probable that only a small proportion of the applied fungicide would have penetrated to the lower infested portion of the culm.

As plants mature, especially post anthesis, translocation of assimilates and increased physiological stress during grain development could favour the saprophytic colonisation of senescing tissues. The increasing proportion of plants colonised above stem base or showing stem-base browning after GS 77-87 supports this view. True systemic growth within the vascular tissues of the culm was only evident in senescent plants at harvest. In these plants fungal growth within either the culm tissues or the pith cavity extended only a short distance above discolouration of the stem. The lack of any colonisation of the ear or upper node, even in senescent plants, strongly suggests that systemic growth is unlikely to contribute to development of *Fusarium* ear blight of winter wheat.

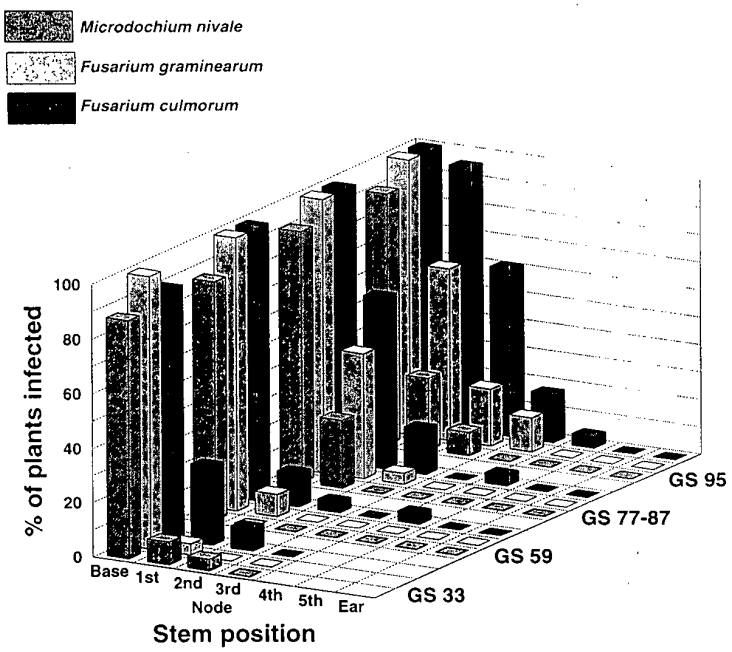


Figure 2.1. Isolation of *Fusarium culmorum*, *F. graminearum*, and *Microdochium nivale* from wheat cv. Cadenza at various growth stages.

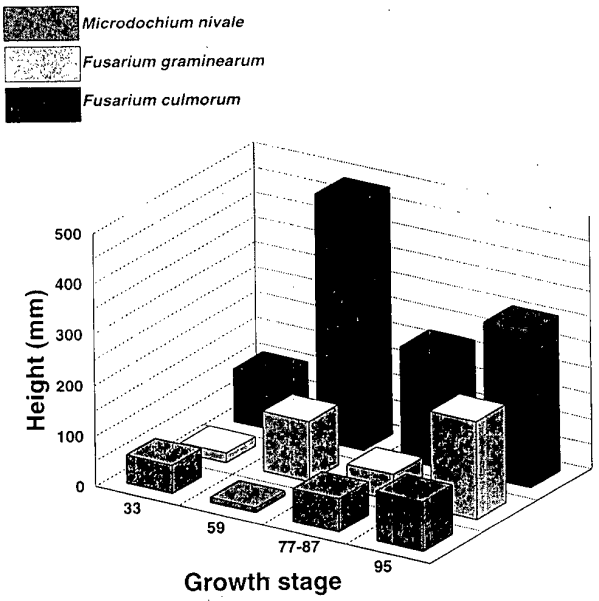


Figure 2.2. Maximum heights at which *Fusarium culmorum*, *F. graminearum*, and *Microdochium nivale* were isolated from culms of wheat cv. Cadenza at various growth stages

Legends to plate

Figures 2.3-2.9: Colonisation of tissues of wheat culm by *Fusarium culmorum*, GS 95.

Figure 2.3: Portion of bisected culm at base of second node. Hyphae within the pith cavity. A dense mycelium (arrows) has formed against the base of the diaphragm of the second node. Bar, 500 μm.

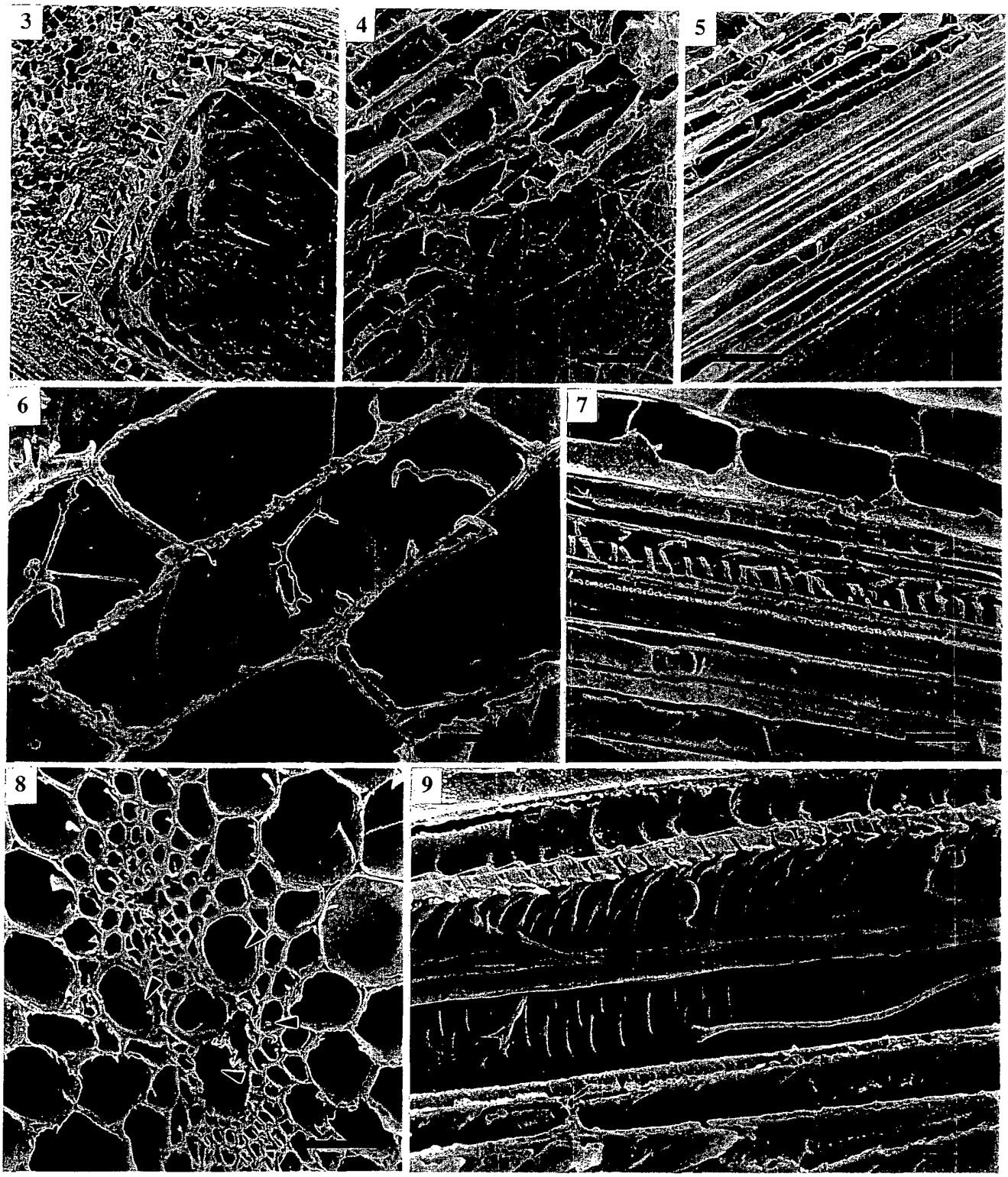
Figures 2.4 & 2.5: Area of bisected culm between first and second node. Bars, 100 μm.

Figure 2.4: Hyphae growing on the wall of pith cavity and within cells of the cortex.
Figure 2.5: Hyphae within the cells of inner cortex but no colonisation of the outer cortex or epidermis. Note stoma in epidermis of culm.

Figure 2.6: Intracellular hyphae in cortex parenchyma. Between first and second node. Bar, 25 μm.

Figures 2.7-2.9: Systemic colonisation of senescent vascular tissue. Hyphae in xylem vessels.

Figure 2.7: Longitudinally bisected vessel in area between first and second node. Bar, 50 μm.
Figure 2.8: Transverse section of vascular bundle above third node with hyphae (arrows). Bar, 50 μm.
Figure 2.9: Hyphae in xylem. Note variation in diameter of hyphae. Bar, 10 μm.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SECTION 3: EFFECT OF HUMIDITY ON DISEASE DEVELOPMENT AND YIELD LOSS CAUSED BY *FUSARIUM* SPECIES

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Objectives

To investigate the environmental conditions which favour *Fusarium* infection of the ears of wheat and subsequent mycotoxin production, and to determine the effects of *Fusarium* infection on grain quality and yield. A further objective was to devise a method whereby field conditions could be manipulated in order to produce ear blight epidemics of the five major ear blight pathogens in order to investigate factors influencing disease development.

Methods

The experiments, carried out on wheat cultivars listed in Table 3.1, were of a split plot design, with three humidity treatments as unreplicated main plots. Computer controlled mist irrigation linked to an in-plot humidity sensor was used to control humidity in two main plots, one maintained at a minimum level of 70% relative humidity and the other at 80% (termed medium and high humidity respectively). Humidity on a third plot was unaltered and thus acted as a control (ambient humidity) plot. Plot misting was initiated immediately after inoculation and continued until medium milk (GS 75) in year one of the experiment and caryopsis watery ripe (GS 71) in the two subsequent years.

Table 3.1. Wheat cultivars used in the experiment

Season	Variety	Dressing	Sowing date
1994	Promessa	Carboxin + thiabendazole	19 April 1994
1995	Avalon	Fludioxonil	4 October 1994
1996	Rialto	Fludioxonil	25 September 1995

Each main plot was divided into four replicate plots, which were further divided into six sub-plots (1.0 m x 1.5 m). In the first year, the six treatments consisted of inoculation with *Fusarium avenaceum*, *F. culmorum*, *F. poae* or *Microdochium nivale*, the two remaining plots were used as uninoculated controls to indicate extent of natural infection of ears. In subsequent years one of the control treatments was replaced by inoculation with *F. graminearum*.

In each year of the experiment meteorological data (humidity, temperature and rainfall levels) were recorded from sowing until harvest using standard recording techniques.

Plot inoculation

Conidia of *F. avenaceum*, *F. culmorum*, *F. poae* and *F. graminearum* were cultured on sucrose nutrient agar (SNA) (Nirenberg 1976). Inoculated Petri plates were incubated at 25°C under near-UV light for 10 days to encourage sporulation. *M. nivale* was cultured on potato dextrose agar (PDA), incubated at 15°C under near-UV light for 10 days.

Conidia were removed from plates by washing with 10 ml of sterile distilled water (SDW).

The washings were filtered through sterile lens tissue and diluted to produce suspensions containing $1x10^5$ conidia ml^{-1}.

Plots were inoculated at early anthesis (GS 60) with 200 ml of the diluted conidial suspension. Control plots were treated with an equivalent quantity of SDW.

Pre-harvest assessments

Stem-base assessments were carried out at flag leaf ligule just visible (GS 39), GS 75 and 85 in the first year of the experiment and at first node detectable (GS 31), GS 39, 75 and 85 in subsequent years. A sample of 25 tillers was taken at random from the guard area (the uninoculated area separating the four replicate plots) of each of the three main plots. Tillers were washed under running water for one hour before surface sterilisation in 10% sodium hypochlorite (NaOCl) for 10 minutes. Stems were examined and any brown lesions removed, placed on PDA and incubated at 25°C for identification of the causal agent.

Visual assessments of ear blight symptoms were carried out on 20 plants per treatment at growth stages 75 and 85. The percentage of each ear showing symptoms was recorded.

Isolation of *Fusarium* species present on the ear was carried out at mid-anthesis (GS 65), GS 75 and 85 to identify the species responsible for the symptoms assessed. From each treatment and control plot, at each assessment, three ears were sampled, surface sterilised in 10% NaOCl for 10 minutes and blot dried. From each ear, four spikelets showing signs of infection were plated onto PDA amended with 0.01% streptomycin; where signs of infection were absent four spikelets were removed at random. Plates were incubated at 25°C under near-UV light for 96 hours. Colonies resembling those of *Fusarium* were transferred to SNA and PDA plates for further identification.

Post-harvest assessments

Grain from fifty individual ears was dried to constant weight (at 80°C) and the individual ear grain weight recorded.

From each treatment and control plot a 100-grain sample was assessed for *Fusarium* infection. Grain were surface sterilised in 10% NaOCl for seven minutes and five grains plated onto each of twenty Petri dishes containing PDA amended with 0.01% streptomycin. Plates were incubated at 25°C under near UV light for five days. Colonies resembling those of *Fusarium* species were transferred to SNA and PDA plates for further identification.

Yield was assessed through comparison of thousand grain weights from treatment and control plots.

A representative 400g sample for each treatment was analysed for the presence of mycotoxins including trichothecenes and zearalenone (1995 and 1996).

Trichothecene

Ground wheat samples were extracted with acetonitrile: 4% aqueous KCl (9:1), filtered and defatted with iso-octane. The extract was cleaned-up by passing through a charcoal/alumina column, evaporated and derivatised with trifluoroacetic anhydride. The derivatised extract were analysed by gas chromatography/mass spectroscopy GC/Ms) using selected ion monitoring (SIM) detection for the following trichothecenes – nivalenol (NIV),

deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxy nivalenol (15-AcDON), fusarenon (FUS-X), monoacetoxyscirpenol (MAS), diacetoxyscirpenol (DAS), HT-2 toxin (HT-2) and T-2 toxin (T-2).

Zearalenone

Ground wheat samples were extracted with acetonitrile:water (3:1) and filtered. The filtered extracts were diluted with PBS buffer and cleaned up using Rhone-Poulenc immuno-affinity columns according to the recommended procedure. Zearalenone (F-2) was determined by HPLC after post column derivatisation with aluminium chloride.

Results

The use of controlled plot-misting enabled daytime field humidity to be maintained at the levels required (Figure 3.1), and significant separation between the three humidity treatments was achieved. During the first year of the project, plants on the high humidity plot showed signs of stress due to over-watering. The introduction of a timer switch to the system reduced the quantity of water applied to the plot and alleviated the problem.

As a result of high levels of take-all caused by *Gaeumannomyces graminis* on the medium humidity plot in the second year of the experiment, statistical analyses of some data were not undertaken.

Stem-base infection

Levels of *Fusarium* spp. at the stem base were higher when winter wheat rather than spring wheat was sown and peaked on winter wheat crops between GS 39 and GS 75 (Table 3.2). On the spring-sown crop this peak appeared to be later between GS 75 and GS 85.

Table 3.2. *Fusarium* spp. isolated from stem base lesions in 1994, 1995 and 1996

Year	Humidity	Percentage stem bases infected at growth stages			
		GS 31	GS 39	GS 75	GS 85
	Ambient	-	16	4	20
1994	Medium	-	0	24	28
(Spring wheat)	High	-	16	40	40
	Ambient	28	84	75	60
1995	Medium	12	45	64	20
(Winter wheat)	High	8	68	55	76
	Ambient	10	40	32	28
1996	Medium	18	60	36	44
(Winter wheat)	High	15	64	12	32

Symptom development

Significant increases in symptom development were recorded between growth stages 75 and 85 (Figures 3.2, 3.3 and 3.4). In general, increased relative humidity led to an increase in ear

blight with highest disease levels recorded on plots inoculated with *F. culmorum* or *F. graminearum*. The exception to this was in 1996 where disease levels caused by *F. culmorum* and *F. graminearum* were higher under medium humidity.

Isolation of species present on ears

In 1994, disease symptoms in individual plots were predominantly caused by the individual *Fusarium* species inoculated, with increasing plot humidity leading to increased frequency of isolation (Figure 3.5). Plots inoculated with *F. poae* or *M. nivale* were more commonly cross-infected or naturally-infected by other species of *Fusarium*, particularly *F. culmorum*, than plots inoculated with other species.

In 1995, *F. avenaceum*, *F. culmorum* and *F. graminearum* were also isolated predominantly in plots where they were inoculated, with earlier and increased levels of isolation resulting from increased humidity (Figure 3.6). Plots inoculated with *F. poae* showed highest levels of infection under medium humidity conditions. Natural infections caused by *F. poae*, were recorded in all plots at all humidity levels, with highest levels found on the medium humidity plot. At GS 85, *M. nivale*, also originating from natural sources, was commonly isolated from all plots at medium and high humidity with levels greatest at high humidity. However, *M. nivale* was not isolated from plots at ambient humidity. In general, recovery of *M. nivale* and *F. poae* from high humidity plots was reduced where *F. avenaceum*, *F. culmorum* or *F. graminearum* had been inoculated.

In contrast to previous results, levels of *F. culmorum* and *F. graminearum* in 1996 were highest on the medium rather than the high humidity plot and levels of *F. avenaceum* were low at all humidity levels. Natural infection by *F. poae* and *M. nivale* again occurred in this year, however the levels isolated were lower than in previous years (Figure 3.7).

Ear grain weight

The effect of inoculum and humidity treatment on ear grain weight for all three years is shown in Table 3. In 1994 a comparison of grain weights showed no significant differences between treatments at ambient and medium humidity. However, at high humidity the mean ear grain weight in plots inoculated with *F. culmorum* were significantly lower than in other treatments. In 1995 and 1996, no significant differences were recorded between inoculated plots at ambient humidity. However, at high humidity in 1995, plots inoculated with *F. culmorum* or *F. graminearum* produced ear grain weights significantly lower than in other treatments. Similarly in 1996, plots inoculated with *F. culmorum* at medium humidity also produced significantly lower ear grain weights.

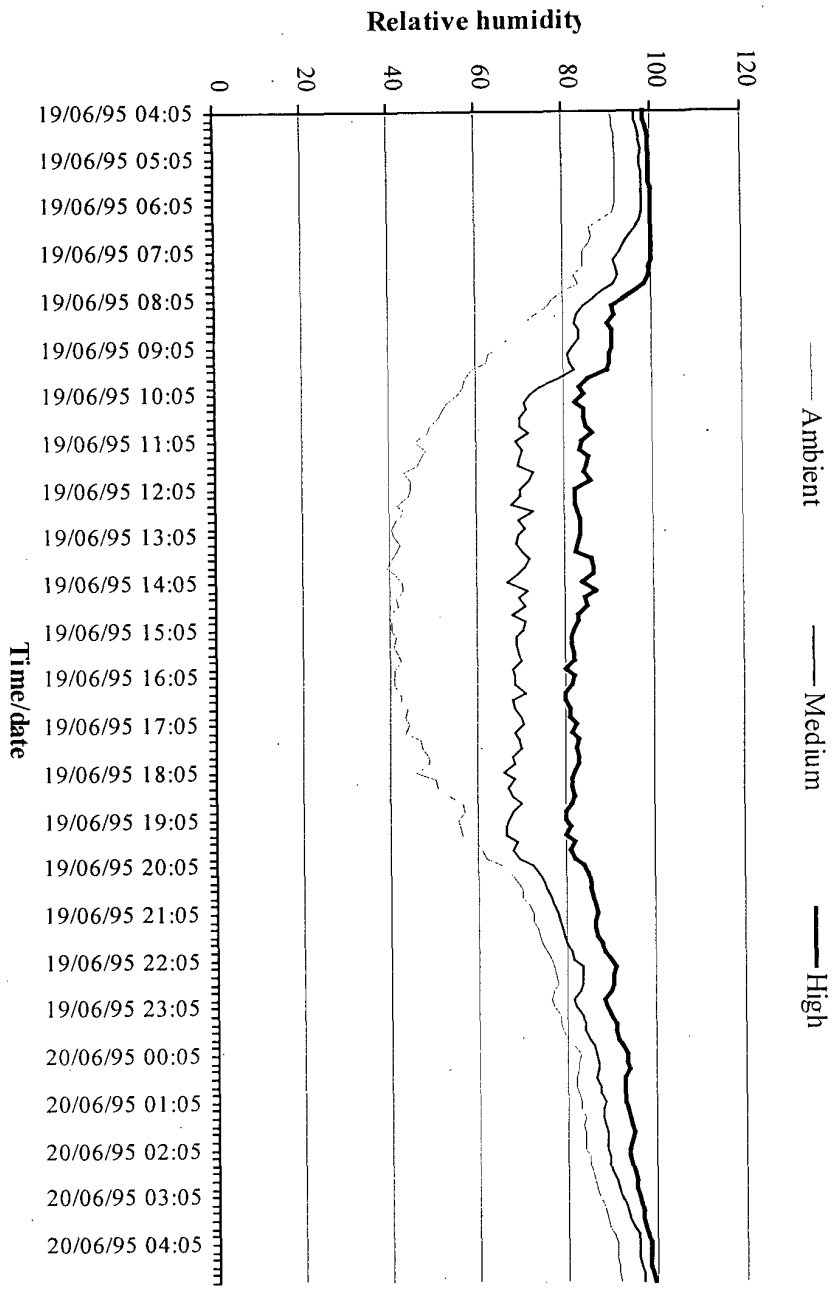

Figure 3.1 The effect of controlled misting on plot relative humidity for a 24 hour period in 1995

Figure 3.2 The effect of inoculum and humidity on ear blight symptoms (1994)

Figure 3.3 The effect of inoculum and humidity on ear blight symptoms (1995)

Figure 3.4 The effect of inoculum and humidity on ear blight symptoms (1996)

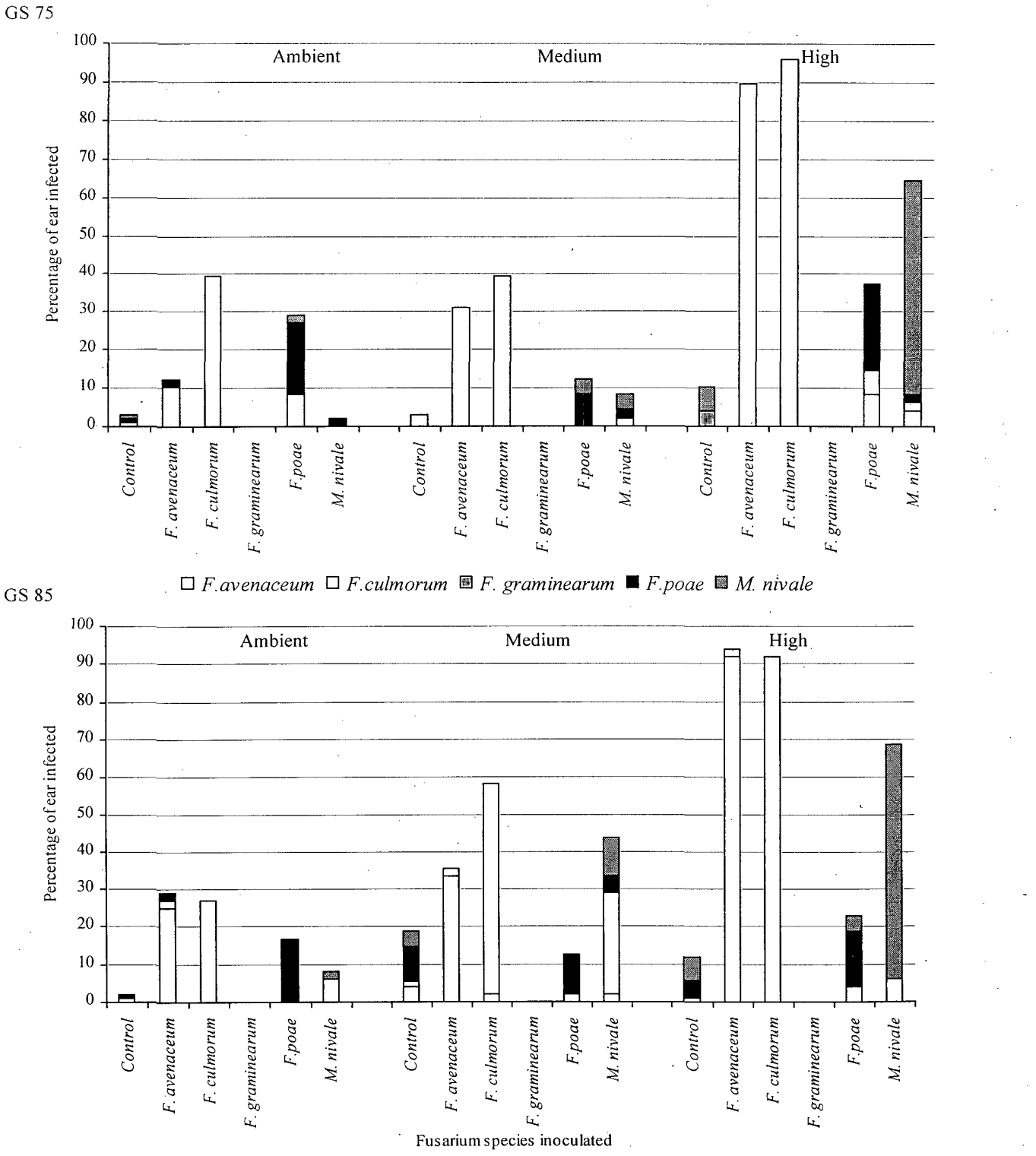

Figure 3.5 The effect of humidity and inoculum on *Fusarium* species isolated from the ear (1994)

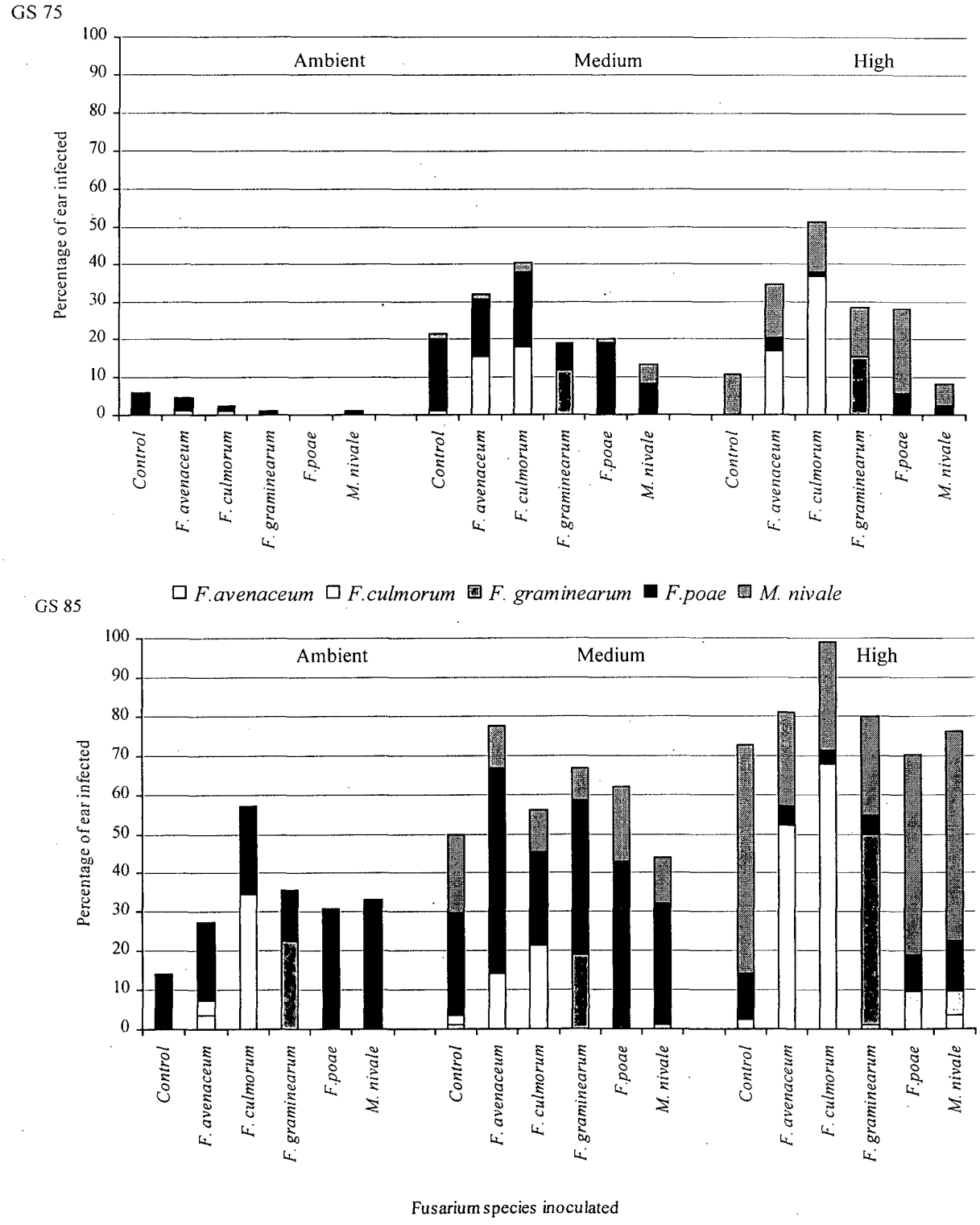

Figure 3.6 The effect of humidity and inoculum on *Fusarium* species isolated from the ear (1995)

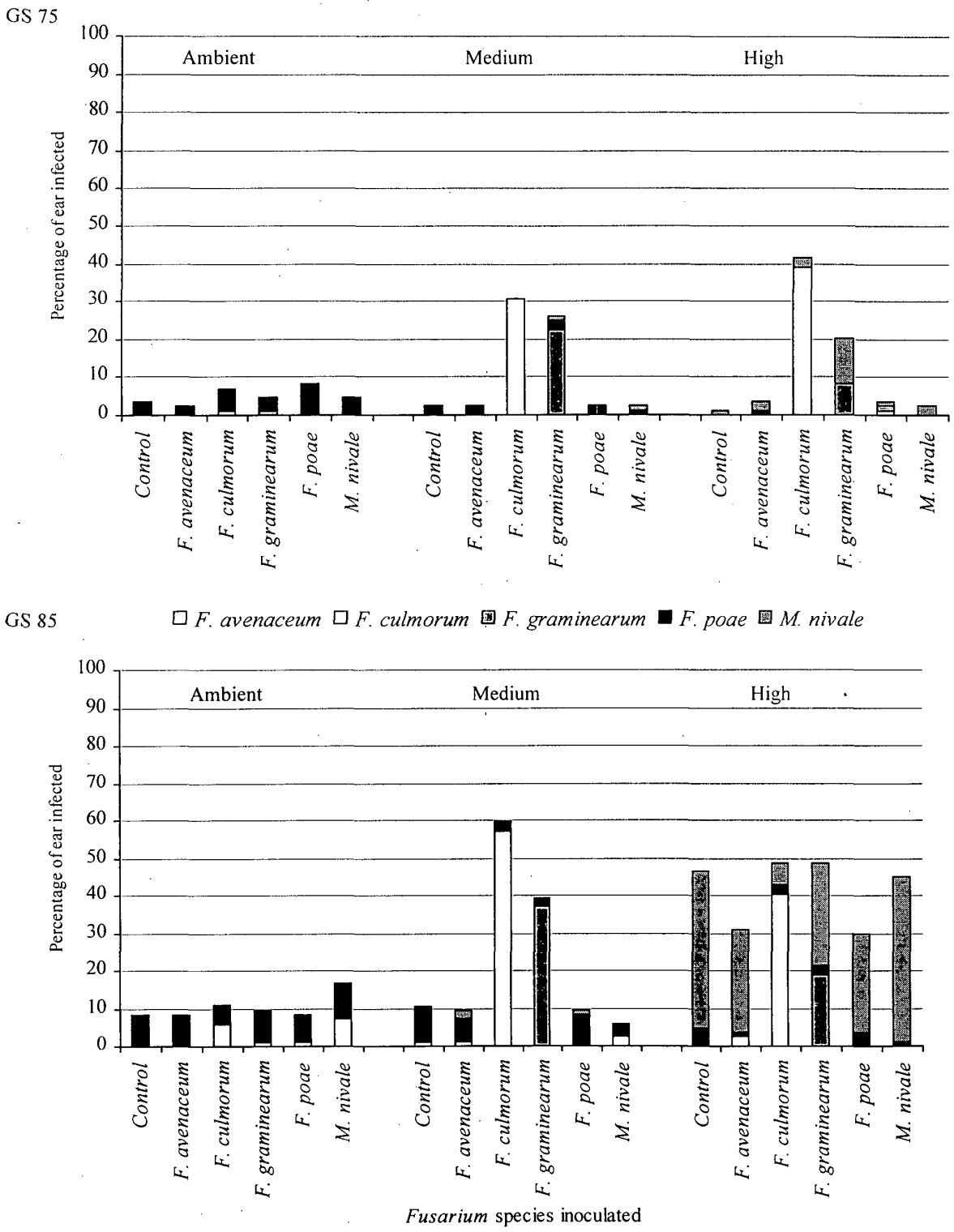

Figure 3.7 The effect of humidity and inoculum on *Fusarium* species isolated from the ear (1996)

Table 3.3. The effect of *Fusarium* inoculum on ear grain weight in 1994, 1995 and 1996.

Year	Humidity	Mean ear grain weight (g) for inoculated and control plots					
		Control	*F. avenaceum*	*F. culmorum*	*F. graminearum*	*F. poae*	*M. nivale*
1994	Ambient	1.91(0.05)	1.78(0.04)	1.54(0.04)	-	1.81(0.04)	1.82(0.05)
	Medium	1.75(0.05)	1.79(0.05)	1.52(0.05)	-	1.84(0.05)	1.68(0.05)
	High	1.16(0.04)	1.09(0.03)	0.81(0.03)	-	1.24(0.04)	1.05(0.03)
1995	Ambient	2.42(0.05)	2.41(0.05)	2.35(0.05)	2.35(0.05)	2.36(0.05)	2.42(0.05)
	Medium	-	-	-	-	-	-
	High	2.09(0.05)	2.12(0.05)	1.68(0.05)	1.71(0.05)	2.03(0.05)	2.10(0.05)
1996	Ambient	2.57(0.05)	2.64(0.05)	2.63(0.05)	2.72(0.05)	2.82(0.05)	2.57(0.05)
	Medium	2.81(0.05)	2.98(0.05)	2.34(0.06)	2.70(0.05)	2.87(0.05)	2.88(0.05)
	High	2.53(0.05)	2.47(0.05)	2.43(0.05)	2.40(0.05)	2.54(0.05)	2.50(0.05)

- indicates no assessment made; standard errors of the mean are shown in parenthesis

Single grain weight data were calculated and subjected to regression analyses to identify relationships with disease severity (Figure 3.8). The following equation described this relationship and accounted for 72% of the variance in the data:

$$y = 47.95 - 0.28x$$

where y = single grain weight and x = disease severity (mean percentage ear affected)

Grain infection

In all three years levels of infection of the grain were similar to levels of ear infection recorded at GS 85 (Table 3.4).

In 1994 the highest levels of grain infection at all humidity levels were observed on plots inoculated with either *F. culmorum* or *F. avenaceum*. The frequency of isolation increased with increasing humidity. Plots originally inoculated with *F. poae* showed the highest levels of infection by other species of *Fusarium*.

In 1995, *F. avenaceum*, *F. culmorum* and *F. graminearum* caused the highest levels of grain infection with levels increasing as humidity increased. *M. nivale,* originating from natural sources, was isolated from all plots at high humidity but no isolates were recovered at ambient humidity.

The levels of *F. culmorum* and *F. graminearum* isolated from the grain in 1996 were highest on the medium humidity plot. Natural infections caused by *M. nivale* were found on all plots at high humidity with levels significantly higher than those recorded for the inoculated species.

Figure 3.8 Relationship between severity and single grain weight

Table 3.4. The effect of humidity and *Fusarium* inoculum on *Fusarium* species isolation from grain in 1994, 1995 and 1996.

Year	Humidity	Plot	Percent grain infected by each *Fusarium* species inoculated				
			F. a	*F. c*	*F. g*	*F. poae*	*M. nivale*
1994	Ambient	Control	0.75		-	0.25	
		F. avenaceum	9.00	0.75	-	5.25	
		F. culmorum		20.75	-	1.75	
		F. graminearum	-	-	-	-	-
		F. poae	0.75		-	4.00	0.25
		M. nivale		0.75	-	2.25	
	Medium	Control	1.00	1.25	-	0.75	
		F. avenaceum	18.75	0.75	-	0.75	0.75
		F. culmorum	1.25	23.00	-	0.25	
		F. graminearum	-	-	-	-	-
		F. poae	1.00	1.00	-	4.75	0.25
		M. nivale	2.25	1.00	-	2.00	3.00
	High	Control	1.25	2.75	-	0.25	4.00
		F. avenaceum	72.75	1.00	-	1.00	
		F. culmorum	0.75	58.00	-		
		F. graminearum	-	-	-	-	-
		F. poae	0.75	1.00	-	7.00	5.25
		M. nivale	0.25	0.75	-		36.75

Year	Humidity	Plot	Percent grain infected by each *Fusarium* species inoculated				
			F. a	*F. c*	*F. g*	*F. poae*	*M. nivale*
1995	Ambient	Control		0.25		5.50	
		F. avenaceum	0.25			4.50	
		F. culmorum		8.00		4.75	
		F. graminearum		0.25	6.00	2.25	
		F. poae		0.25		4.00	
		M. nivale		0.25		4.75	
	Medium	Control	-	-	-	-	-
		F. avenaceum	-	-	-	-	-
		F. culmorum	-	-	-	-	-
		F. graminearum	-	-	-	-	-
		F. poae	-	-	-	-	-
		M. nivale	-	-	-	-	-
	High	Control	0.50		0.25	1.50	16.25
		F. avenaceum	11.25			2.75	9.25
		F. culmorum	0.25	18.00		2.00	11.75
		F. graminearum			8.75	1.50	13.75
		F. poae				2.75	15.50
		M. nivale	0.25	0.25		4.25	17.50

F. a = Fusarium avenaceum, *F. c = F.* culmorum, *F. g = F. graminearum*
- indicates no assessment made.

Table 3.4 (continued).

Year	Humidity	Plot	Percent grain infected by each *Fusarium* species inoculated				
			F. a	*F. c*	*F. g*	*F. poae*	*M. nivale*
		Control		0.25		2.00	
		F. avenaceum		0.25		3.00	
	Ambient	*F. culmorum*		0.75		3.00	
		F. graminearum		0.25	2.00	1.75	
		F. poae		1.25		4.00	
		M. nivale				4.00	
		Control		2.25		1.00	
		F. avenaceum		1.75			
1996	Medium	*F. culmorum*		24.25		1.75	1.00
		F. graminearum		1.00	12.75	2.00	0.75
		F. poae		0.25		2.00	0.25
		M. nivale		1.00		0.75	0.25
		Control	0.25	0.25			79.75
		F. avenaceum		0.75			68.00
	High	*F. culmorum*		18.00		0.25	38.00
		F. graminearum			5.75		45.75
		F. poae					67.75
		M. nivale		0.25			50.75

F. a = *Fusarium avenaceum*, *F. c* = *F.* culmorum, *F. g* = *F. graminearum*
- indicates no assessment made.

Yield

In 1994 (Figure 3.9) none of the plot treatments gave a significant reduction of yield at ambient humidity. At increased humidity, yield losses were highest on plots inoculated with *F. culmorum*; yield loss increased from 9% to 22% as plot humidity increased from medium to high. Plots inoculated with *F. avenaceum* gave yield losses of 9% at high humidity.

Losses in 1995 ranging from 3.5 to 8% were recorded on inoculated plots at ambient humidity, with *F. culmorum* producing a significantly greater loss than the other treatments (Figure 3.10). Plots inoculated with *F. culmorum* or *F. graminearum* at high humidity produced yield losses of 15 and 17 % respectively.

In 1996, as in 1994, there were no significant differences in yield loss at ambient humidity (Figure 3.11). At medium humidity, *F. culmorum* and *F. graminearum* produced yield losses of 18 and 8% respectively. At high humidity, only plots inoculated with *F. graminearum* or *M. nivale* produced significant yield losses ($P<0.05$).

Data on yield were subjected to regression analyses to identify relationships with disease severity (Figure 3.12). The following equation described this relationship with yield loss and accounted for 70% of the variance in the data:

$$y = 4.74 + 0.54x$$

where y = yield loss (based on mean 1000 grain weight) relative to the control and x = disease severity (mean percentage ear affected). The equation was derived using data from all inoculated plots irrespective of species but describes well relationships for individual species.

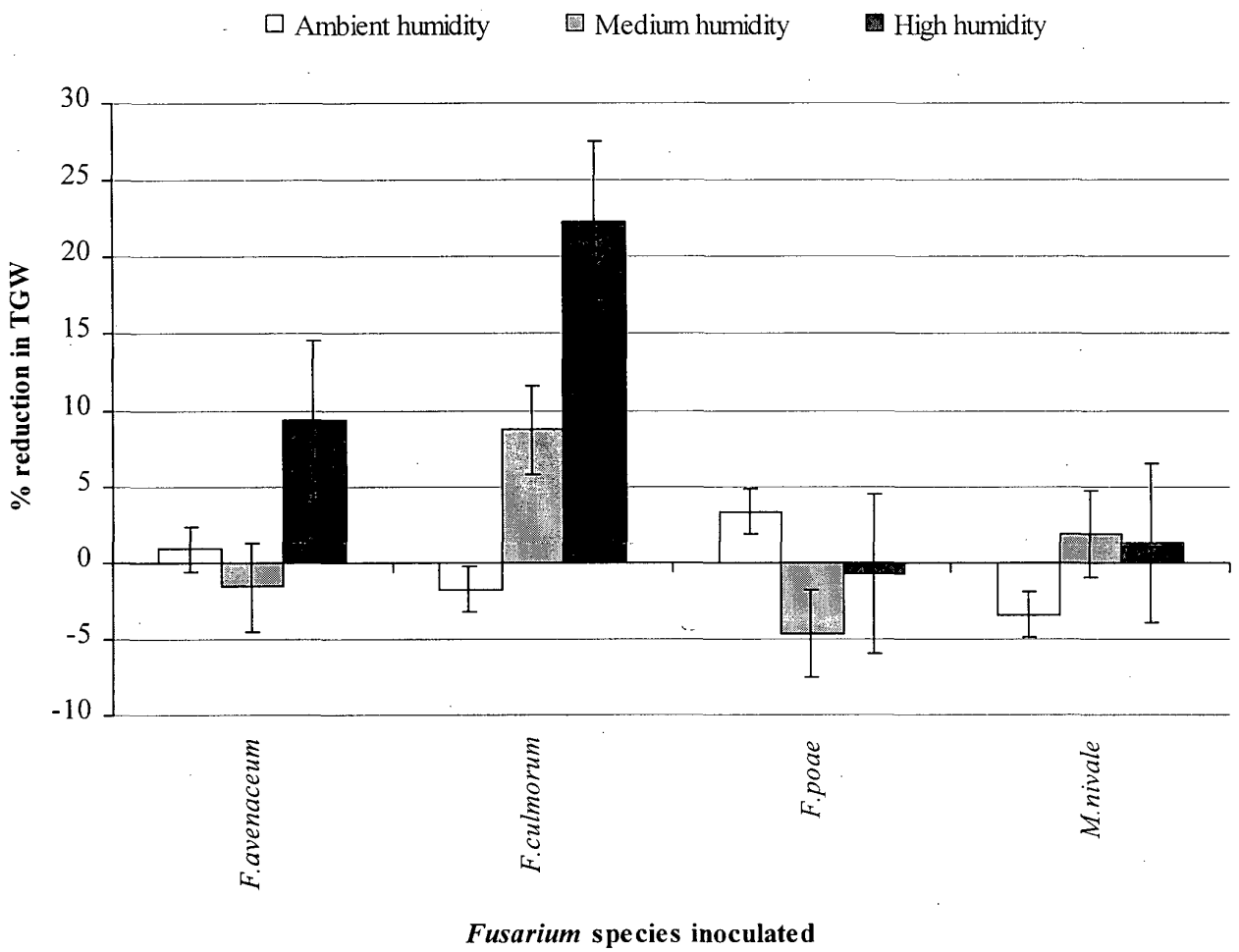

Figure 3.9 The effect of humidity and inoculum on yield loss (1994)

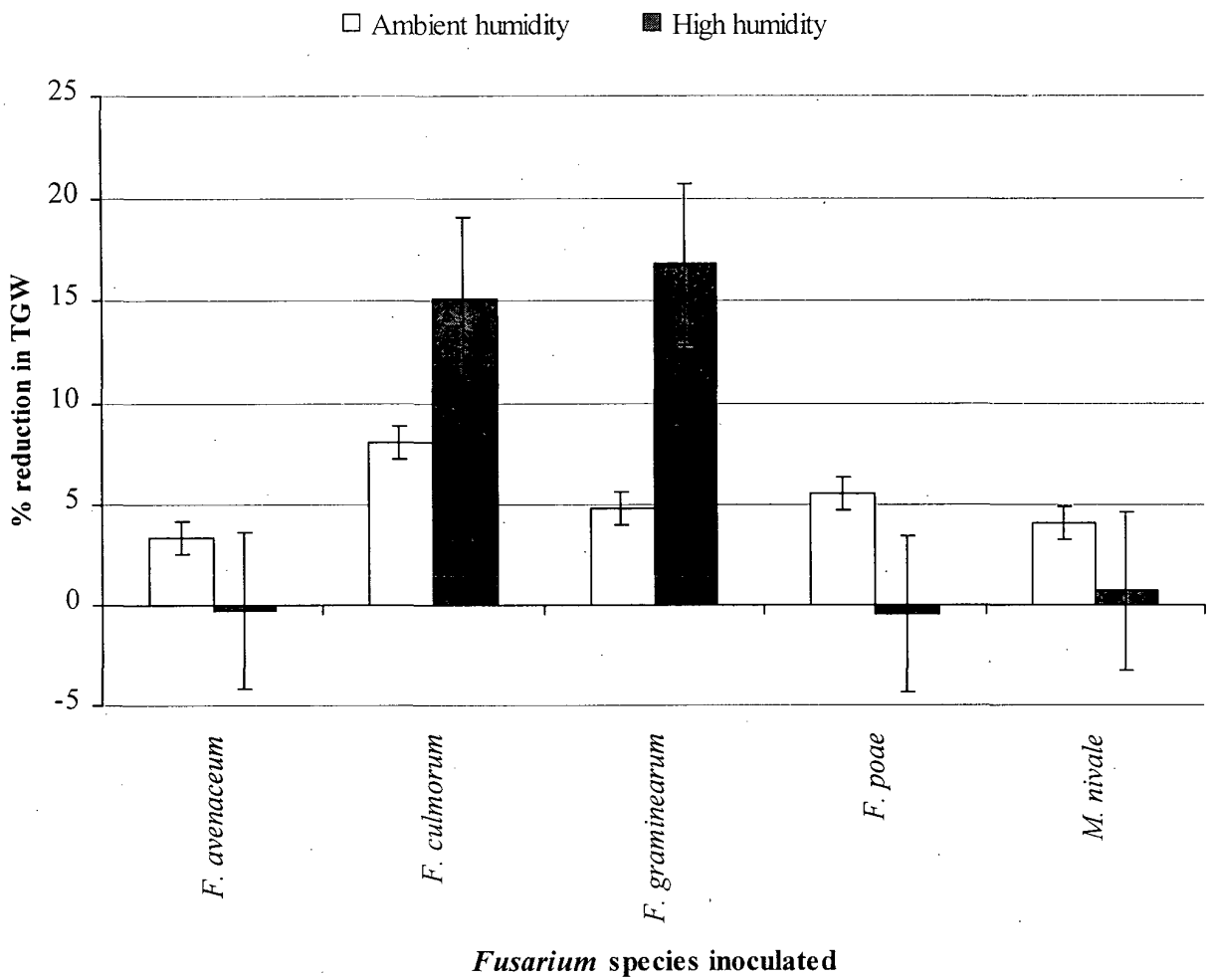

Figure 3.10 The effect of humidity and inoculum on yield loss (1995)

Figure 3.11 The effect of humidity and inoculum on yield loss (1996)

Figure 3.12 Relationship between severity and yield loss (related to thousand grain weight)

Mycotoxin analyses

The results of mycotoxin analyses for control samples in 1995 indicated low level trichothecene contamination; higher levels were found only in samples from plots inoculated with *F. culmorum* or *F. graminearum* (Table 3.5). Overall, levels of toxin were higher in samples from high humidity treatments. Grain from plots inoculated with *F. culmorum* showed significant production of nivalenol and DON, and low levels of 3-acetyl DON. These were also the only samples found to contain zearalenone. Ambient plots inoculated with *F. graminearum* showed significant production of DON; at high humidity, low levels of 15-acetyl DON and nivalenol were also produced. The highest levels of nivalenol, the most toxic mycotoxin, were produced by *F. culmorum* and *F. poae*.

In 1996, overall levels of mycotoxin were higher than in 1995, with highest levels of toxin produced on plots inoculated with either *F. culmorum* or *F. graminearum* (Table 3.6). Levels of mycotoxins produced were generally related to the level of *Fusarium* infection of the grain. Highest levels of nivalenol were found in grain infected with *F. culmorum*, *F. poae* or *M. nivale*. As in 1995, highest levels of DON occurred in grain infected with *F. culmorum* or *F. graminearum*.

Table 3.5. Effect of humidity and *Fusarium* treatment on mycotoxin production (1995).

Humidity	Inoculation treatment	Mycotoxin (μg kg^{-1})					
		Trichothecene					
		DON	3-acetyl DON	15-acetyl DON	Nivalenol	MAS	Zearalenone
Ambient	Control	20			12	5	
	F. avenaceum				7		
	F. culmorum	385	15		286		6
	F. graminearum	419					
	F. poae				8		
	M. nivale				8		
High	Control	21			14		
	F. avenaceum				12		
	F. culmorum	776	22		588		10
	F. graminearum	786		14	22		
	F. poae				12		
	M. nivale				25		

DON = deoxynivalenol, MAS = monoacetoxyscirpenol.

Table 3.6. Effect of humidity and *Fusarium* treatment on mycotoxin production (1996).

Humidity	Inoculation treatment	Mycotoxin (μg kg^{-1})					
		Trichothecene					
		DON	3-acetyl DON	15-acetyl DON	Nivalenol	MAS	Zearalenone
Ambient	Control		-	21	47	10	*
	F. avenaceum		-	15			*
	F. culmorum		-				*
	F. graminearum	18	-		32		*
	F. poae		-		89		*
	M. nivale	23	-		35		*
Medium	Control		-		81		*
	F. avenaceum		-				*
	F. culmorum	2091	-	41	2163	19	*
	F. graminearum	850	-		56		*
	F. poae	24	-		83		*
	M. nivale	53	-		233		*
High	Control		-		32		*
	F. avenaceum		-				*
	F. culmorum	198	-		186		*
	F. graminearum	23	-		113		*
	F. poae	22	-		222		*
	M. nivale	75	-	13	130		*

DON = deoxynivalenol, MAS = monoacetoxyscirpenol.
- Calibration failed
* Not tested

Discussion

A system was successfully developed which allowed manipulation of in-crop humidity levels in the field. As a direct result of plot inoculation and humidity control, differential epidemics of *Fusarium* ear blight were observed on plots in each year of the experiment. For the first time, the potential of different *Fusarium* spp. to cause disease and yield loss in the UK has been quantified in the field. In addition, yield loss relationships have been established for individual species of *Fusarium* associated with ear blight diseases, and the factors influencing the infection and development of ear blight have been elucidated. The dataset generated from the trials, illustrating a particularly wide range of disease severities, also affords an original and unique tool for the investigation of relationships between incidence and severity.

Yield losses of over 20% were recorded on inoculated plots under conditions of increased humidity. Such high losses were observed with the more aggressive pathogens *F. culmorum* and *F. graminearum*. The increased impact of these two species may be attributed to differences in disease establishment between species after infection. A single site infection on the ear caused by either *F. culmorum* or *F. graminearum*, given favourable conditions, can lead to a total loss of grain production above the point of infection. However, a single site infection by either of the other three species seems to remain confined to the infected spikelet, thus having less influence on the development of grain above the site of infection within the infected ear. The high yield losses recorded in 1994 may have resulted from the longer duration of humidity control, possibly indicating a relationship between the duration of increased humidity and yield loss.

F. culmorum was shown to be the most aggressive ear blight pathogen with the potential to cause the greatest effect on yield loss and production of mycotoxins. *F. graminearum* also caused a significant yield loss under favourable conditions. In general, *F. culmorum* and *F. graminearum* were the most aggressive colonisers of the ear seeming to reduce the presence of any other species which may have been present. *F. culmorum* is commonly found on wheat crops in the UK and thus, given favourable conditions, may pose a major threat to crop yields. *F. graminearum* is less commonly isolated from wheat crops and poses less of a threat to yield and grain quality. However, current farming practice involving growing maize either in monoculture, or in rotation with wheat, grass and barley may lead to an increase in inoculum of *F. graminearum* in the soil and thus could lead to increased risk from this disease in the future. Sayer (1992) demonstrated that *Fusarium* infection, including *F. graminearum*, on maize crop debris in New Zealand in 1989 was high and, combined with conducive weather conditions at anthesis, led to high disease incidence on the following barley crop.

Differences in disease development related to humidity were observed between *Fusarium* species. Most *Fusarium* species, the more aggressive species in particular, produced highest levels of disease and yield loss under conditions of increased humidity. Highest incidence of visual infection by *M. nivale* were also recorded at high humidity. These findings were confirmed by results of monitoring the level of incidence of natural infections which also showed highest incidence at high humidity. This suggests there may be a threshold level below which significant disease caused by *M. nivale* will not develop on the ear. *F. poae*, which is generally regarded as the weakest of the *Fusarium* pathogens of wheat, produced lowest levels of infection at high humidity. Natural infection by *F. poae* was also lowest under high humidity conditions. However, the potential of *F. poae* to produce nivalenol and its ability to infect under ambient conditions mean that this species should not be overlooked when assessing the risks of *Fusarium* ear diseases.

Mycotoxin analyses indicated that, in 1995, only plots infected by *F. culmorum* or *F. graminearum* showed increased levels of mycotoxin compared to control plots, and that these levels increased with humidity. Levels of mycotoxin contamination also increased relative to the level of grain infection in both years with levels higher in 1996. Increased levels of mycotoxin production in 1996 may have been due to lower temperatures prior to harvest. Evidence from *in vitro* experiments suggests that some *Fusarium* strains produce higher levels of mycotoxin after lower temperature treatment (Zhu & Zhang, 1991). Production of mycotoxins can also be influenced by differences in tolerance to mycotoxin accumulation in different wheat cultivars (Chelkowski, Pers. comm.). Unofficial guidelines issued by authorities in Canada suggest a tolerance limit of 2000 $\mu g\ kg^{-1}$ for uncleaned soft wheat (Van Egmond, 1989). Mycotoxin levels in plots inoculated with *F. culmorum* at medium humidity slightly exceeded this limit. These results demonstrate the potential risk of significant mycotoxin production in severely infected wheat crops under favourable conditions. However, the incidence of the conditions required to promote significant toxin production is currently rare in the UK.

In conclusion, this work has illustrated that, under certain conditions, a range of *Fusarium* species can cause significant yield loss through reduced grain production per ear and reduced thousand grain weight. Subsequent mycotoxin contamination can affect grain quality. The conditions conducive to ear infection and symptom development have been more clearly defined and methodologies for inducing epidemics developed. However, factors influencing mycotoxin production remain unclear and further work utilising the techniques developed here is essential before the true risks of *Fusarium* ear diseases can be precisely identified.

SECTION 4: RELATIONSHIP BETWEEN INCIDENCE OF *FUSARIUM* EAR BLIGHT AND METEOROLOGICAL FACTORS

J A Turner & P Jennings, Central Science Laboratory

Objective

To use historic disease and meteorological data to investigate the relationships between *Fusarium* ear blight incidence and weather conditions.

Methodology

Disease surveys of winter wheat crops have been conducted annually, with the exception of 1983 and 1984, since 1970. The survey of leaf, ear and stem-base diseases was carried out each year when crops were at the early- to medium-milk growth stage (GS 73-75) when the dry matter of the grain was accumulating most rapidly. Data generated from these surveys was stored on a powerful INFORMIX relational database at CSL.

Fusarium ear diseases were divided into two categories:

(i) ear blight - areas of the ear showing bleaching
(ii) glume spot - symptoms resembling those caused by *Fusarium poae*

The period of years over which analyses could be carried out was limited by the availability of raw data on the number of plants affected (from 1988 onwards) and availability of meteorological data (up to 1995). The analyses were therefore carried out for the 8 year period from 1988 to 1995. Meteorological data were obtained from the Monthly Weather Reports published by the Meteorological Office. Weather factors selected for analysis were mean temperature, rainfall and rain days (the number of days when rain occurred). These data were expressed for each weather factor as the difference from the 30 year mean e.g. mean temperatures were recorded as plus/minus x° compared to the mean and rain days were expressed as the number of days rain in comparison to the mean.

Variation in disease incidence was investigated on a national and regional level. Data were subjected to regression analyses to identify the subsets of weather variables which accounted for the highest proportion of the variation in incidence of all *Fusarium* diseases over the eight-year period. Incidence of ear blight was calculated as the percentage plants showing symptoms of ear blight. Incidence of *Fusarium* ear diseases was calculated as the percentage of plants with symptoms of ear blight or glume spot.

Results

The national incidence of *Fusarium* ear blight ranged from 0.1 to 2.0% plants affected between 1988 and 1995, whereas incidence of all *Fusarium* disease ranged from 0.8 to 15.6% plants affected (Table 4.1). Incidence of ear blight alone fluctuated markedly between 1988 and 1995 with the disease being particularly prevalent between 1991 and 1993. Lowest incidence of the

disease occurred in 1995 and 1996. National incidence of all *Fusarium* ear diseases was also lowest in 1995 and 1996 but highest levels occurred in 1988, 1990, 1991 and 1993. Levels of *Fusarium* ear diseases were relatively low in 1992.

Table 4.1. National incidence of *Fusarium* ear diseases (mean percentage plants affected)

Year	*Fusarium* ear blight	All *Fusarium* ear diseases
1988	1.1	15.6
1989	0.7	8.1
1990	0.4	10.7
1991	1.4	11.1
1992	1.7	5.28
1993	2.0	10.4
1994	0.4	2.8
1995	0.1	0.8

Over the eight-year period, regional incidence of *Fusarium* ear blight alone ranged from 0 to 5.6% plants affected (Figure 4.1) whereas incidence of all *Fusarium* diseases ranged from 0.3 to 23.5% plants affected (Figure 4.2). Regional disease levels also fluctuated from year to year but the annual level of disease in each region did not change proportionally in relation to national disease levels. This probably reflects differences between weather patterns at the regional level.

Statistical analyses of data on ear blight alone showed poor correlation with meteorological factors. The use of the best subset of five variables accounted for less than 34% of the variation. Regression analyses on ear disease data incorporating assessments of glume spot identified four weather variables which accounted for 70% of the variation in disease incidence. The regression equation for the relationship between these factors was as follows:

Disease incidence = - 4.47 + 1.52(temp Jan) + 0.102(rain Jun) + 0.810(rain days July) –0.653(rain days Nov)

Graphical representation of the data illustrates that in 1988, when disease levels were highest, the climate followed the pattern described the equation i.e. a drier than average November was followed by a warmer than average January and this preceded wetter than average weather in June and July (Figure 4.3). Use of the same factors to examine the incidence of ear blight alone showed little relationship between selected variables and disease incidence (Figure 4.4). Use of the same four variables accounted for 22% of the variation.

Regression analyses using disease severity data showed no clear relationship between severity and weather factors. A maximum of only 27% of the variation was accounted for with analyses suggesting that rain in March and June with warm weather in April and May were most important.

Discussion

The regression equation generated indicates that warm, wet conditions in the winter and early

spring followed by rain in June and July were most conducive to development of *Fusarium* ear diseases. Drier weather in November and warm weather in January could encourage sporulation. There is evidence in the literature to support the role of dry soils and warmer temperatures on growth and development of *Fusarium* infections. In greenhouse experiments, Colhoun & Park (1964) have shown that post-emergence death of seedlings caused by *Fusarium* species was most severe in dry soils and that an increase in the number of plants killed occurred with increasing temperature. Duthie & Hall (1987) also reported that warm dry weather is conducive to spread of *F. graminearum* from seed to the stem-base. Wetter weather during June and July would favour spread of the inoculum onto the ear. These results also agree well with those of Inglis & Cook (1981), Snijders (1990c) and Jennings & Turner (1996), which indicated that wet or humid conditions at anthesis (June) favoured development of *Fusarium* ear diseases. Species such as *F. culmorum* and *F. graminearum* are typical splash-borne pathogens and are therefore most likely to be favoured by wet conditions

The degree of disease severity will depend on the level of infection during anthesis and weather conditions between anthesis and harvest. A model to forecast *Fusarium* ear diseases would therefore need to consist of two elements (i) a prediction of build-up of inoculum and risk of infection (ii) prediction of conditions after infection which would lead to significant development of the disease. Experimental data show that *Fusarium* species can often be isolated from ears at anthesis but the disease will not develop due to absence of conditions conducive to disease development.

The equation for the relationship between *Fusarium* ear disease and environmental factors now needs further development using analyses of data generated from unsprayed trials with concomitant weather data to validate and refine the precision of the model. Use of the survey data alone may not provide a model which is robust enough to be used for prediction of epidemics. Trends in the data may be confounded by the effects of agronomic factors and the use of fungicide sprays. In addition the growth stage at which assessments were carried out was not optimal for assessment of *Fusarium* ear blight. Full expression of symptoms may only occur just prior to crop senescence, several weeks after the growth stage at which samples were assessed (GS 73-75). At GS 75, it is possible that some ear blight infections were expressed as symptoms of glume spot. In later years the introduction of newer cultivars and the particularly warm summers have resulted in crops being harvested approximately two weeks earlier than in for example 1988. With crops reaching GS 75 earlier in July the influence of July rainfall will be less and *Fusarium* ear disease at this stage of development reduced as a consequence.

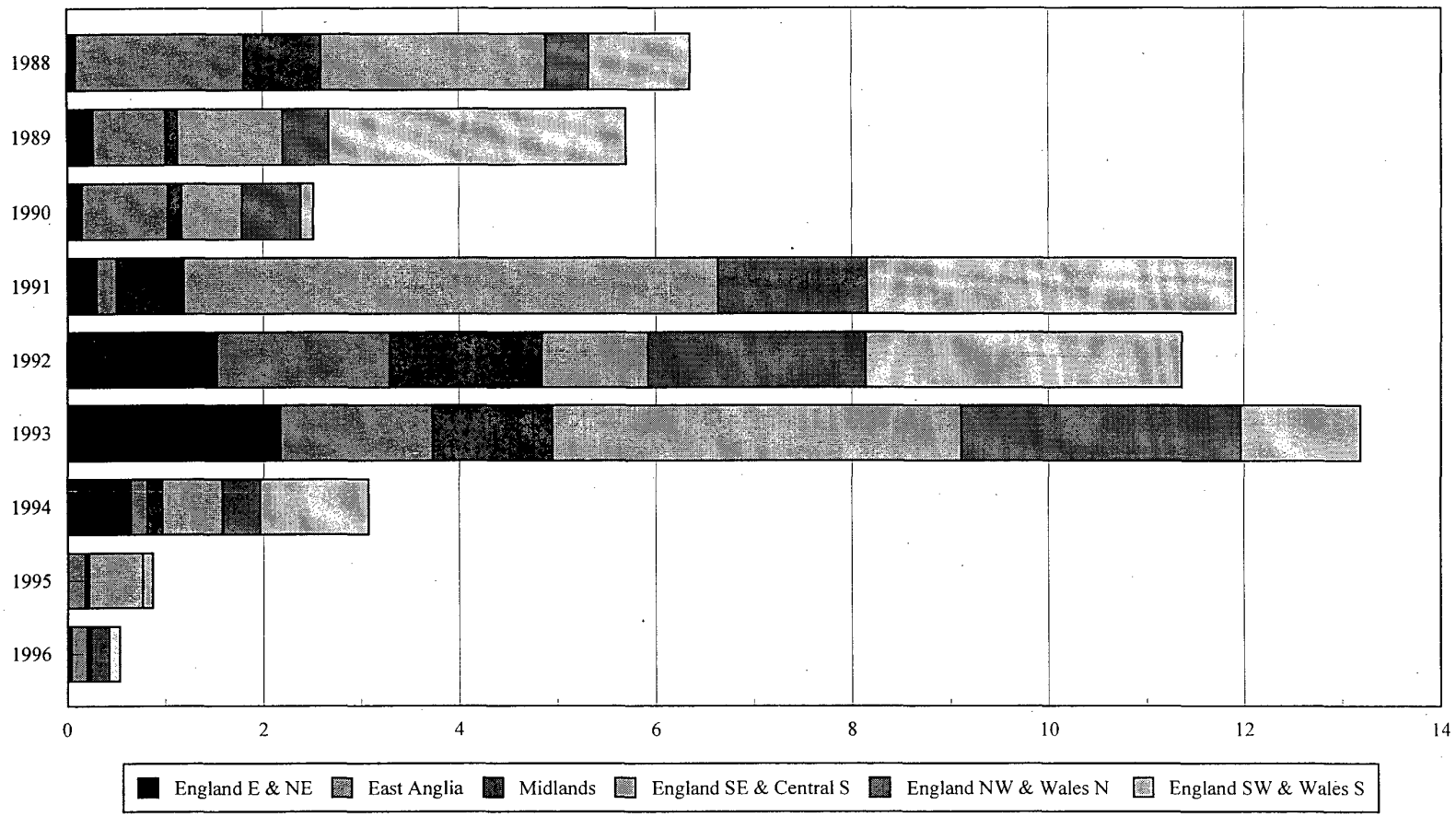

Figure 4.1 Percentage plants affected by fusarium ear blight (1988-1996)

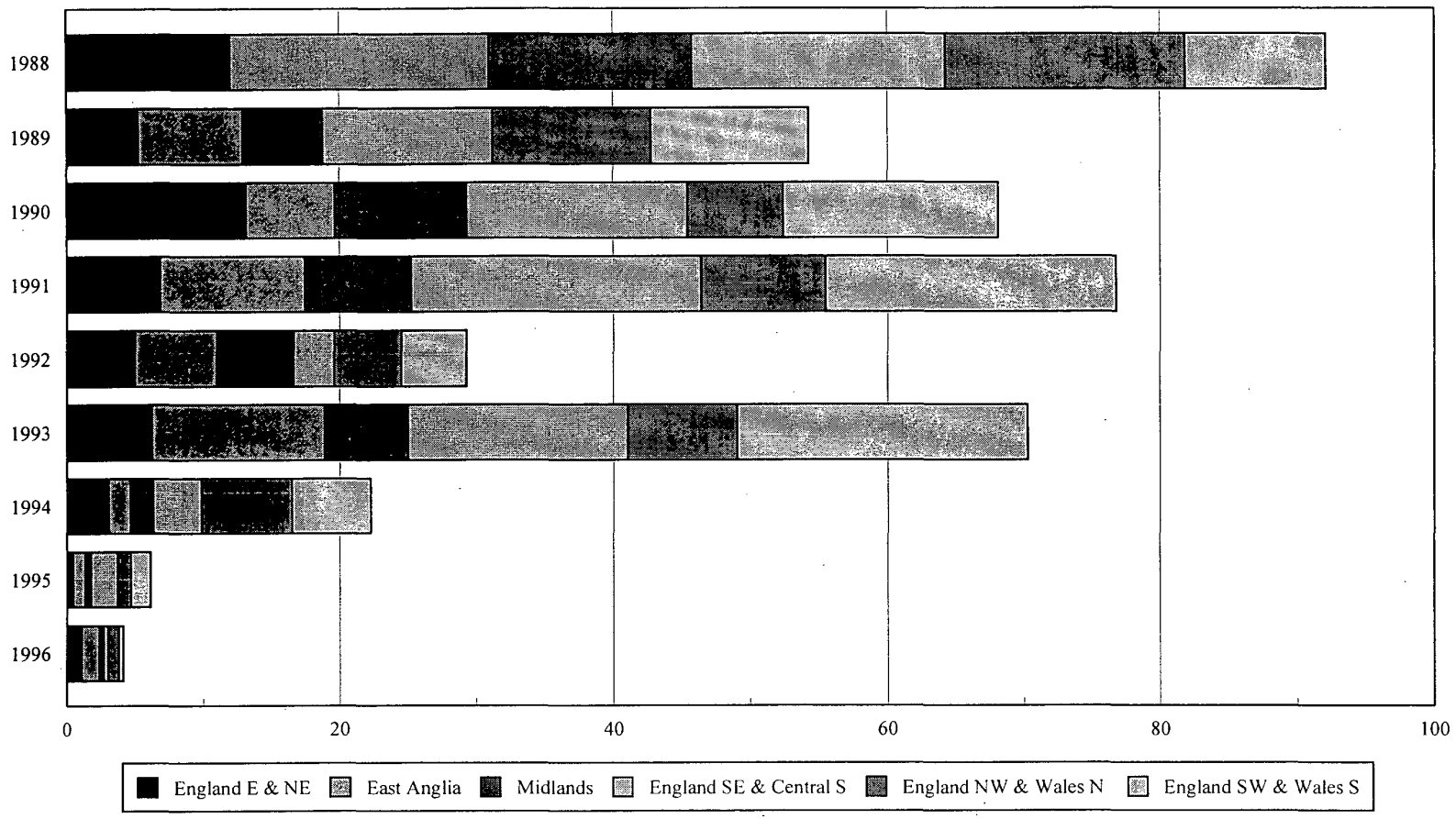

Figure 4.2 Percentage plants affected by fusarium ear diseases 1988-1996

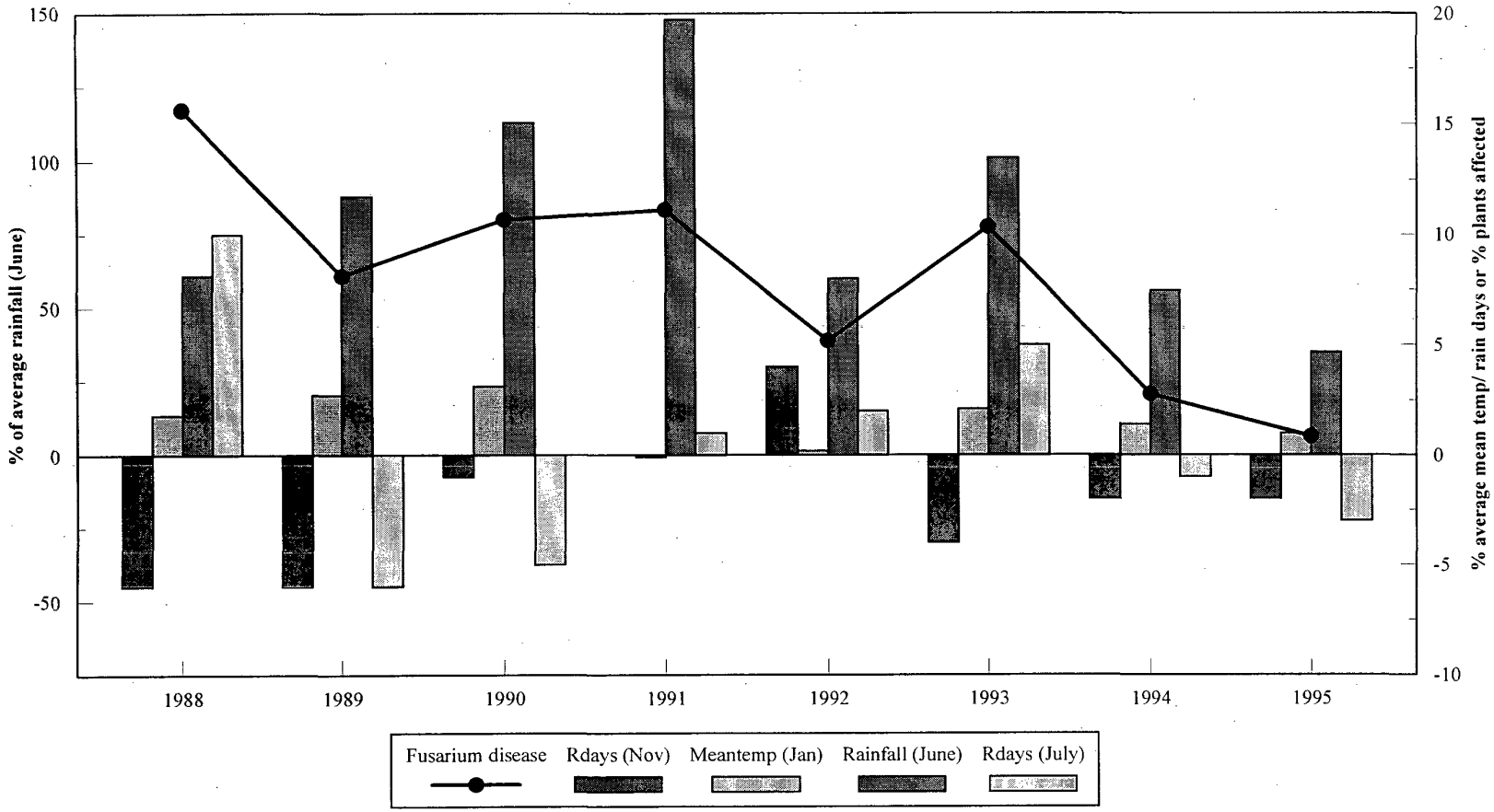

Figure 4.3 Relationship between incidence of fusarium ear diseases and climate (1988-1995)

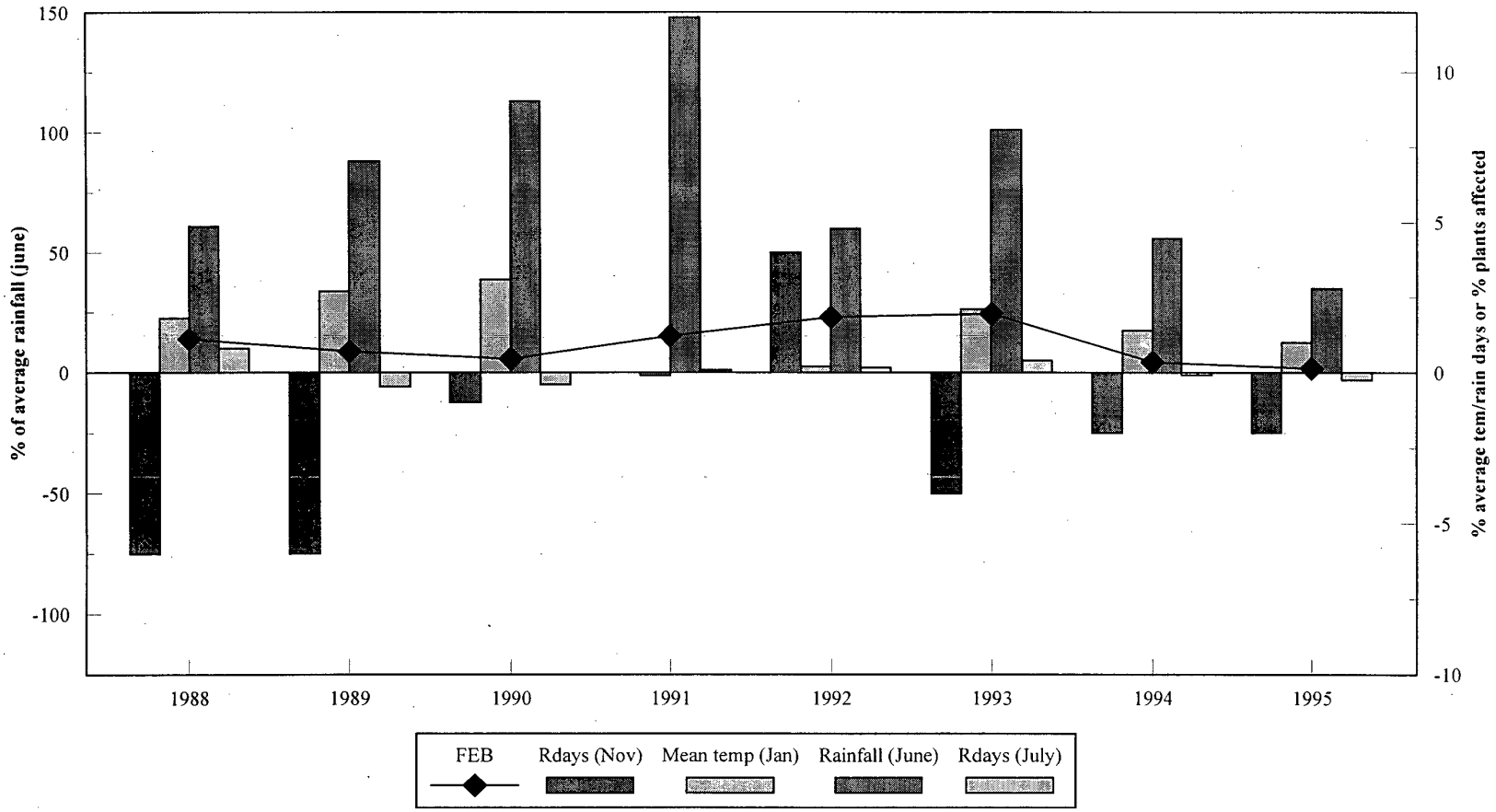

Figure 4.4 Relationship between incidence of fusarium ear blight and climate (1988-1995)

SECTION 5: WINTER WHEAT: FUNGICIDE EVALUATION AND TIMING FOR CONTROL OF EAR BLIGHT

D R Jones & P Gladders, ADAS

Objectives

To evaluate fungicides for activity against *Fusarium* ear blight and black point (caused primarily by *Alternaria alternata*), and to investigate the protectant and eradicant activity of fungicides against *Fusarium* ear blight, in order to determine the 'spray window' available for treatment.

Materials and methods

Fungicide activity against both *Fusarium* ear blight and black point was investigated in two experiments at ADAS Arthur Rickwood, in 1993 and 1994. In each, a range of fungicides was applied once or twice during the period from GS 59 to GS 75; full details of the fungicide products are given in the tables of results (Tables 5.2 and 5.3). Active ingredients and application rates are given in Table 5.1. The design of each experiment was a split plot with three replicates, with irrigation as the main plot factor and fungicide treatments on sub-plots. In 1993, irrigation was provided for a short period following each fungicide application, whereas irrigation was provided for two days after each application in 1994.

The protectant and eradicant activity of tebuconazole against *Fusarium* ear blight was investigated by two methods. Some sites were irrigated to provide a period highly conducive to *Fusarium* infection. Others relied on precipitation to facilitate natural infection. Plot sizes were in the range 36-72 m^2, and all experiments were on cv. Avalon except where indicated below.

In the first irrigated experiment on fungicide activity, on cv. Admiral at ADAS Gleadthorpe in 1994, each irrigated treatment received only one short period of irrigation and there were no inoculated plots. Ear disease was minimal. Experience from other sites in 1994 indicated that the duration of irrigation at Gleadthorpe was too short to provide optimal conditions for infection. No conclusions could be drawn from this experiment.

Further irrigated sites were at ADAS Gleadthorpe and ADAS Arthur Rickwood in 1995 and 1996. Each experiment received a substantial period of irrigation at early anthesis, with single applications of tebuconazole on various dates within a week either side of the irrigation. At Gleadthorpe in 1995, and at both sites in 1996, an additional factor was inoculation, with half of each block inoculated with a mixture of three isolates of *Fusarium culmorum* supplied as cultures on potato dextrose agar by Harper Adams Agricultural College. Inoculation was achieved by preparing a spore suspension with a concentration in the range 10^4 to 10^5 spores ml^{-1}. Spore suspensions were applied by farm sprayer at Gleadthorpe (in 330 l ha^{-1} water) and knapsack sprayer at Arthur Rickwood (in 200 l ha^{-1} water), with the pressure set as low as possible (*c.* 100 kPa) to minimise physical damage to spores. Integrity of spores after passage through the sprayer was checked at both sites in 1996, and no physical effects were observed, but viability was not determined. Inoculation was done at early anthesis, 4-6 hours prior to irrigation. Irrigation was provided over a period of about 36 hours; it was not continuous but several short periods of irrigation were provided during this period to keep the crop continuously wet through the 36 hours. Each experiment had four replicates except for that at Arthur Rickwood in 1996 which had three. Dates of fungicide application, inoculation

and irrigation are given in the tables of results (Tables 5.4 to 5.6). To minimise the effects of foliar diseases on yield, each experiment received one foliar fungicide application at GS37, using a fungicide which would not be expected to have a significant effect on the *Fusarium* ear blight epidemic. Fenpropidin plus propiconazole was used at ADAS Gleadthorpe, and triadimenol at ADAS Arthur Rickwood.

There were four identical experiments reliant on natural infection, at ADAS Bridgets in 1994 (on cv. Rialto) and at ADAS Rosemaund in 1994-96. Each consisted of eight treatments replicated three times which received a single application of tebuconazole, on a series of dates at 3 or 4 day intervals starting at GS 57-59. An additional factor investigated was whether *Thrips* could act as a vector for *Fusarium* spp.. This was not investigated in great detail, but one treatment received a single GS 57-59 application of chlorpyrifos to control *Thrips*, but no fungicide. Each experiment received an application of propiconazole plus fenpropidin at GS 37-39 to control foliar diseases.

In all experiments, ear diseases were assessed at GS 75 and GS 83-85 on 10 ears per plot. At ADAS Arthur Rickwood in 1996, additional assessments were done to give a weekly series of assessments from GS 71 to GS 83. Experiments were harvested, and yield, specific weight and thousand grain weight determined, and data calculated for 85% dry matter. At ADAS Arthur Rickwood, grain samples were examined for black point and disease severity recorded on a 100 grains per plot sample using the following index :-

0 - no symptoms
1 - district darkening at the embryo end
2 - darkening extending beyond the embryo, up to one third of grain affected.
3 - discolouration affecting more than one third and up to two thirds of grain.
4 - severe discolouration: more than two thirds of grain surface discoloured.

Table 5.1. Fungicides: active ingredients and products

Active ingredient(s)	Product	Rate of a.i. in product and formulation	Application rate ha^{-1}*
cyproconazole	Alto 100 SL	100 gl^{-1} SL	0.80 l
chlorothalonil	Bravo	500 gl^{-1} EC	2.00 l
mancozeb	Dithane 945	800 g kg^{-1} WP	2.00 kg
chlorpyrifos	Dursban	480 gl^{-1} EC	1.00 l
anilazine	Dyrene	480 gl^{-1} SC	2.00 l
tebuconazole	Folicur	250 gl^{-1} EC	1.00 l
fenpropimorph +propiconazole	Glint 500 EC	375 + 125 gl^{-1} EC	1.00 l
carbendazim + maneb	Multi W FL	50 + 320 gl^{-1} SC	5.00 l
fenpropidin	Patrol	750 gl^{-1} EC	0.75 l
difenoconazole	Plover	250 gl^{-1} EC	0.30 l
carbendazim + flusilazole	Punch C	125 + 250 gl^{-1} EC	0.625 l
iprodione	Rovral Flo	250 gl^{-1} SC	2.00 l
tebuconazole + triadimenol	Silvacur	250 + 125 gl^{-1} EC	1.00 l
triadimenol	Spinnaker	250 gl^{-1} EC	0.50 l
cyproconazole + prochloraz	Sportak Delta	48 + 320 gl^{-1} EC	1.25 l
propiconazole	Tilt	250 gl^{-1} EC	0.50 l

* Fungicides were applied at these rates except where stated otherwise in tables of results

Results

Fungicide evaluation, ADAS Arthur Rickwood 1993

There were no effects of irrigation on foliar or ear disease, nor on yield or grain quality, so mean data for the two irrigation treatments are presented below (Table 5.2). *Fusarium* ear blight affected a mean incidence of 9.2 ears per plot by 16 July (GS 77). Effects of fungicides on *Fusarium* ear blight were not quite statistically significant ($P = 0.06$), with the greatest reductions were given by tebuconazole plus triadimenol treatments. Sooty moulds developed on the ears during early August but treatment differences were not significant. The one foliar disease to reach significant levels was brown rust. Tebuconazole plus triadimenol treatments provided good control, and useful activity against brown rust was also apparent for cyproconazole plus prochloraz and difenoconazole (at GS 69).

Black point developed appreciably in this experiment. It was aggravated by the irrigation regime, with a significantly higher ($P < 0.05$) mean level (9.0%) in irrigated plots than non-irrigated plots (8.0%). However, no fungicide treatment controlled black point. No relationships could be detected between black point and various factors (e.g. yield, specific weight, green leaf retention, brown rust control). Black point was consistently associated with the largest grains. Discoloured, shrivelled grain resulting from damage by wheat blossom midge was present in all samples and affected 7.3% of grain overall. There were no effects of treatment on incidence of midge damage. Foliar disease levels were negligible.

There were small but significant yield responses to tebuconazole plus triadimenol plus anilazine and the split application of tebuconazole plus triadimenol. Treatments had no effect on thousand grain weight but there were statistically significant ($P < 0.05$) increases in specific weight from tebuconazole plus triadimenol (1.0 l ha^{-1}) and the tebuconazole plus triadimenol split application.

Fungicide evaluation, ADAS Arthur Rickwood 1994

In 1994, the overall incidence of ear blight was very low, but the use of irrigation lead to a 15 fold increase in ear blight incidence. There was very little ear blight in non-irrigated plots, so data are presented for irrigated plots only (Table 5.3). Tebuconazole plus triadimenol plus anilazine gave the most effective control of ear blight, significantly better than all other treatments in irrigated plots. Carbendazim plus maneb, cyproconazole plus prochloraz, tebuconazole plus triadimenol (both rates), fenpropimorph plus propiconazole and cyproconazole at the lower rate also gave control of ear blight. *Fusarium culmorum* was the predominant pathogen isolated from 92% of affected ears but 8% ears were infected by *Fusarium graminearum.*

Black point affected 7.4% grains overall and there was a three fold increase with the irrigation treatments. Control of black point with tebuconazole plus triadimenol plus anilazine was superior to most other treatments except cyproconazole plus prochloraz and the higher rates of cyproconazole and tebuconazole plus triadimenol. There was a lower incidence of black point in one block which was affected particularly severely by drought stress. Further analysis excluding this block revealed a significant effect of irrigation on black point index (mean 3.8 irrigated, 1.1 non-irrigated) whilst fungicide differences approached significance ($P = 0.052$). There were no significant differences in weight of healthy or affected grain between treatments. Grains with black point were consistently heavier than healthy grain; affected grain had an overall mean weight of 15% above that of unaffected grain. Nearly all grain with black point were Index 1 (7.3% grain) with a low incidence at Index 2 (1.0%) and only occasional grains at Index 3. Ten immature grains (1 per ear) were collected from the middle of non-irrigated and irrigated control plots with forceps, cut in half and the embryo end was placed on potato dextrose agar amended with the antibiotic streptomycin. This was carried out on 1 July and repeated on 12 July. On 1 July, *Alternaria* was isolated from one grain from the non-irrigated control only whilst *Cladosporium* was present on 90% of both irrigated and non-irrigated grain. By 12 July, *Alternaria* was present on 30% of grain from non-irrigated and 50% grain from irrigated controls. *Cladosporium* was common affecting 60% and 50% of non-irrigated and irrigated controls respectively. *Fusarium culmorum* was found on 10% of grain in the irrigated control on each assessment.

Sooty moulds developed on the ears from late July onwards and all tebuconazole plus triadimenol treatments had conspicuously brighter ears than other treatments on 2 August. By the 7 August, however tebuconazole plus triadimenol treatments could only be differentiated in the non-irrigated blocks.

There were no significant effects of treatment on yield, but significant block effects were noted after some plots ripened prematurely because of drought stress. There were no treatment effects on specific weight or thousand grain weight.

Fungicide timing in relation to infection, ADAS Arthur Rickwood 1995

In 1995, the incidence of *Fusarium* ear blight was low. All treatments had a lower incidence of ear blight than the untreated control, with the percentage control in the range from 45% to 88% (Table 5.5). Black point developed on the grain, and treatments 4 days before irrigation and 6 days after irrigation reduced black point severity. Glume blotch (*Septoria nodorum*) and mildew were controlled by all treatments and sooty moulds by most. There were indications

that sprays applied 2 or 4 days after irrigation were the most effective, and the spray shortly before irrigation was the least effective.

There were no effects of treatments on yield or specific weight, but all treatments except the earliest gave significant increases in thousand grain weight.

Fungicide timing in relation to infection, ADAS Arthur Rickwood 1996

On 10 July (GS 75) inoculated control plots showed 5.3% ears affected with infection largely confined to a single spikelet on each ear. There was significantly lower incidence of ear blight in all treated plots except 4 days pre-inoculation but no differences in the severity per ear. A whole plot assessment of ears showing obvious bleaching of sections of the ear revealed very low numbers of severely affected ears.

The incidence of ear blight almost doubled between 10 and 17 July (GS 77). There were differences in disease severity with tebuconazole plus triadimenol treatments having the lowest number of affected spikelets per affected ear (Table 5.5). A count of the total number of severely blighted ears on 17 July showed very large treatment differences and highly significant control (>95%) by several treatments. Treatments applied 2 days before inoculation up to 7 days after inoculation were most effective. Treatment immediately before inoculation was less effective than those applied 2 days before or 2-7 days after inoculation. Tebuconazole applied at half rate to both sides of the ear appeared to be more effective than a single spray at full rate. Ears ripened quickly after this assessment and further whole plot counts were no longer reliable.

Ear blight continued to increase in incidence and severity up to 24 July (GS 83) (Fig. 5.1). Control plots had 17.8% ears affected and similar control was achieved with treatments applied during the period 2 days before inoculation up to 7 days after inoculation. A treatment applied 6 days before inoculation was not effective. Examination of disease severity on individual ears highlighted more clearly the benefits of treatments applied after inoculation (Fig. 5.2). The lowest incidence of ear blight was found after the two spray programme of tebuconazole plus triadimenol plus anilazine.

There were no significant effects on yield although the difference between the mean yields for inoculated and uninoculated plots (8.54 t ha^{-1} and 8.72 t ha^{-1} respectively) approached statistical significance (P = 0.054). There was an effect of inoculation on thousand grain weight but no effect of fungicide.

Black point development on the grain and treatment differences were not significant on incidence (P = 0.093 for fungicide) or disease index (P = 0.083 for fungicide). The covered control area showed only 10.1% grains with black point (in a 1000 grain sample) which suggested that the irrigation regime had contributed to the high incidence of black point infection.

Sooty moulds developed on the ears in late July and early August. On 31 July (GS 85) plots which had received tebuconazole plus triadimenol plus anilazine at GS 60+71 had the brightest ears (i.e. least sooty mould development). This effect was still obvious on 7 August (GS 92) and there were weak effects from the single tebuconazole plus triadimenol plus anilazine spray. Even at this late stage the more effective treatments showed 90% green leaf area on the top two leaves.

Fungicide timing in relation to infection, ADAS Gleadthorpe 1995 and 1996

In 1995, disease levels were low at Gleadthorpe. There was not a statistically significant effect of inoculation on disease, but all fungicide timings gave significant reductions in both ear blight and sooty moulds (Table 5.6). Although there were no significant differences between fungicide timings for either disease, there were indications that the last spray date was less effective than others against ear blight and that the last two were less effective against sooty moulds. Yields were low, due to the effect of the drought, and there were no significant differences in yield, specific weight or thousand grain weight.

In 1996, *Fusarium* ear blight incidence was even lower than in 1995, but there was a significant further reduction in ear blight from all fungicide timings (Table 5.6). Sooty mould severity was also reduced by all fungicide timings except the earliest. Treatments did not have any significant effect on yield, specific weight or thousand grain weight.

Fungicide timing in relation to infection, ADAS Rosemaund 1994-1996, ADAS Bridgets 1994

In the experiments which relied on natural infection at Rosemaund and Bridgets, there was very little ear disease. *Fusarium* severity was well below 1%, so data are not presented. There was some development of mildew and sooty moulds on the ears at Rosemaund, but virtually no ear disease at Bridgets. At Rosemaund, all tebuconazole treatments and chlorpyrifos reduced ear mildew in each of the three years, but the greatest effects were generally from sprays between 3 and 11 days after the first treatment, i.e. at GS 61-65 (Table 5.7). Sooty moulds in 1995 and 1996 were also reduced by all treatments. Foliar disease at all sites was negligible. There were no significant effects of treatments on yield or grain quality in any of the experiments.

Table 5.2. ADAS Arthur Rickwood 1993: Effect of fungicide treatments on brown rust ,ear blight, black point, yield and specific weight; mean of irrigated and non-irrigated plots.

Treatment	Timing		Brown rust % area (angular transformation)‡	*Fusarium* ear blight - no. affected ears per plot (log transformation)‡	Black point % grains	Yield (t ha^{-1})	Specific weight (kg hl^{-1})
	GS 59 7 June	GS 69 17 June					
Untreated	-	-	3.1 (10.1)	9.2 (2.2)	6.9	9.43	79.0
Chlorothalonil	+	-	4.1 (11.6)	10.7 (2.3)	7.8	9.45	78.8
Mancozeb	+	-	3.6 (10.8)	7.0 (1.8)	7.0	9.24	78.8
Carbendazim + maneb	+	-	3.7 (10.9)	9.2 (2.0)	8.9	9.61	79.3
Carbendazim + flusilazole	+	-	2.6 (9.0)	7.3 (1.9)	7.9	9.46	78.9
Cyproconazole + prochloraz	+	-	1.3 (6.3)	8.2 (1.7)	9.4	9.67	79.3
Tebuconazole + triadimenol	+	-	0.5 (3.0)	6.3 (1.7)	8.2	9.63	79.4
Tebuconazole + triadimenol	+	+	0.1 (1.7)	6.7 (1.7)	10.3	9.85	79.4
Tebuconazole + triadimenol + anilazine	+	-	0.9 (4.8)	6.2 (1.5)	9.6	9.73	79.2
Difenoconazole	+	-	2.6 (9.0)	10.3 (2.3)	8.0	9.59	79.2
Difenoconazole	-	+	1.2 (6.0)	15.0 (2.3)	9.0	9.17	79.1
Iprodione	+	-	3.4 (10.6)	12.3 (1.9)	9.5	9.56	79.2
Iprodione	-	+	3.9 (11.3)	10.0 (2.2)	8.5	9.48	78.9
SED (between fungicides) (48df)			0.90***	0.28	1.37	0.143**	0.19*

‡transformed data analysed

Significant differences ***$P < 0.001$, * $P < 0.05$; other effects not significant.

Table 5.3. ADAS Arthur Rickwood 1994: *Fusarium* ear blight incidence at GS 77, yield and black point.

Treatment	Timing		*Fusarium* ear blight - number of infected ears per plot at GS 77 (square root transformation)‡	Yield (t ha^{-1})	Black point %grains	Black point index
	GS 59	GS 75				
	13 June	6 July	Irrigated	Mean	Irrigated	Irrigated
Untreated	-	-	8.3 (2.9)	8.35	13.6	4.5
Chlorothalonil	+	+	6.3 (2.5)	8.47	11.5	3.6
Mancozeb	+	+	6.3 (2.5)	8.40	13.5	4.8
Carbendazim + maneb	+	+	2.7 (1.6)	8.45	11.2	3.7
Carbendazim + flusilazole	+	+	4.3 (2.1)	8.28	11.9	4.0
Cyproconazole + prochloraz	+	+	3.3 (1.8)	8.29	11.2	3.6
Tebuconazole + triadimenol	+	+	2.7 (1.6)	8.54	9.6	3.4
Tebuconazole + triadimenol (125 + 62.5 g a.i. ha^{-1})	+	+	2.7 (1.6)	8.22	9.6	3.4
Tebuconazole + triadimenol + anilazine	+	+	0.0 (0.0)	8.05	5.5	1.5
Difenoconazole	-	+	4.7 (2.1)	8.22	12.9	4.3
Difenoconazole	-	+$^{+}$	8.0 (2.7)	8.44	11.5	4.0
Iprodione	-	+	6.0 (2.4)	8.47	10.7	3.5
Iprodione	-	+$^{+}$	5.3 (2.2)	8.16	15.1	5.3
Fenpropimorph + propiconazole	+	+	4.0 (1.6)	8.42	11.2	3.9
Cyproconazole	+	+	5.0 (2.0)	8.21	9.4	3.1
Cyproconazole (40 g a.i. ha^{-1})	+	+	3.3 (1.5)	8.01	12.3	4.0
SED (60df)			0.49*	0.24	2.40	0.86

$^{+}$applied 13 July, i.e. 7 days after GS 75

‡transformed data analysed

Significant differences ** $P < 0.01$, * $P < 0.05$; other effects not significant

Table 5.4. ADAS Arthur Rickwood 1995: ear disease and yield

Tebuconazole timing relative to irrigation (days)	*Fusarium* ear blight ears per plot	Black point index	*Septoria nodorum* % ears affected	Sooty moulds % ears affected	Mildew % ear area (angular transform)‡	Yield (t ha^{-1})	Thousand grain weight (g)
Untreated	12.25	2.2	4.25	3.50	2.4 (8.8)	7.12	33.9
-6	6.75	1.7	1.52	1.55	0.4 (3.1)	7.25	35.1
-4	4.50	1.3	2.00	1.08	0.1 (1.4)	7.19	36.4
-3	3.25	1.7	0.65	1.52	0.1 (1.7)	7.21	36.4
0	2.25	2.1	2.03	2.62	0.3 (2.9)	7.07	36.0
+2	3.00	2.0	0.90	0.80	0.2 (2.6)	7.18	36.0
+4	1.50	1.9	0.33	0.35	0.4 (3.7)	7.61	35.6
+6	3.00	1.5	1.90	2.55	0.6 (4.1)	7.12	36.8
SED (21 df)	1.825***	0.27*	0.875**	0.842*	0.82***	0.181	0.70*

‡transformed data analysed

Significant differences *** $P < 0.001$ ** $P < 0.01$, * $P < 0.05$; other effects not significant

Table 5.5. ADAS Arthur Rickwood 1996: ear disease (inoculated plots only) and yield

Fungicide and timing relative to irrigation (days)	*Fusarium* ear blight GS 77 no. spikelets per affected ear	*Fusarium* ear blight GS 77 no. ears per plot (transform)‡	*Fusarium* ear blight GS 83 no. spikelets per affected ear	*Fusarium* ear blight GS 83 % ears per plot	Yield (t ha^{-1})
	Inoculated	Inoculated	Inoculated	Inoculated	Mean
Untreated	2.41	182.7 (2.26)	6.72	17.67	8.65
Tebuconazole + triadimenol -6	3.21	74.7 (1.83)	6.90	12.67	8.65
Tebuconazole + triadimenol -4	1.52	52.3 (1.70)	6.72	9.00	8.55
Tebuconazole + triadimenol -2	1.00	7.0 (0.88)	4.04	4.00	8.64
Tebuconazole + triadimenol 0	1.78	25.7 (1.33)	5.94	5.00	8.56
Tebuconazole + triadimenol +2	1.00	6.0 (0.84)	1.92	7.33	8.67
Tebuconazole + triadimenol +4	1.00	6.3 (0.53)	2.92	3.33	8.51
Tebuconazole + triadimenol +7	1.28	0.3 (0.10)	2.06	4.67	8.67
Tebuconazole + triadimenol +2+	1.17	0.3 (0.10)	3.96	6.37	8.68
Tebuconazole + triadimenol + anilazine +2	0.70	4.3 (0.38)	1.63	4.33	8.73
Tebuconazole + triadimenol + anilazine +2 & GS 71	0.54	4.3 (0.41)	0.74	1.04	8.64
cv (%)	35.2	38.3	45.0	60.4	0.175
SED (20 df)	0.407*	0.295*	1.453*	3.405	2.5

+sprayed twice at 0.5 l ha -1, once from each direction along the plot

‡transformed data (Log_{10} (n+1))analysed

Significant differences, * $P < 0.05$; other effects not significant

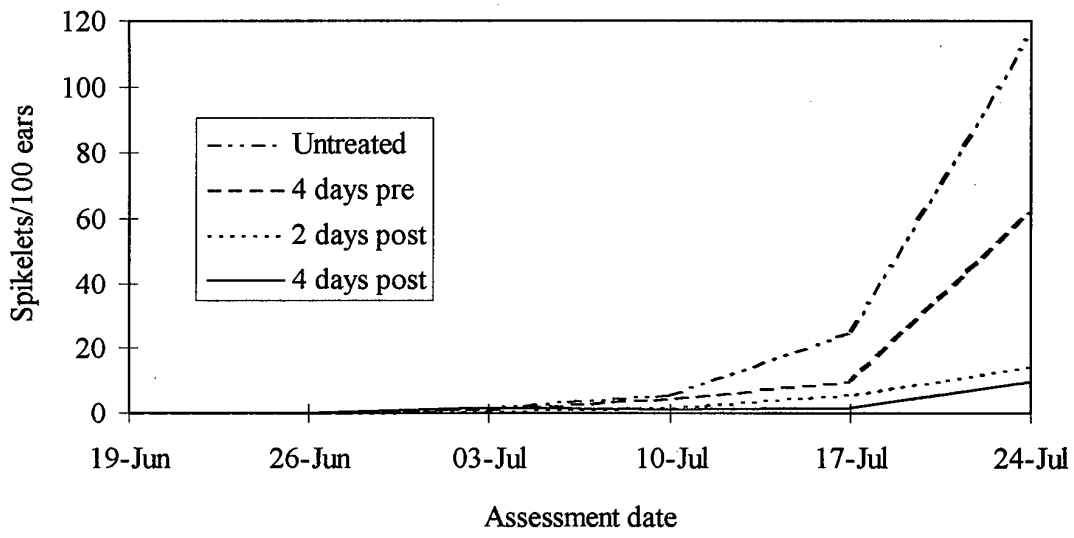

Figure 5.1. Fusarium spikelet infection in untreated control and selected spray treatments

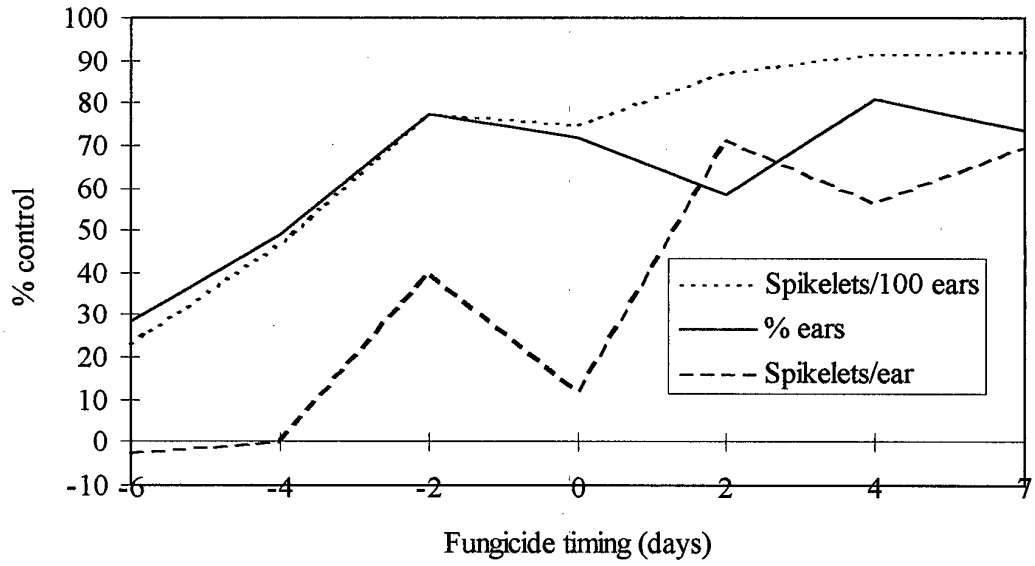

Figure 5.2. Control (%) of ear and spikelet infection caused by Fusarium culmorum with tebuconazole at various timings before and after inoculation (Day 0), 24 June 1996

Table 5.6. ADAS Gleadthorpe 1995 and 1996: ear disease at GS 85 and yield (mean of inoculated and non-inoculated)

Tebuconazole timing relative to irrigation (days)	1995			1996		
	Fusarium ear blight % ear area	Sooty moulds % ear area	Yield (t ha^{-1})	*Fusarium* ear blight % ear area	Sooty moulds % ear area	Yield (t ha^{-1})
Untreated	2.1	9.6	4.11	0.8	3.8	6.13
-6	0.3	1.8	4.10	0.3	2.6	6.24
-4	0.5	2.6	4.03	0.2	1.9	6.19
-3	0.6	2.9	3.91	0.2	1.5	6.19
0	0.6	3.2	4.28	0.4	2.2	6.03
+2	1.0	1.9	4.19	0.3	1.8	6.28
+4	0.8	2.7	4.12	0.3	1.6	6.22
+6	1.4	3.1	4.15	0.3	1.7	6.02
cv (%)	10.5	17.0	8.3	16.5	51.7	7.1
SED (42df)	0.22***	1.19***	0.243	0.11***	0.45***	0.171

Significant differences *** $P < 0.001$; other effects not significant

Table 5.7. ADAS Rosemaund 1994, 1995 and 1996; ADAS Bridgets 1994: ear disease at GS 85 and yield.

Treatment	Rosemaund 1994		Bridgets 1994	Rosemaund 1995			Rosemaund 1996		
	Mildew % ear area	Yield (t ha^{-1})	Yield (t ha^{-1})	Mildew % ear area	Sooty moulds % ear area	Yield (t ha^{-1})	Mildew % ear area	Sooty moulds % ear area	Yield (t ha^{-1})
Untreated	5.8	7.72	10.18	1.5	5.5	7.74	2.0	1.1	7.81
Tebuconazole, day 0	2.1	7.61	10.36	0.5	1.8	8.06	0.7	0.6	7.88
Tebuconazole, day 3/4	1.6	7.74	10.22	0.3	1.3	7.62	0.5	0.3	8.03
Tebuconazole, day 7	1.5	7.85	10.29	0.2	1.1	8.09	0.2	0.3	8.02
Tebuconazole, day 10/11	2.3	7.79	10.18	0.2	0.7	7.88	0.5	0.1	8.13
Tebuconazole, day 14	2.8	7.63	10.02	0.3	1.3	7.98	0.8	0.2	7.95
Tebuconazole, day 17/18	3.9	7.56	10.17	0.7	1.1	8.02	0.7	0.3	7.91
Tebuconazole, day 21	3.0	7.79	10.08	0.7	1.9	7.58	0.9	0.4	8.05
Tebuconazole, day 24/25	3.3	7.46	10.12	0.5	1.9	8.00	1.2	0.5	7.80
Chlorpyrifos, day 0	2.4	8.00	10.15	1.0	2.7	8.22	1.3	0.5	8.09
SED (27 df)	0.66**	0.218	0.228	0.13**	0.69**	0.278	0.21***	0.18***	0.143

Dates of first spray application: Rosemaund 1994 10 June
Bridgets 1994 7 June
Rosemaund 1995 1 June
Rosemaund 1996 14 June

Significant differences *** $P < 0.001$; other effects not significant

Discussion

Fungicide efficacy against *Fusarium* ear blight

The experiments at ADAS Arthur Rickwood in 1993 and 1994 showed that few fungicides had activity against *Fusarium* ear blight, even though the disease pressure was not particularly severe. In these experiments, fungicides were applied shortly before the irrigation which would have provided conditions conducive for infection, so the protectant activity of the fungicides was being tested. In both years, tebuconazole plus triadimenol gave the greatest reduction in disease, particularly when mixed with anilazine. Several other fungicides gave some control of ear blight, but insufficient to suggest that they would be of commercial interest. Following discussions with the manufacturer, Bayer, it was decided that tebuconazole alone should be used in later experiments, since it was thought to be unlikely that the triadimenol component was contributing much to *Fusarium* control.

In the later experiments on fungicide timing in relation to infection, the efficacy of tebuconazole was demonstrated further. Although it did not give complete control, the effect was sufficient to indicate that it might be commercially useful, provided that the risk of disease could be assessed and the fungicide timed accurately. Tebuconazole was generally more effective when applied shortly after the infection event than when applied before and, in 1996, there was evidence from Arthur Rickwood that there was good activity even when applied seven days after infection. This finding offers the prospect that, if periods of high infection risk can be identified, then fungicidal control may be possible within the following week. Further work is needed under conditions of greater disease severity to confirm whether this is a practicable control strategy, and the period within which tebuconazole can give worthwhile control needs to be determined more precisely.

The greater activity of tebuconazole as an eradicant than as a protectant fungicide suggests that it may be worth re-examining some of the other azole fungicides for short-term eradicant activity. From the work reported above, cyproconazole would appear to be the most likely candidate, although others such as difenoconazole may be worth investigating for eradicant activity. In addition, epoxiconazole has become available since this work was undertaken, and several new azole and other fungicides are likely to become available within the next few years. Of particular interest are the strobilurin analogues, kresoxim-methyl (from BASF) and azoxystrobin (from Zeneca), since these fungicides have already been shown to have very long-term foliar disease effects and the possibility of using them for ear disease control is worth invesigating. However, these fungicides are primarily protectant in their activity, so may be less flexible in use against ear blight than tebuconazole or any other azole which has eradicant activity.

The improvement in disease control from using tebuconazole plus triadimenol plus anilazine compared with tebuconazoel alone, at Arthur Rickwood in 1996, merits further investigation, as does the finding that spraying at half rate from each direction improved disease control. This latter result suggests that part of the problem in controlling ear blight with fungicides is in achieving adequate coverage of the ears with fungicides which are unlikely to show much, if any, systemic movement within the ears.

In view of the very dry weather in mid and late June in each of the three years of the study, it is not surprising that no useful results on *Fusarium* ear blight were obtained from the experiments at ADAS Rosemaund and Bridgets which relied on natural infection. The only conclusion that can be drawn is that, under dry conditions and with foliar disease already

controlled, there was no yield benefit from using tebuconazole at or after ear emergence. These experiments were on varietes with relatively good resistance to the major foliar diseases, in years when the weather was hot and dry in mid and late June. From previous work on yield responses to fungicides applied at different growth stages (Cook & Thomas, 1990; Cook *et al.*, 1995), such conditions would be expected to result in small yield effects from a fungicide applied at GS 59.

The overall conclusion from this work is that it may be possible to achieve commercially acceptable control of *Fusarium* ear blight using tebuconazole during anthesis, but that the benefits from such a treatment are likely to be small or nil if ear disease does not develop. This highlights the need to predict ear blight, and to understand the eradicant properties of fungicides, so that treatments can be applied at the correct time to those crops where treatment is likely to be beneficial.

Fungicide efficacy against black point

Control of black point was achieved with tebuconazole plus triadimenol plus anilazine, and is thought to be the first successful control with fungicides in the UK. Trends in the data suggested small effects from tebuconazole plus triadimenol, but the main activity appears to have come from anilazine. Cyproconazole (in Sportak Delta and Alto) also merits further investigation for black point control activity, since the black point scores for both products were among the lowest recorded. GS 75 and GS 75 + 7 days timing with difenoconazole or iprodione were unsuccessful. Whilst these products have known activity against *Alternaria* spp., multiple rather than the single spray treatments may be required for black point control.

Black point incidence was substantially increased (approximately 3 fold) by irrigation in 1994 which contrasted with a small, but significant, increase from 8% to 9% in 1993. This may be attributed to the use of 2 successive days with irrigation and the rather late timing (*c.* GS 77) in mid July which were used in 1994. Black point developed to a similar degree overall in both 1993 and 1994, despite different rainfall patterns, but it was less common in non-irrigated plots in 1994 than in 1993. In 1994, there was less frequent rainfall in mid June and again in mid-late July than in 1993. Irrigation was applied during both these periods in 1994. However, there was rather more rain in late June/early July in 1994 than in 1993 but this did not greatly favour black point which affected only 3.5% of grain in non-irrigated plots. When disease assessments were made on 11 July some plots were already senescing with drought stress and these failed to respond to irrigation in terms of higher black point incidence. This could indicate that grain was beyond the vulnerable stage at which rain or high humidity can promote black point development. Circumstantially, this points to early or mid-July as the critical time for black point development.

Since treatment for black point control has to be applied even later than for *Fusarium* ear blight control, it is unlikely that the fungicides applied at this stage could have any beneficial effect on crop yield. Clearly, it is important to identify crops where there is a significant risk of black point if treatment is to be attempted. Further field data are required to identify more clearly the period and conditions which favour black point development.

GENERAL CONCLUSIONS

The experiments on yield losses associated with *Fusarium* ear blight at Harper Adams and CSL confirmed that ear blight does have a significant effect on yield. Although the methods of disease assessment differed between sites (the percentage of infected spikelets was counted at Harper Adams, and the percentage area affected was assessed visually at CSL), the relationships between disease and yield were similar at the two sites. The yield losses that would result from 25% infection of ears were estimated to be in the range 10-15%. The loss in yield was due almost entirely to reduction in thousand grain weight, so grain quality was affected in addition to the reduction in yield. Other aspects of grain quality (such as grain nitrogen content and effects on breadmaking quality) were outside the scope of the present study. Potential for mycotoxin production on infected grain is clearly another important grain quality issue. The analyses of samples by CSL showed that mycotoxins were produced but that, in all but one of the samples, the levels were below the thresholds set for human or animal consumption. However, mycotoxin production is a complex issue in which ear blight severity is just one component. Crop lodging, which increases the risk of direct contamination of grain by soil-inhabiting pathogens, and grain storage conditions, particularly temperature and humidity, also have a profound influence on mycotoxin production. With increasing concern about food safety issues, an understanding of factors which influence mycotoxin production is clearly an important research need.

The disease survey data in Section 4 confirm that the incidence of *Fusarium* ear blight in the UK is extremely erratic, with some years in which the disease is common and severe, but others in which it is of negligible importance. This leads to the question of whether the severe outbreaks can be predicted and action taken to prevent them. The analysis of survey data highlighted the importance of rainfall in June, and indicated that there may be important effects of the weather earlier in the year. The epidemiological studies at CSL also showed the importance of high humidity for most of the *Fusarium* species which are important for ear blight (although *F. poae* was favoured by lower humidity). However, even in years in which *Fusarium* ear blight is widespread, there is great variation in disease severity from field to field within one locality as well as on a larger geographical scale. This could be due to several factors, including (1) local microclimatic variation, (2) differences in varietal susceptibility, (3) differences in growth stage between varieties and between crops of the same variety sown on different dates resulting in only a proportion of crops being at the critical growth stage for infection when weather conditions are suitable, and (4) differences between crops in the amount of inoculum available. Of these, information on varietal susceptibility is available from NIAB. The critical growth stage for infection has been shown previously to be early anthesis (Parry *et al.*, 1995). Environmental conditions, particularly rainfall and subsequent duration of high humidity, at early anthesis are clearly of fundamental importance to epidemic development, particularly in relation to dispersal of conidia from infected stem-bases to the ears. It is generally considered that inoculum is rarely limiting, since *Fusarium* spp. are common pathogens of cereal stem-bases. Parry *et al.* (1995) considered that sporulation on stem-bases was encouraged by dry conditions in May and early June, but there is little quantitative information on whether inoculum can be a limiting factor in *Fusarium* ear blight epidemics.

The possibility that *Fusarium* spp. may infect ears by systemic growth from infected stem-bases cannot be discounted. However, the detailed study of systemic development of the pathogen within the present study failed to show any evidence of systemic infection. Until clear evidence of systemic infection is produced, it can be concluded that it is unlikely to be a

significant factor in the epidemiology of the disease, and that splash-dispersal of spores is the key mechanism for ear infection.

From a practical consideration, improvements in ear blight prediction are of benefit to the industry only if they allow control measures to be implemented. The fungicide evaluation at ADAS Arthur Rickwood in 1993 and 1994 confirmed previous findings that few fungicides have useful activity against ear blight, but the performance of tebuconazole was encouraging. The later work on fungicide timing indicated that tebuconazole is more effective as an eradicant than as a protectant, and may have activity when applied up to 7 days after infection. However, tebuconazole applied after ear emergence would be unlikely to make a significant contribution to foliar disease control and, in the experiments at ADAS Rosemaund and Bridgets in the present study in which ear disease was negligible, there were no statistically significant effects on yield of tebuconazole applied from GS 57 onwards. This emphasises the importance of being able to predict where such treatments are likely to be of economic benefit.

Several new fungicides are under development for the UK cereal market. The efficacy of these against foliar pathogens of cereals is under investigation in HGCA project 0027/01/95. Of particular interest are the strobilurin analogues kresoxim-methyl and azoxystrobin, which have been found to have long-lasting effects on foliar diseases. The possibility that these fungicides, when applied in the period when they will be most beneficial against foliar diseases (i.e. up to GS 39), may have some effect on ear diseases is worth investigating at sites where irrigation is available to provide conditions conducive to *Fusarium* ear blight.

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