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THE IMPORTANCE AND CONTROL OF STEM-BASE DISEASES OF CEREALS

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THE IMPORTANCE AND CONTROL OF STEM-BASE DISEASES OF CEREALS

by

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This is the final report of a four year project which started in September 1992. The work was co-ordinated by Dr D J Royle, IACR-Long Ashton Research Station, in collaboration with Dr D W Hollomon, IACR-Long Ashton, Dr R A Bayles, NIAB, Cambridge, Dr M W Hims, Central Science Laboratory, Harpenden, Dr S Oxley, SAC-Edinburgh, Dr D R Jones, ADAS Rosemaund, Mr R J Cook, Morley Research Centre, Norfolk, Dr D W Parry, Harper Adams Agricultural College, Newport, Shropshire and Dr P Nicholson, John Innes Centre, Norwich. The work was funded by a grant of £486,644 from the Home-Grown Cereals Authority (Project no. 0015/1/91).

CONTENTS

SUMMARY

Eyespot

1. Immunodiagnostics, used to chart eyespot epidemics in different wheat cultivars over a four-year period, confirmed that diagnostic kits provide a simple, robust and reproducible way to monitor progress of this disease quantitatively.

2. Eyespot antigen levels varied between cultivars but once eyespot symptoms appeared, antigen levels correlated well with the disease levels assessed visually. Antigen levels provided a better measure of eyespot severity than visual assessment and exposed variation in factors affecting eyespot development which were not apparent from the visual assessments.

3. Immunodiagnostics detected eyespot before symptoms appeared. Antigen levels in the eyespot-resistant Rendezvous were often similar or even greater than in susceptible cultivars. Resistance to eyespot in Rendezvous appears to be expressed not by blocking infection, but by delaying and suppressing symptom expression.

4. Throughout the field experiments at IACR-Long Ashton and NIAB, uninoculated plots had high levels of eyespot, even in first wheats, indicating that the eyespot pathogen is considerably more mobile than was previously thought. Wind-blown ascospores of the recently-discovered sexual stage, *Tapesia yallundae*, may initiate eyespot and explain the widespread and uniform distribution of antigen units throughout the crops. First-wheat crops, previously considered to be of low risk from eyespot attack, therefore need to be monitored carefully, and where early infections are detected in susceptible cultivars, fungicides should be applied.

5. As a consequence of the occurrence of eyespot in uninoculated plots, attempts to manipulate eyespot levels in the Long Ashton experiments was only partially successful. Although the Rye (R)-pathotype dominated in plots inoculated with either the R- or the Wheat (W)-pathotype, the W-pathotype appeared to be more invasive and damaged crops earlier than the R-pathotype.

6. Correlations between antigen levels and either final disease levels or yield were erratic and dependent on cultivar. Occasionally, antigen levels at GS24-39 were related to yield loss and a threshold level of disease could be suggested. However, this was not normally the case and no obvious spray threshold based on either visually-determined symptoms or antigen levels could be proposed.

7. The experiments at NIAB showed that symptoms produced by both W- and R-pathotypes can become severe by the time of grain-filling. However, a given level of symptoms caused by R-type eyespot may be less damaging to yield than the same level caused by W-type eyespot. The implication is that eyespot-related yield losses may be lower today, with an eyespot population dominated by the R-type, than they were before 1980, when the population was almost entirely W-type. Disease/yield loss relationships derived from W-type eyespot need re-defining for R-type.

8. In some cases of severe eyespot, there may be little or no response to eyespot control by fungicides. This underlines the difficulty of predicting whether fungicide application is likely to be cost-effective.

9. Cultivars rank similarly for resistance to eyespot, whether infection is caused by W- or R-type. A single rating should be adequate to describe a cultivar's resistance to either pathotype and a grower does not need to be aware of the composition of the eyespot population in a field before choosing a suitable cultivar.

10. The resistance of cultivars to W-type infection, as indicated by DNA-diagnostics, was in close agreement with symptom expression. However, PCR detected higher levels of R-type eyespot in the two most resistant cultivars than would have been expected by their low symptom expression. PCR also detected R-type eyespot in all cultivars in sprayed plots which were without symptoms.

11. In the experiments described in section 1.3 DNA-diagnostics appeared to be able to confirm with reasonable accuracy the presence of pre-symptomatic eyespot and to quantify W- and R-type infections. However, there is still no accurate, early-disease threshold upon which to predict the degree of risk for individual crops.

12. Overall, prochloraz gave better yield maintenance, associated with reductions in the eyespot index and, where it occurred, lodging, compared to flusilazole and cyprodinil.

13. The optimum time for a single treatment varied markedly across the 14 site/year combinations studied. At some sites, especially Markle Mains, (the same location for four years), it was possible to distinguish the mid-tiller (GS23-27) stage and the first node (GS31) as the best times for fungicide application, numerically but not statistically. This was possible at none of the other sites. At Markle Mains, the optimum times for a split treatment were early tillering (GS23-25), then up to first node (GS20-31) but not GS30 nor GS32. The best timing for application of prochloraz thus remains uncertain.

2. Fusarium

1. Eleven field experiments, over three years and at five sites, were carried out to determine the effect of fusarium on grain yield and quality, and to establish the timing of any suitable fungicide application for disease control. The experiments depended upon natural development of *Fusarium* spp. and *Microdochium nivale* and the efficacy of the Ciba Geigy fungicides fenpiclonil (applied as a seed treatment) and fludioxonil (a protectant fungicide, applied in programmes of foliar sprays) to generate different development patterns and amounts of fusarium.

2. Both nodal and internodal fusarium symptoms developed severely in only a few experiments; levels were mostly moderate or slight. In each year, *M. nivale* was the predominant species encountered during the winter and spring, but *F. culmorum* became prevalent by GS75 at most sites.

3. Fludioxonil gave some control of fusarium though it was unreliable: spray

programmes starting any time up to April gave variable control of nodal fusarium, from 75% to almost none, and was also variable but much less effective on internodal fusarium. Fenpiclonil, applied as a seed treatment, had little, if any, effect on stem-base fusarium.

4. There was a relationship between crop yield and nodal fusarium only in one experiment, at Morley in year 2, where fusarium levels were only moderate. At some other sites treatments reduced fusarium levels but without any effect on yield. Yield increases occurred in four experiments that were unrelated to fusarium control.

5. Regressions overall, and from the Morley experiment in year 2, suggest that there may be a relationship between stem-base fusarium, especially nodal infection, and yield. However, greater confidence in this relationship requires more sites with severe fusarium and fungicides with greater efficacy, which are not currently available.

6. To parallel the field approach, investigations were carried out in years 2-4 using a partially-controllable experimental system in which fusarium levels in wheat, grown outdoors in plastic containers, were varied by applying different quantities and timings of *M. nivale* and *F. culmorum* inoculum and, in year 2 only, by applications of fludioxanil. This system allowed for good development of stem-based fusarium and meaningful yields.

7. Disease levels at GS31 and GS75 increased with increasing amounts of *M. nivale* and *F. culmorum* inoculum applied. Yields were reduced by up to 45% after application of inoculum of these pathogens separately and in mixture.

8. In 1994, disease levels and yield loss were greater after inoculation in March than in April and May, whilst in 1995 no effect of inoculation timing was observed.

9. Whilst the results of DNA diagnostics, used in the 1995 experiment, broadly confirmed the effects of inoculum amounts and timing on disease levels, they could not be related to yields, possibly because the analysis was performed only at two growth stages.

GENERAL INTRODUCTION AND AIMS

On the basis of ADAS surveys, eyespot (*Pseudocercosporella herpotrichoides*) has consistently been regarded as among the most damaging fungal diseases of cereals. However, judgment of its precise importance has been impaired because of the presence at the stem base of other pathogens, notably several *Fusarium* or related species and *Rhizoctonia cerealis* (sharp eyespot), with which the eyespot pathogen interacts in unknown ways. This leads to difficulties in both diagnosis and in estimating eyespot intensities at critical stages of growth of the pathogen and crop.

Since the first indication of resistance to mbc-fungicides in UK populations of the eyespot fungus in 1981 (Brown, Taylor & Epton, 1984), the structure of the eyespot population has changed markedly. Surveys during 1983-1988 indicated that the so-called rye (R) pathotype had become predominant in populations in which there was a high level of resistance to mbc. Where the wheat (W) pathotype occurred together with mbc-resistance, this pathotype represented only 20-30% of the eyespot population. As far as we know, this is the situation at the present day.

The criteria which relate eyespot severity to crop loss, and the ADAS spray threshold criteria of 20% plants/tillers severely affected with eyespot between GS30-37, were both derived when the W-pathotype predominated. Evidence has been accumulating which suggests that differences in the epidemiology of the R- and W-types may invalidate these criteria for populations now dominated by the R-type. For example, there is evidence that eyespot lesions often develop later during the grain-filling period than before. Such late development may have reduced the amount of crop loss caused by eyespot. In addition, the criteria themselves are insufficiently explicit to be able to suggest the best timing of fungicides for eyespot control. They are likely to be influenced by the disease resistance characteristics of particular cultivars and it is important that fungicide regimes should be tailored accordingly. Much of the difficulty in improving this situation in recent years has occurred because of confused diagnosis and poor quality measurement of eyespot which occurs especially when it is in the frequent presence of fusarium and sharp eyespot.

Modern immunological techniques based on ELISA (enzyme-linked immunosorbent assay) were developed in the late 1980s to a state where they allow a different approach to be applied to this problem. These techniques have been used to identify accurately *P. herpotrichoides* without cross-reactivity towards other important cereal pathogens, and are being augmented by "user-friendly" assays. The eyespot pathogen can be detected as early as tillering, before symptoms appear, and in stems where fusarium and sharp eyespot symptoms otherwise mask eyespot. Experience gained from field testing in France, Germany and the UK during 1989-90 suggests that immunology may provide quantitative measurements of the eyespot pathogen present in crops, and has the potential to contribute towards identifying the need for eyespot control measures (Smith *et al.*, 1990). However, regional differences in the nature and development of the stem-base disease complex in cereals suggest that when to sample, and any threshold values derived from ELISA, will need to be developed specifically for the UK, if growers are to benefit from this technology.

Damaging eyespot occurs widely in northern France, and a good correlation has been

observed between pre-symptomatic, immunodiagnostic levels measured at tillering, and final disease levels. Some success has been achieved, therefore, in identifying crops that would benefit from an early, well-timed eyespot treatment. In Bavaria, eyespot develops later during crop growth, and ELISA has been directed towards identifying where fungicide applications may be delayed or even omitted. In the UK, the stem-base disease complex is more significant, and the presence of fusarium often masks eyespot symptoms. Results suggest that immunology may have the potential to improve eyespot control in the UK if threshold values and sample timing can be identified.

Present immunological methods do not distinguish between R- and W-pathotypes of the eyespot fungus. However, where potted plants are inoculated with known pathotypes, ELISA has demonstrated that R- and W-pathotypes penetrate the stem tissue at different rates. If this can be shown also to be true in field situations then it should help to quantify factors that influence the epidemiology of R- and W-pathotypes and perhaps explain why fungicides, such as prochloraz and flusilazole, differ in their ability to control the two pathotypes.

During the early stages of this project, separate work at John Innes identified a number of DNA markers for the major pathogen components of the stem-base complex. These were developed to produce primer sequences for use in the polymerase chain reaction (PCR) which enables sensitive detection and identification of the pathogens present in individual plants. Sequences were developed to detect the eyespot pathogen and to identify W- and R-pathotypes directly in extracts from plant tissue. These methods were introduced to the project in year 4 when greater emphasis was given to examining the opportunity which a number of detection technologies might provide to fulfil the objectives listed below.

Surveys funded by the Authority (1989-90) have identified the range of *Fusarium* and related species present on stem bases, ears and grain of UK winter wheat. They have also shown that *F. nivale* is the most common coloniser of stem bases. Other species are, however, often present also and may dominate in years with different climatic conditions. Whilst the surveys provided valuable data on incidence and frequency of species, they provided no information to indicate the impact and significance of fusarium on yield and quality of wheat in the UK.

Aims

This project addressed three general objectives:

1. To investigate the use of immunology based on ELISA for providing estimates of eyespot in stem bases that would allow thresholds of disease for fungicide application at GS31 to be defined.

2. To establish novel field experiments (a) to investigate relationships between progress of eyespot symptoms, fungicide and yield loss, and (b) to look for evidence of cultivar x pathotype interactions. In view of the previous evidence for a shift in predominance from W- to R-pathotype in eyespot populations and likely differences in the epidemiology of the

two pathotypes, previous estimates of spray thresholds may not be valid.

3. To investigate the relationships between disease levels and yield loss in order to establish whether the widespread occurrence of stem-base fusarium presents a problem in UK wheat production. At the outset new fungicides became available which were considered to be active against fusarium and could be used to manipulate levels of *Fusarium* species.

1. EYESPOT

1.1 ESTIMATION BY IMMUNOLOGY

D.W. Hollomon, H. Baggett and D.J. Royle (IACR-Long Ashton)

1.1.1 Introduction

The main aim of the contribution by IACR-Long Ashton was to determine whether ELISA-based immunology was able to quantify eyespot in wheat plants more accurately than visual assessments. Current spray recommendations for eyespot are based on visual symptom expression and the field experiments carried out at IACR-Long Ashton and NIAB investigated the potential of more accurate spray thresholds, based on immunology. The specific aims of these field experiments were:
(i) To monitor the progression of R- and W-pathotypes in susceptible and resistant winter wheat cultivars.
(ii) To compare visual and immunological assessment techniques.
(iii) To investigate the extent of correlation between visual and immunological assessments and yields in order to establish the relationships between disease levels and yield loss.
(iv) To generate different yields by manipulating disease levels with supplemented eyespot inoculum applied to cultivars with different degrees of susceptibility, and to investigate the effects of fungicides.
(v) To compare correlations between visual and immunological measurements of disease, early and late in the season, and with yield, in an attempt to define spray thresholds for eyespot control.

1.1.2 Materials and methods

IACR-Long Ashton experiments: In the first field experiment, in 1992, Apollo was the susceptible cultivar (NIAB rating 5), but due to its susceptibility to mildew it was replaced by Hereward (also NIAB 5) in the following years' experiments. In 1992 and 1993, Rendezvous (NIAB 8) was included as a resistant cultivar. However, in subsequent experiments only one cultivar was used in order to enable the treatments to include a prophylactic application of prochloraz, (Sportak 45, at full-rate), at GS13, in an attempt to reduce high background eyespot antigen levels.

In 1992, 93 and 94 some plots were inoculated with either the R- or the W-pathotype. In 1995, inoculations were made with a mixture of these pathotypes. Prochloraz (Sportak 45) and flusilazole (Sanction) were applied at full field dose rates in the spring of each year with additional pre-treatments of prochloraz applied in the winter in 1994 and 1995. The growth stages (Zadoks *et al.*, 1974) at which inoculum or fungicide was applied each year were:
1992: inoculated at GS12, treated with flusilazole and prochloraz at GS37-39
1993: inoculated at GS21, treated with flusilazole and prochloraz at GS31
1994: inoculated at GS21, pre-treated with prochloraz at GS13, then treated with flusilazole and prochloraz at GS33

1995: inoculated at GS21, pre-treated with prochloraz at GS13, then treated with flusilzole, flusilazole + silwet and prochloraz at GS31

Samples of 30 plants were randomly selected at several growth stages throughout the season, the main tiller (young plants), or main stem (older plants), cut to 4cm and the roots removed. Each stem was assessed visually for eyespot to generate an Eyespot Severity Index - ESI, (Scott & Hollins, 1974), before being crushed in buffer for ELISA analysis to determine the antigen content as Eyespot Antigen Units (EAU). Samples of plants were taken at the following growth stages.
1992: GS22-24, GS37-39 (before fungicides applied) and GS80-85
1993: GS24, GS30-31 (before fungicide application), GS37, GS65-69 and GS80-85
1994: GS24, GS30-31, GS37, GS65 and GS80
1995: GS21, GS23, GS30, GS39, GS65 and GS92

Isolations on agar media were made from lesions on additional stems gathered at the final sampling stage each year. The colonies obtained were classed according to their growth on potato dextrose agar as rapid growth with even-edged colonies (W-type), slow growth with feathery-edged colonies (R-type).

Samples from NIAB experiments: In 1992, 1993 and 1994 the cultivars Beaver, Riband, Talon and Rendezvous were used. In 1995, Beaver and Rendezvous were replaced by Lynx and Andante (*see Section 1.2 for details* and prochloraz (Sportak 45 at full field rate) was applied to uninoculated plots in an attempt to reduce the high levels of background antigen levels which had occurred in previous experiments. Half of the uninoculated plots were sprayed in 1994 and all uninoculated plots were sprayed in 1995. Plant-stem samples were sent to IACR Long Ashton for immunological evaluation at the following growth stages:
1992: GS22, GS30-31, GS37 and GS75 (5 stems per plot)
1993: GS22, GS30-31, GS37-39 and GS80 (20 stems per plot)
1994: GS20, GS30, GS37, GS65 and GS80 (20 stems per plot)
1995 GS22, GS30, GS37, GS65 and GS80 (20 stems per plot)

At Long Ashton,, the stems were both visually assessed and analyzed via ELISA. Yield data was provided by NIAB each year to enable correlations to be investigated.

1.1.3 Results

The progression of eyespot epidemics was monitored visually and immunologically, and the effects of both pathotype and fungicide on disease levels and yields were investigated. Correlations were made to compare the accuracy of visual symptoms and antigen levels in quantifying eyespot. Correlations between early levels of disease and disease levels at the final sampling explored the potential of early disease levels as predictive tools and determined whether visual symptoms or antigen levels gave the best estimates of final disease levels. Correlations with yields showed the effects of a range of disease levels in each field experiment. Comparing fungicide-treated and non-treated plots (LARS only) provided an opportunity to determine thresholds for fungicide application to control eyespot.

1.1.3.1 Eyespot development each year

At LARS, weather conditions were conducive for eyespot development and eyespot antigen was detected pre-symptomatically from GS21-24. Visual symptoms were present on all cultivars by GS24, except on Hereward in 1994 and 1995 when symptoms failed to appear before GS37.

Eyespot antigen and visual symptoms were detected by GS22 in all cultivars used in the NIAB field experiments from 1992 to 1994. In 1995, symptoms appeared by GS30 on all cultivars except Lynx on which symptoms did not appear until GS37. In the NIAB samples it was not possible to ascertain if eyespot antigen was present before GS22 and prior to the expression of visible symptoms.

Eyespot symptom severity and antigen levels increased steadily in all cultivars as the season progressed. Any temporary reduction in antigen levels in the absence of fungicide treatment could be attributed to the loss of leaf sheaths.

1.1.3.2 The effect of cultivar on disease levels

At LARS, antigen levels and visual symptoms on Apollo were consistently greater than on Rendezvous and, in 1993, visual symptoms on Hereward were also greater than on the resistant cultivar. However, antigen levels revealed that eyespot levels were much greater in Rendezvous at early growth stages than was suggested by visual symptoms.

The NIAB experiments revealed no consistent cultivar effects, with disease levels between cultivars varying according to growth stage and with each separate eyespot epidemic created from 1992 to 1995. Trends were apparent from the analysis of final disease levels and, in Rendezvous, both antigen levels and visual symptoms were much less compared to levels in the more eyespot-susceptible cultivars. From 1992 to 1994, Talon had the greatest antigen levels at the final sampling date but visual symptoms on this cultivar were much less severe than was suggested by the level of antigen present. Riband and Beaver appeared to express eyespot symptoms more readily than either Talon or Rendezvous. In 1995, Talon had the greatest antigen levels and visual symptoms whereas Lynx showed the least disease. In general, the expression of eyespot symptoms showed greater variability between cultivars than did antigen levels.

1.1.3.3 The effect of pathotype on disease levels

The major finding of both the LARS and NIAB experiments was the presence of very high levels of naturally occurring eyespot in uninoculated plots. It was not possible to determine the pathotype constitution of the background inoculum for the NIAB experiment, although in the later experiments, background levels were reduced substantially by an early application of prochloraz during the winter, 1994 and 1995.

At LARS, in 1992 and 1993, the R-type predominated in the background inoculum. In 1994, despite a prophylactic application of prochloraz at GS13, background inoculum remained at a high level and was composed of a mixture of both R- and W-types. These observations indicate that pathotype effects on disease levels must be viewed with caution,

although the effect of the plots of Apollo, Hereward and Rendezvous inoculated with the W-pathotype could be investigated during 1992 and 1993.

In Rendezvous, there were no effects of pathotype on visual eyespot but infection generated from the W-type produced the greatest antigen levels in this cultivar. In Apollo, visual symptoms and antigen levels generated from the W-type were significantly greater than those generated from the R-type. There were no effects of pathotype on eyespot symptoms in Hereward in 1993, but antigen levels revealed that infection had been greatest in response to the W-type. In 1994, the effects of pathotype were more difficult to determine but it appeared that R-type infection had produced the most disease in this cultivar by the end of the season.

At NIAB, the effects of pathotype varied with the different eyespot epidemic created each year. In 1992 and 1994, all eyespot-susceptible cultivars showed more disease, (antigen levels and visual symptoms), in response to W- than R-type infection. In 1993, all susceptible cultivars had more disease in response to the R-pathotype (Fig. 1). Rendezvous, although much less affected by pathotype than the more susceptible cultivars, showed similar responses to pathotype. In 1995, the effects of pathotype seen at early growth stages were much reduced by GS80 but by this time the W-type still produced the most disease (Fig. 2).

1.1.3.4 The effects of fungicide on disease levels

Fungicides consistently reduced visual symptoms and antigen levels in Apollo, Hereward and Rendezvous. Prochloraz, applied from GS31-GS37, was the most effective. The effects of fungicide in Apollo and Rendezvous in 1992 are shown in Fig. 3. A prophylactic application of prochloraz to Hereward at GS13 reduced disease levels early in the season, and in uninoculated plots this treatment was more effective against the background W-types than the R-types. The benefits of a prophylactic application of prochloraz to Hereward were very variable however: In 1994, early treatment improved the efficacy of a second prochloraz application applied at GS33 and this fungicide combination provided the best control of eyespot antigen levels seen this year (Fig. 4). In 1995, the effects of prochloraz at GS13 were much reduced and a single application of prochloraz at GS31 gave the most effective control of eyespot antigen levels (Fig. 5).

1.1.3.5 Treatment effects on yields

At LARS, lodging in Apollo and Rendezvous in 1992 was responsible for extensive yield losses and benefits from the application of fungicides were seen in Apollo only. Prochloraz raised yields more effectively than flusilazole in this cultivar (Fig. 6).
Yields of Rendezvous were, as expected, consistently less than those of either Apollo or Hereward. In 1993, when no crop lodging occurred, fungicides applied at GS31 raised yields in all plots of Rendezvous whereas no benefits were seen from applying any fungicide to Hereward in 1993, 1994 or 1995.

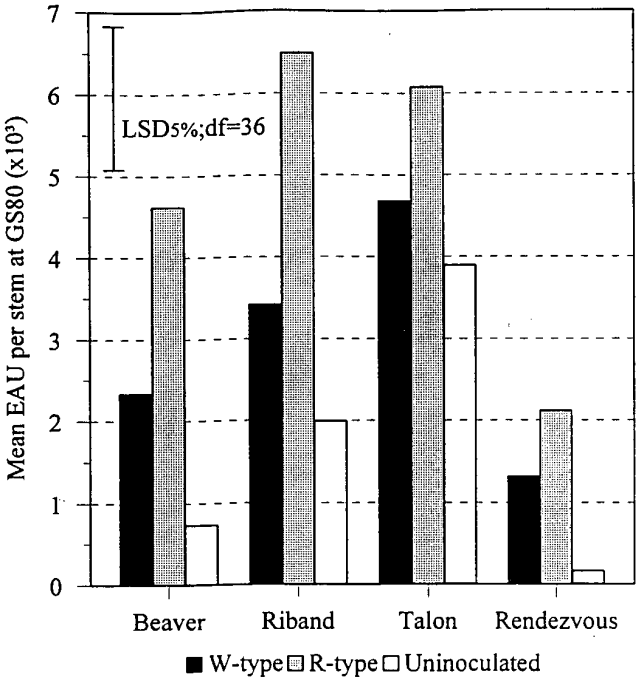

Fig. 1. The effect of pathotype on eyespot in four winter wheat cultivars at NIAB in 1993

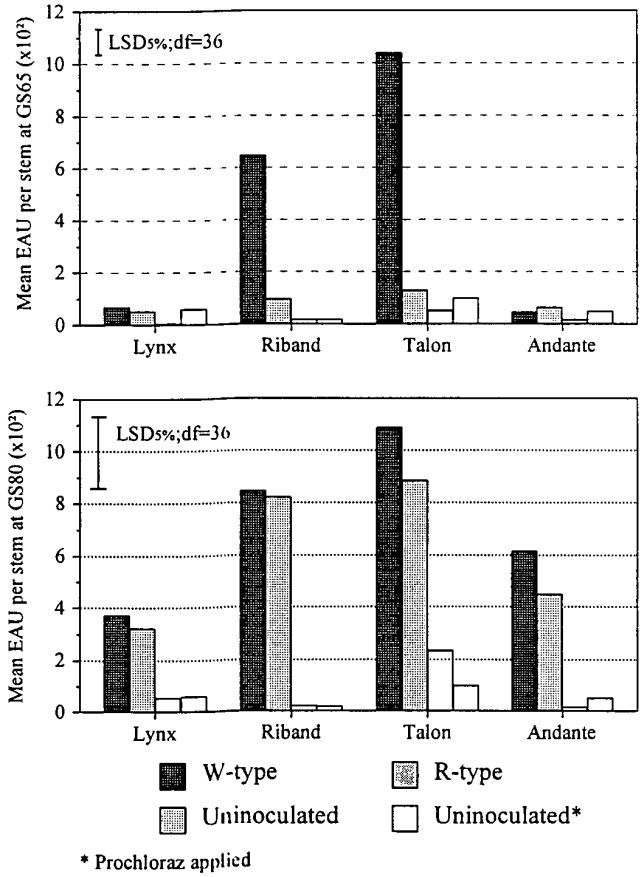

Fig. 2. The effect of pathotype on eyespot in four winter wheat cultivars at NIAB in 1995

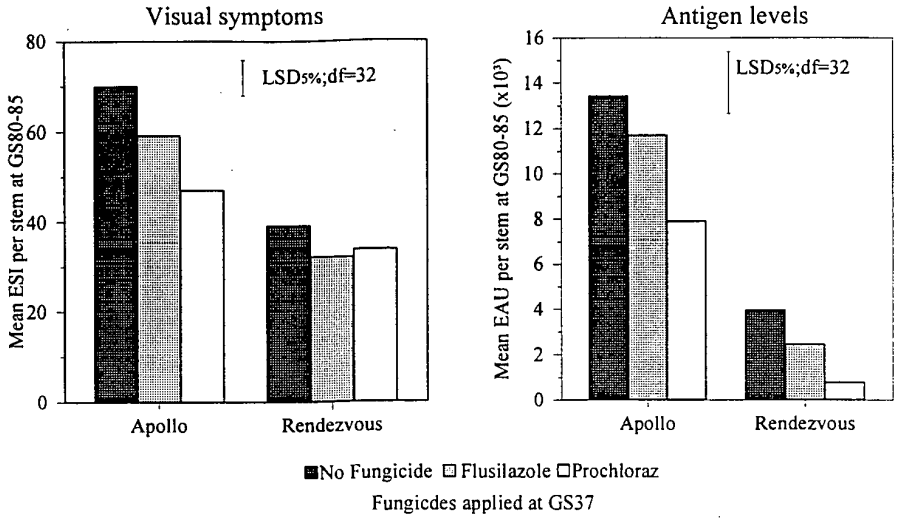

Fig. 3. The effect of fungicides on eyespot levels in Apollo Rendezvous at GS80-85 at LARS in 1992

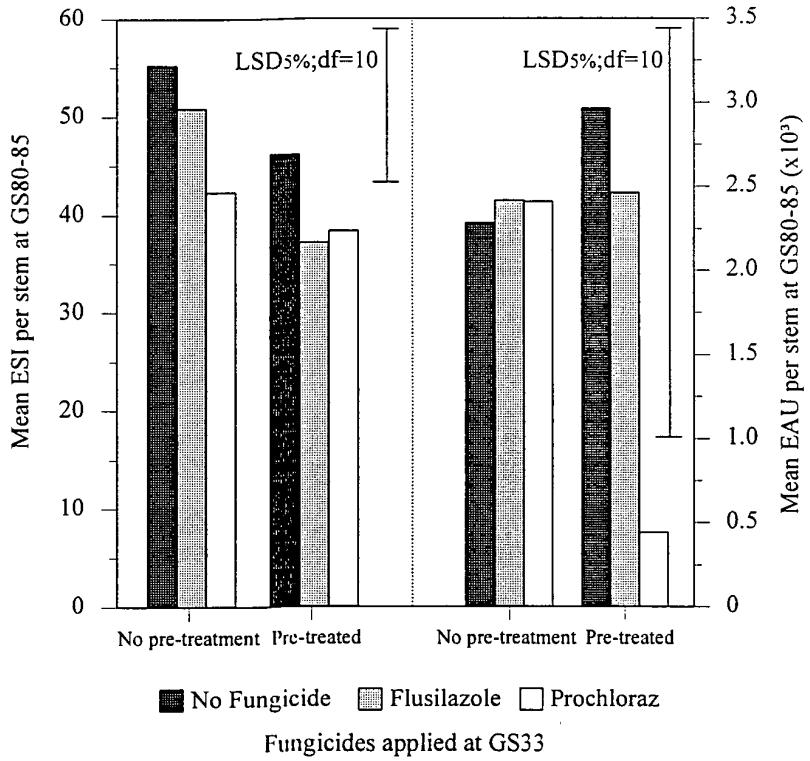

Fig. 4. The effects of fungicide on eyespot levels in Hereward at LARS in 1994

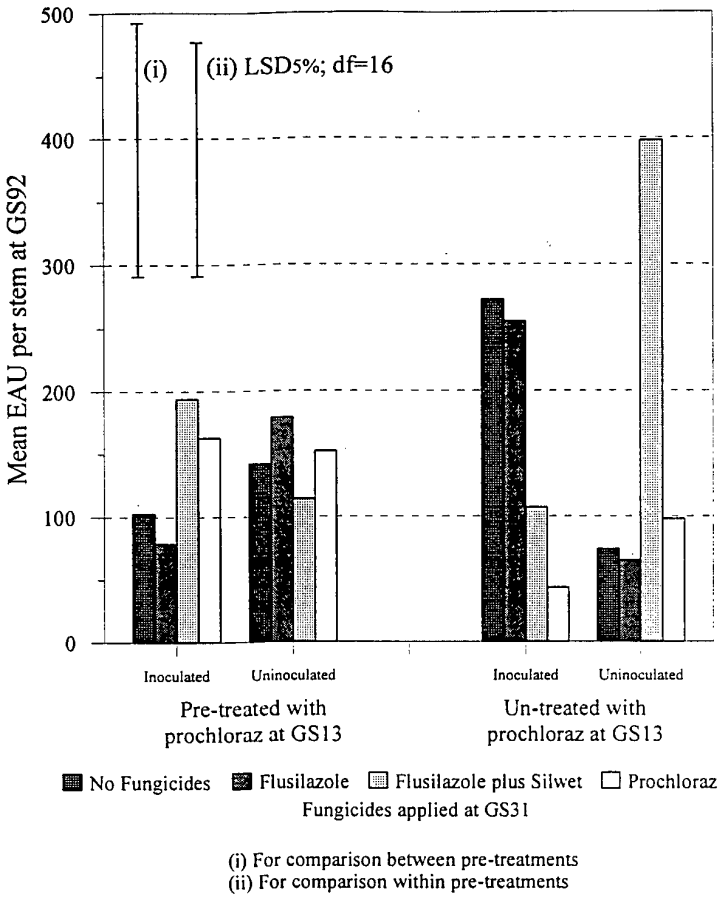

Fig. 5. The effect of fungicide on antigen levels in Hereward at GS92 at LARS in 1995

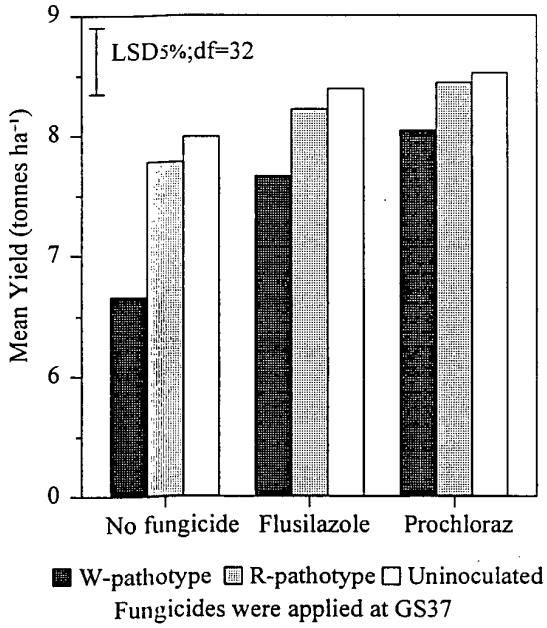

Fig. 6. The effects of fungicides on the yields of Apollo at LARS in 1992

1.1.3.6 Relationships between disease levels and yield

Correlations between visual symptoms and antigen levels. In Apollo at GS24, eyespot symptom severity did not correlate with antigen levels, whereas from GS37-39 there was a significant relationship. There was also a good correlation between visually-determined disease levels at GS37-39 and at GS80, but only in plots which had not been treated with fungicides (Fig. 7). It was difficult to determine the precise benefits of applying fungicides but fungicides applied to plots with severity indices greater than 20 at GS37-39 did appear to provide better eyespot control.

Whilst at any growth stage in Rendezvous visual symptoms and antigen levels were not related, at GS30-31 and GS80 there was a relationship (Fig. 8). Fungicides applied when antigen levels were greater than 50 EAU per stem at GS30-31 clearly reduced disease levels, whereas fungicides applied below this threshold were of no benefit. It was not possible to relate either visual symptoms or antigen levels at any growth stage in Hereward and, in addition, disease levels at early growth stages were not related to final levels of disease in this cultivar.

In the NIAB data there were good correlations between antigen levels and eyespot severity indices for all eyespot-susceptible cultivars from GS30, during 1992, 1993 and 1994. In Rendezvous, however, correlations were consistently poor but they improved from GS75-80. In 1995, correlations between visual symptoms and antigen levels in Riband and Talon were good from GS30 onwards, whereas in Lynx and Andante, correlations were poor before GS65. There were correlations between early and final antigen disease levels only in Beaver, Riband and Talon (Fig. 9).

Correlations between disease levels and yield. In Apollo, both visual symptoms and antigen levels at GS37-39 correlated well with yields (Fig. 10): Plots with severity indices of 15 or below at GS37-39 generated yields between 8- 9 tonnes ha^{-1} and these were not improved by the application of fungicides; plots with severity indices greater than 20 at GS37-39 benefitted from fungicides and yields were raised by more than 0.5 tonnes ha^{-1} to 7.5 tonnes ha^{-1} or above. Plots with antigen levels greater than 2000 EAU at GS37-39 showed increases of 1.5 tonnes ha^{-1} in response to fungicides, whereas below this threshold, there were no yield benefits.

For Rendezvous and Hereward, there were no relationships between disease levels and yields and it was therefore not possible to determine spray thresholds based on yield benefits in either of these cultivars.

In the NIAB experiments there were correlations between visual symptoms and yields. It was only possible to estimate a disease threshold for Riband where the relationship between antigen levels at GS24 and yield suggested a spray threshold of approximately 3000 EAU per stem (Fig. 11).

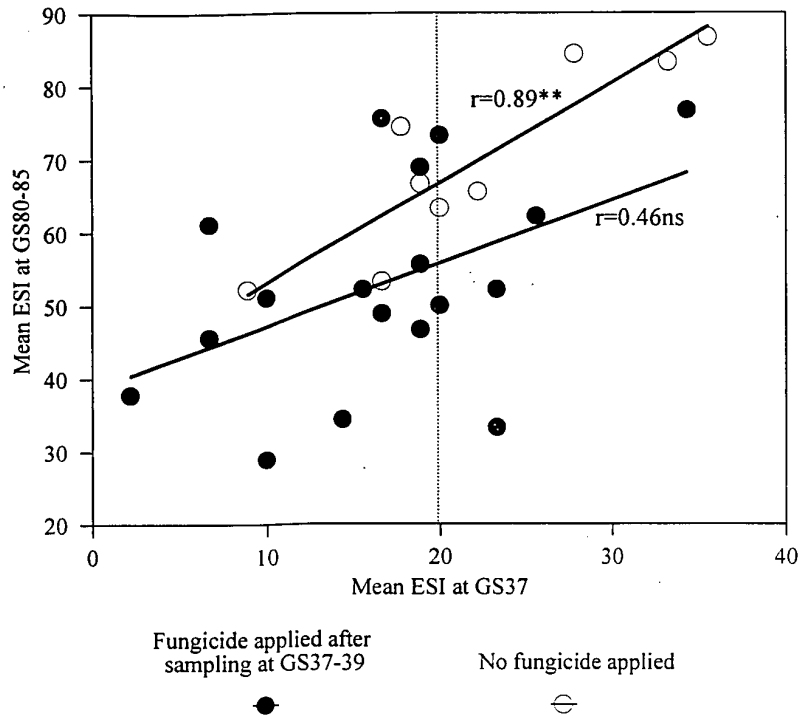

Fig. 7. Correlations between visually-determined eyespot symptoms at GS37-39 and GS80-85 in Apollo

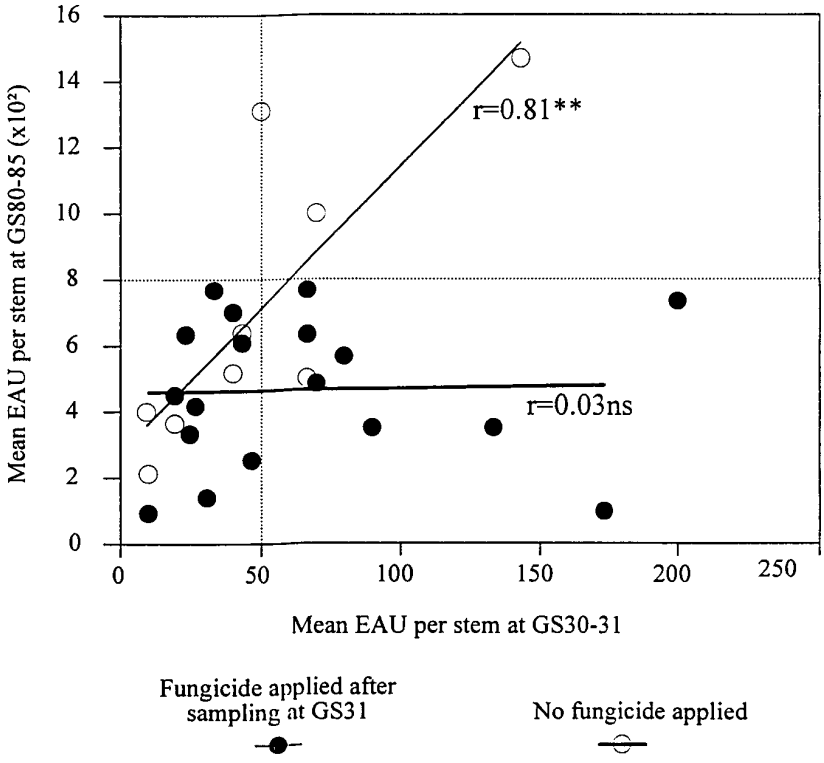

Fig. 8. Correlation between antigen levels at GS30-31 and GS80-85 in Rendezvous

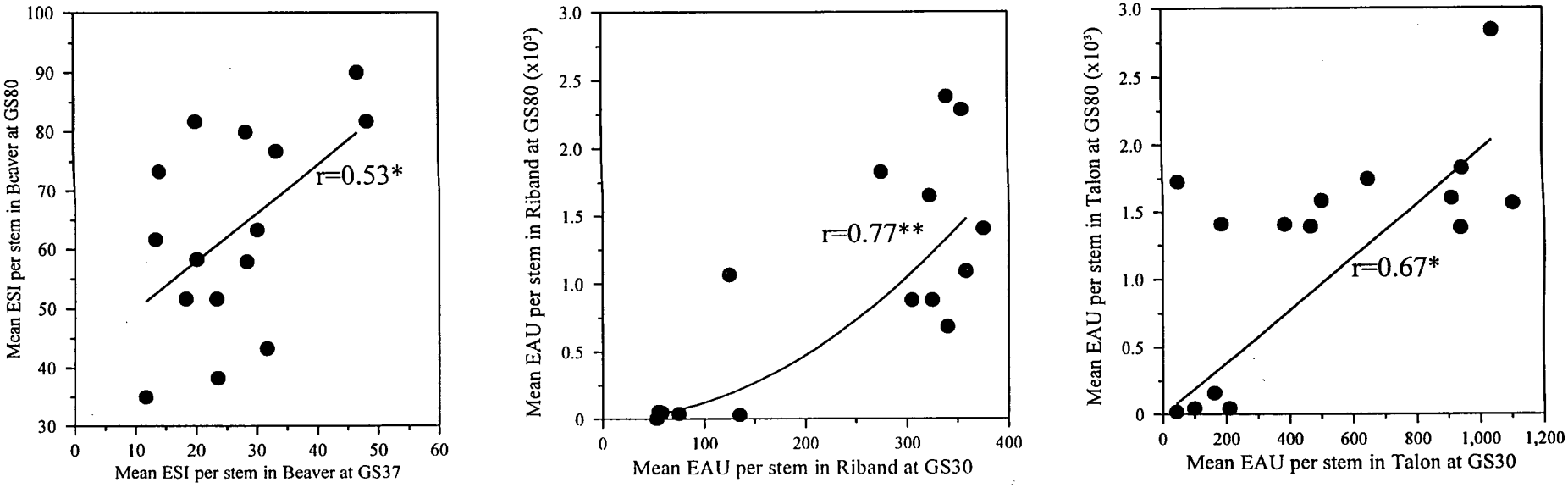

Fig. 9. Correlations between antigen levels at GS30/37 and GS80 in Beaver, Riband and Talon

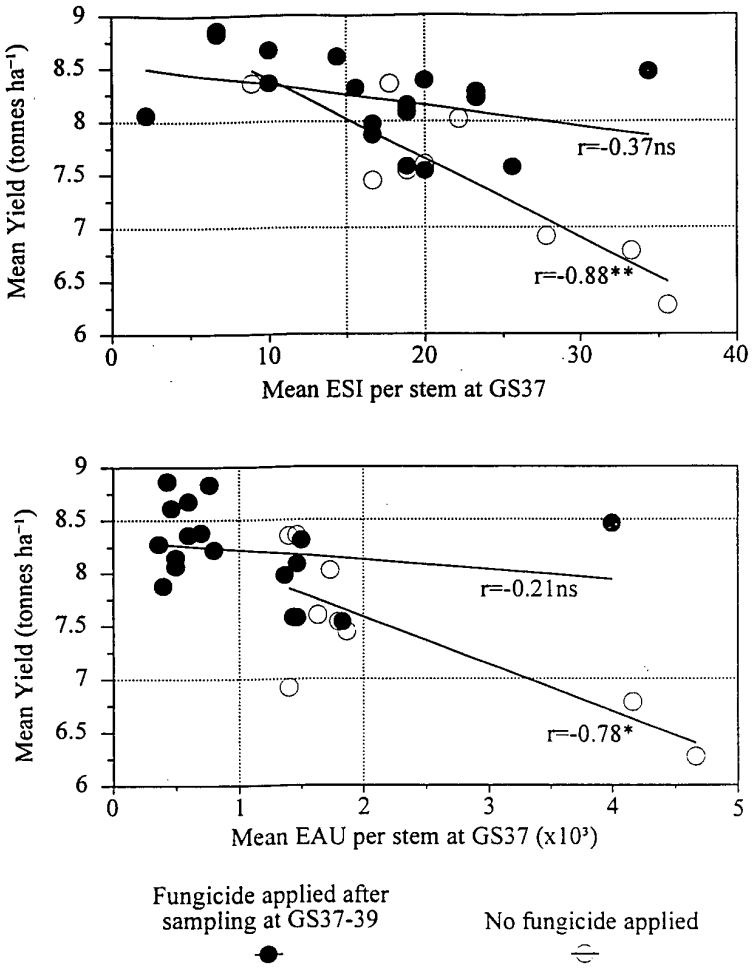

Fig. 10. Correlations between disease levels at GS37 and yield in Apollo

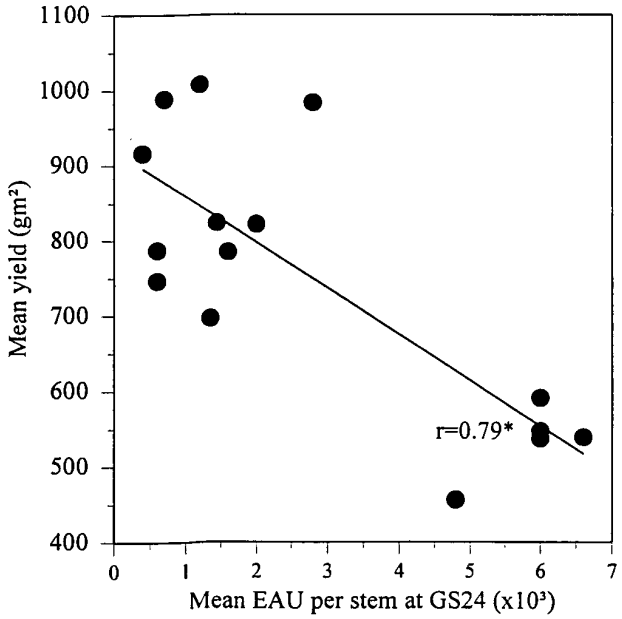

Fig. 11. Correlation between antigen levels at GS24 and yield in Riband

1.1.4 Discussion

Immunodiagnostics provide new opportunities to chart the development of plant diseases. Using this technology to follow eyespot epidemics in different wheat cultivars over a four year period, has confirmed that diagnostic kits provide a simple, robust and reproducible way to quantitatively monitor progress of this disease. Although actual antigen levels varied between cultivars, once eyespot symptoms were visible, antigen levels and disease levels were always well correlated at each sampling time. As each epidemic progressed, and especially after GS65, antigen levels progressed much more than changes in the severity index would suggest. Consequently, immunology has exposed variation in a number of factors affecting eyespot development, which were not realized when disease development could only be followed visually.

Each year eyespot was detected pre-symptomatically, regardless of the wheat cultivar used. Up to GS37-39 antigen levels in the eyespot resistant cultivar Rendezvous were similar, or sometimes even higher, than in susceptible cultivars. Subsequently, antigen and disease levels increased far more in susceptible cultivars than in Rendezvous. It seems, therefore, that resistance is expressed in Rendezvous, not by blocking infection, but by delaying and suppressing symptom expression. These results provide an explanation as to why yield benefits often follow from fungicide treatment of Rendezvous in situations where eyespot is the main disease found in untreated crops.

It also emerged from these studies that a background level of eyespot was always present in uninoculated plots, even when these were in a first wheat crop. Antigen levels in uninoculated plots were at least half those found in inoculated plots, and in some cases actually exceeded levels found after artificial inoculation (eg in the susceptible cultivar Apollo grown at LARS in 1992). The recent identification of the sexual stage of eyespot in stubble in the UK (Hunter, 1989), and the realization that wind dispersed ascospores might initiate eyespot, helps explain the widespread and uniform distribution of antigen units throughout these crops, in a disease hitherto thought to be dispersed only by rainsplash. The practical implications of this finding are significant. First wheat crops, previously considered to be of low risk from eyespot attack, need to be monitored carefully, and where early infections are detected in susceptible cultivars, fungicides should be applied.

Unfortunately, early infection by eyespot from apparently wind blown sources countered the attempts to monitor the progress of wheat and rye eyespot pathotypes, by creating epidemics through artificial inoculation of plots with either W- or R-type only. Despite inoculating plots with W-types, R-types always predominated in isolations made from infected stems at GS75, reflecting the dominance of R-types in inoculated plots, and in the airspora. Nevertheless, the evidence available from this study, suggests that the W-type is more invasive than the R-type, and damages the crops earlier than do R-types.

Significant background antigen levels in all plots severely restricted attempts to generate a wide range of eyespot levels at GS30-31, either by varying inoculum timing, or cultivar use. Consequently, only a narrow range of antigen levels were available to assess the significance of correlations between antigen levels at GS30-31, and final disease and yield, and to identify thresholds to guide fungicide timing. Autumn application of prochloraz did

generate a wider range of antigen levels at GS30-31, but still did not provide the positive correlations needed to predict disease levels some two or three months later. On some occasions in susceptible cultivars, antigen levels at GS37-39 were correlated well with final (GS75) disease levels and yield, but this is too late to provide a useful guide for fungicide timing. For the resistant cultivar, Rendezvous, antigen and final disease levels were only correlated at the final sampling date (GS75). Background antigen levels in uninoculated plots and yield increases obtained from treating plots in the same experiment, spray thresholds were derived but were erratic, and varied between 50 units per stem in 1993 and 2000 units per stem the following year. These large differences between years, and possible differences between cultivars, means that the practical use of immunodiagnostic kits to guide the management of fungicide use against eyespot is limited, perhaps to only the most susceptible cultivars.

Whenever significant correlations were obtained between eyespot antigen units and final disease levels and yields, these correlations were always better when based on antigen units, rather than visual symptoms. This suggests that immunodiagnostics do provide a better measurement of eyespot than do visual symptoms, but despite the improved qualification of eyespot levels at GS 30-31, disease levels at this stage are not the major factor limiting damage and yield in UK wheat crops. Clearly the weather in May and June has a big impact on the ability of eyespot to subsequently penetrate cereal stems and cause yield losses. If fungicides could be developed which effectively controlled eyespot from applications at GS37-39, when correlations between eyespot antigen levels and yield are generally significant, and predicting of yield losses more reliable, eyespot diagnostic kits would be more useful in guiding spray decisions.

1.2 EFFECT OF CULTIVAR ON EYESPOT DEVELOPMENT AND CROP LOSS

Rosemary A. Bayles (NIAB, Cambridge)

1.2.1 Introduction

The objective of this part of the project was to compare disease progress and crop loss in epidemics of W-type and R-type eyespot. Artificial inoculation was used to create separate infections of the two pathotypes in a range of wheat cultivars with different levels and sources of genetic resistance.

DNA diagnostics for the W- and R-types of *Pseudocercosporella herpotrichoides* have been developed at the John Innes Centre. In 1995, they were used to detect and quantify the presence of the two pathotypes and to compare the levels of resistance with symptom expression in four of the cultivars.

1.2.2 Materials and Methods

Field experiments: A single trial was carried out in each of four years, 1992 to 1995, on the NIAB trial ground at Cambridge. Trials were situated on land following a one year break (1992) or a two year break (1993 - 1995) from cereals in order to reduce the risk of carry over of eyespot from the previous crop.

In each trial, four wheat cultivars, with varied genetic resistance to eyespot, were inoculated either with a mixture of W-type or a mixture of R-type isolates of *Pseudocercosporella herpotrichoides*. Uninoculated plots were included to give disease-free controls for yield comparisons. In 1994, additional uninoculated plots were sprayed repeatedly with prochloraz between early March and May to control naturally occurring eyespot. In 1995 the uninoculated treatment without prochloraz was dropped and replaced by the uninoculated treatment with prochloraz.

Cultivars were:

Cultivar	Resistance	Resistance source	Year
Talon	Susceptible	None	'92-'95
Beaver	Intermediate	Cappelle-Desprez	'92-'94
Riband	Intermediate	Cappelle-Desprez	'92-'95
Rendezvous	Resistant	Cappelle-Desprez + VPM	'92-'95
Lynx	Resistant	Cappelle-Desprez + VPM	'95
Andante	Resistant	VPM	'95

The trial design was a randomised split-plot, with 5 replicates. Inoculation treatments were allocated to main plots and cultivars to sub-plots. Plots measured 4m x 1m and inoculation treatments were separated by a 5m barrier of barley. Trials were drilled in early to mid October and inoculated at GS11, in late October to early November. Inoculation was by means of distributing 35 artificially infected straw segments evenly within each plot.

Eyespot symptoms were assessed up to five times between GS 22 and GS 85, using either the seedling infection key of Macer (1966) or the adult plant infection key of Scott and Hollins (1974). 20 tillers were sampled from the front 2 m of each plot for assessment.

Grain yield was determined from ear samples taken from four 0.5m lengths of row in the rear 2m of each plot.

DNA-diagnostics: Samples of the four cultivars used in the 1995 trial were taken for DNA analysis at GS 22, 31, 37, 65 and 85. DNA was extracted from 30 stem-base samples after visual assessment at NIAB. The samples were diluted to a standard representing 0.25mg of dried plant material per ml and each sample was tested for the presence of W- and R-type of eyespot and also for *Fusarium culmorum, F. graminearum, F. avenaceum, Microdochium nivale* and *Rhizoctonia cerealis*. The W- and R-type eyespot was quantified on a 4-point scale: 0, absent; 1, detected at low level; 2, detected at moderate level and 3, detected at high level. This scale has been found to give good correlation with competitive PCR if the conditions of DNA extraction and PCR amplification are constant for all samples. Attempts to maintain these conditions were made in the analyses carried out in this work. More precise quantification is possible using competitive PCR and will be carried out on these samples at a later date when competitor templates have been refined for the two eyespot pathotypes.

1.2.3 Results

1.2.3.1 ***Field experiments:*** Eyespot disease indices for each cultivar inoculated with W and R pathotypes are shown in Figs. 12-15.

In all four years eyespot infection established successfully. Early season symptoms at GS 30/31 were more severe in 1992 and 1993 than in the following two seasons. At the final assessment of the season, between GS 75 and GS 85, eyespot symptoms were most severe in 1992 and least severe in 1995, with 1993 and 1994 being intermediate. The maximum disease index for the susceptible cultivar Talon reached a value of 93.0 in 1992, compared with 78.0 in 1995.

Eyespot severity in uninoculated, unsprayed plots was variable. In 1992, following only a one-year break from cereals, infection indices in uninoculated plots reached approximately 85% of their mean level in inoculated plots. In 1993 and 1994, following 2-year breaks, indices in uninoculated plots were much lower, at about 50% of the inoculated level. Treatment of uninoculated plots with prochloraz in 1994 and 1995 virtually eliminated eyespot symptoms.

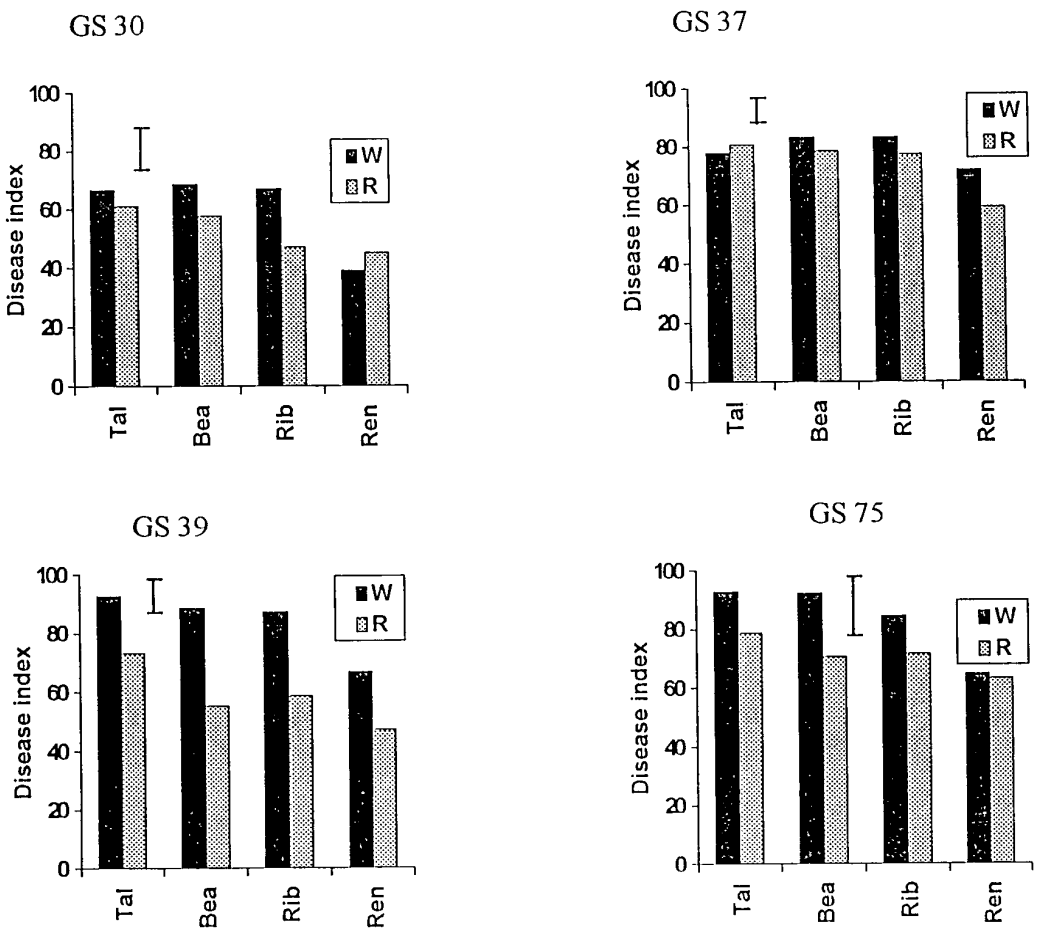

Fig. 12. Disease indices for 4 wheat varieties inoculated with W or R pathotypes of *P. herpotrichoides*, 1992

Bar = lsd (0.05)

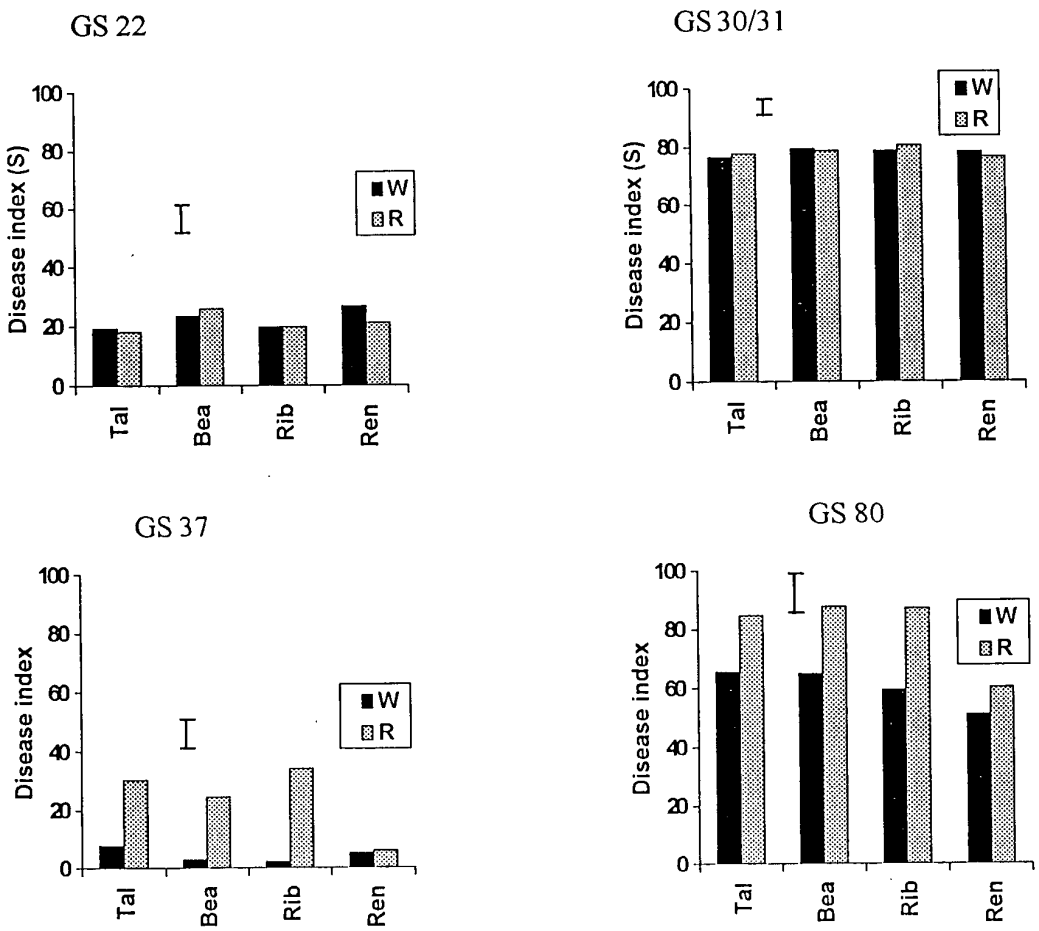

Fig. 13. Disease indices for 4 wheat varieties inoculated with W or R pathotypes of *P. herpotrichoides*, 1993

[(S) = seedling disease index] Bar = lsd (0.05)

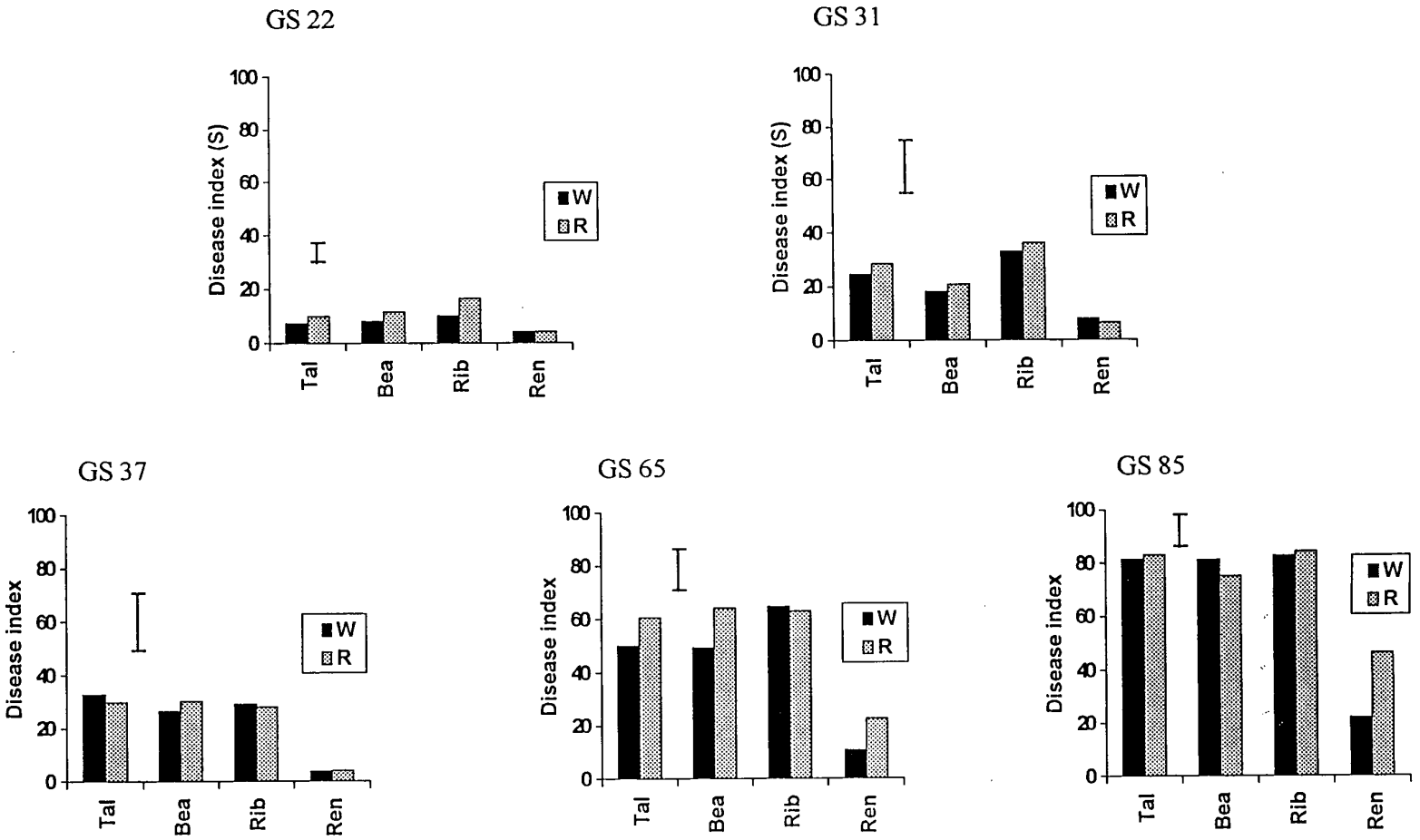

Fig. 14. Disease indices for 4 wheat varieties inoculated with W or R pathotypes of *P. herpotrichoides*, 1994

[(S) = seedling disease index] Bar = lsd (0.05)

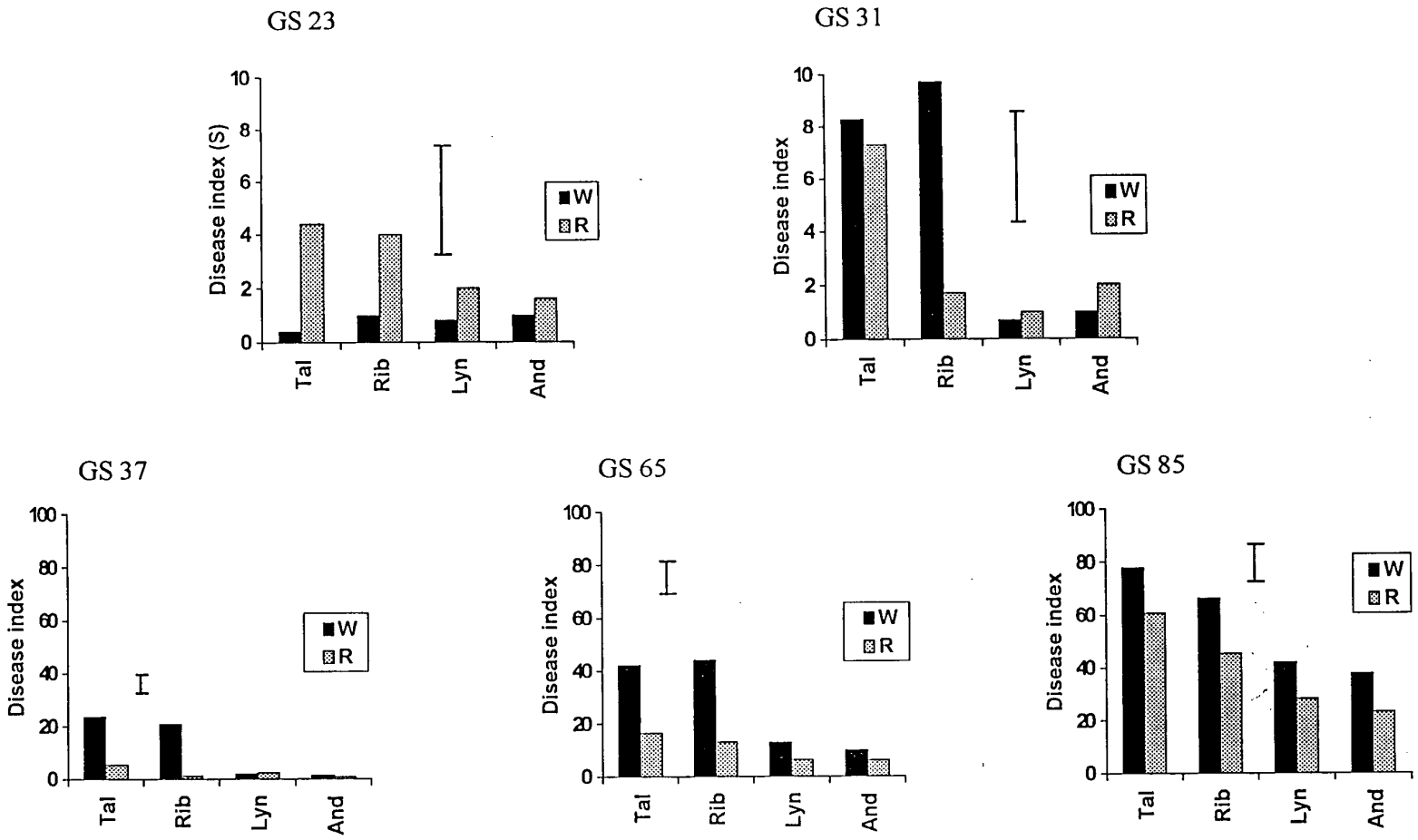

Fig. 15. Disease indices for 4 wheat varieties inoculated with W or R pathotypes of *P. herpotrichoides*, 1995

[(S) = seedling disease index] Bar = lsd (0.05)

The relative severity of eyespot symptoms in plots inoculated with W and R pathotypes varied between years. In two years, 1992 and 1995, inoculation with W-types produced the more severe symptoms on all except the resistant cultivars (Rendezvous in 1992; Lynx and Andante in 1995). In 1993, symptoms resulting from R-type inoculation were the more severe. In 1994, inoculum type had no effect on eyespot severity except in the resistant cultivar Rendezvous, which was most severely effected by R-types.

Grain yields are given in Table 1. In 1992 and 1995, W-type inoculation reduced yield compared with both R-type inoculation and no inoculation in at least some cultivars. In 1992 all cultivars except Rendezvous responded in this way, whereas in 1995, with lower overall levels of eyespot, only the susceptible cultivar Talon incurred a significant yield loss. Inoculation treatment had no effect on yield in either 1993 or 1994.

1.2.3.2 ***DNA-diagnostics.*** DNA diagnostics showed that up until, and including, GS 65, W-type eyespot was isolated almost exclusively from plots inoculated with W-type, and R-type eyespot from plots inoculated with R-type (Table 2). At GS 85 however, R-type eyespot was also detected at moderate levels in plots inoculated with W-type and in the sprayed control plots. The resistance of cultivars to W-type infection, as indicated by PCR, was in close agreement with symptom expression. However, PCR detected higher levels of R-type eyespot in the cultivars Andante and Lynx than would have been expected from their relatively low level of symptoms. PCR also detected R-type eyespot in all cultivars in sprayed plots, which were virtually without symptoms.

High levels of *M. nivale* were detected at all growth stages. *F. culmorum* and *F. avenaceum* were detected in some plots at later growth stages, but only low levels of *R. cerealis* were detected in a very few plots.

1.2.4 Conclusions

Artificial inoculation of field plots was used as a means of creating discrete epidemics of the W and R pathotypes of eyespot. Evidence gained by re-isolating the fungus from plants during 1994 and from DNA diagnostics during 1994 and 1995 indicated that this was largely successful, although a low level of contamination of one pathotype with another could be detected. Eyespot symptoms were also recorded in uninoculated plots, even following a two-year break from cereals. Contamination may have been due to incoming ascospores early in the season, conidia from inoculated plots within the trial or conidia from outside the trial. Except in 1992, when the trial followed a non-cereal break of only one year, soil-borne inoculum is a less likely explanation.

Starting from comparable inoculum loads, the development of epidemics of W- and R- type eyespot differed from season to season. In 1992, W-type produced relatively severe symptoms throughout the season. In 1993, infection in R-type plots became relatively severe from GS 37 onwards. In 1994 there was little difference in symptom development between the two pathotypes, except in the cultivar Rendezvous, which was more heavily infected by R-type towards the end of the season. During 1995, there was a change from early spring, when symptoms caused by R-type were more severe, to the main summer period, when W-type appeared the more damaging. The relationship between early and late

eyespot levels for each pathotype was variable, presumably being influenced by seasonal variation in weather and other factors.

Although DNA diagnostics may indicate the presence of R-type eyespot, the distribution of the fungus on and within the tissue of the stem is unknown and it is therefore possible that it may not be damaging or produce recognisable symptoms. In addition, it has been observed in other trials that where *Michrodochium nivale* is present, as here, R-type eyespot does not produce typical symptoms.

Table 1. Grain yields (gm/m2)

	1992			1993			1994				1995		
	W	R	O	W	R	O	W	R	O	OS	W	R	OS
Talon	412	710	670	517	580	578	928	831	920	926	983	1261	1126
Beaver	554	852	858	534	557	605	984	910	905	959			
Riband	481	738	802	462	452	403	922	892	939	916	1148	1118	1190
Rendezvous	770	834	788	742	768	810	910	848	830	884			
Lynx											1166	1207	1169
Andante											973	959	941
lsd (P=0.05) (inoculum mean within variety)	123.7			152.0			157.8				117.1		

Inoculum
W = wheat pathotype
R = rye pathotype
O = uninoculated
OS = uninoculated and sprayed with prochloraz to control eyespot

Table 2. **PCR-estimations[1] of W- and R-type eyespot present in samples, taken at five growth stages, of four winter wheat cultivars, differing in susceptibility to eyespot.**

Cultivar	Growth stage	Treatment: W-type		R-type		Sprayed	
		W-type	R-type	W-type	R-type	W-type	R-type
Talon	22	1.2	0	0	2.2	0	0
Riband	"	1.8	0	0	2.4	0	0
Andante	"	1	0	0	2.2	0	0
Lynx	"	1.8	0	0	1.2	0	0
Talon	31	1.6	0	0	2	0	0
Riband	"	2.2	0	0	2	0	0.2
Andante	"	1	0	0	2.2	0	0
Lynx	"	1.4	0	0	1.8	0	0
Talon	37	1.8	0	0.4	1.2	0.2	0
Riband	"	1.4	0	0.4	1	0	0
Andante	"	0.4	0	0	1.6	0	0
Lynx	"	0.2	0	0	1	0	0
Talon	65	2.2	0.4	0	2	0.4	0.8
Riband	"	2	0	0	2	0	0.2
Andante	"	1	0	0	1.6	0	0
Lynx	"	2.2	0	0	2	0	0
Talon	85	3	1.6	0.4	2.8	0.4	2.2
Riband	"	3	2	0	3	0	0.4
Andante	"	1.2	1.4	0	2.8	0	0.6
Lynx	"	1.8	1.4	0	2.8	0	1

[1] Mean of 5 replicate samples, each consisting of 30 stem-bases, based on 4-point scale (see text)

The apparent effect of eyespot on yield was also variable. Severe W-type eyespot, culminating in final disease indices in the range 75-95, often, but not always, resulted in a loss in yield compared with uninoculated plots (i.e. cultivars Talon, Beaver and Riband in 1992 and Talon in 1995). However, similarly high levels of R-type eyespot did not appear to reduce yield in any of the trials (i.e. cultivars Talon in 1992; Talon, Beaver and Riband in 1993 and 1994). This suggests that, for a given level of symptoms, eyespot may be less damaging to yield if caused by the R pathotype than the W pathotype. The implication is that, since the UK eyespot population became dominated by the R-type in the mid 1980's, eyespot-related yield losses may now be lower than when the eyespot population consisted predominantly of the W-type. Further similar experiments, in a greater range of environments, would be needed to confirm this conclusion.

There was a striking lack of response to fungicide in the 1994 and 1995 trials, despite the fact that repeated applications of prochloraz had virtually eliminated eyespot symptoms. Control of eyespot at levels as high as index 84.3 in 1994 and index 66.3 in 1995 produced no yield benefit, indicating that the ability of the crops to respond to disease control was limited by other factors, such as environmental conditions. These results serve to emphasise the difficulty of predicting whether fungicide application for eyespot control is likely to be cost effective.

Cultivars ranked similarly for eyespot symptoms whether inoculated with W- or R-type isolates and there was no evidence to suggest that they differed in their resistance to the two pathotypes. The only possible exception to this was in the 1994 experiment, in which Rendezvous appeared relatively susceptible to the R-type. However, this was not confirmed in other years, nor for other cultivars carrying the VPM resistance. It appears that cultivar resistance evaluated in situations where one pathotype dominates is relevant where the other dominates. For artificially inoculated trials, a sensible approach is to use a mixture of the two pathotypes. The grower can continue to choose cultivars with good eyespot resistance without having to know the composition of the local eyespot population.

1.3 EYESPOT DEVELOPMENT, CROP LOSS AND CONTROL

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1.3.1 Introduction

The objectives of this part of the project were:

(i) to attempt to engineer differential development of eyespot epidemics caused by the R- and W-pathotypes, using fungicides as manipulative tools,
(ii) to monitor eyespot development associated with the two pathotypes, using visual diagnosis and confirmatory isolations on artificial media, (the isolations were replaced with DNA diagnostics in 1995),
(iii) to re-examine the timing of fungicide application in relation to eyespot development associated with the two pathotypes and to attempt to re-define treatment thresholds for fungicide treatment,
(iv) to establish new disease severity/crop loss relationships for eyespot caused by the two pathotypes and the growth stage at which such relationships are best used to calculate crop loss.

1.3.2 Materials and Methods

Experiments were carried out at 14 sites over the 4 years of the project (Table 3). Two experiments in each of the first 3 years and four experiments in 1995 were funded by H-GCA; MAFF also funded two experiments in each of the first 2 years. All sites used a second wheat crop of an eyespot-susceptible cultivar.

In 1992, 1993 and 1994 (Tables 4 & 5), a randomised block design was used consisting of 24 treatments, 2 untreated controls and 3 replicates (total of 78 plots in 'stacked blocks'). Minimum plot dimensions were 2.5 - 3.0 m x 35 - 40 m with 1.5 x normal plot length to allow 33% area for destructive eyespot sampling. This area was not taken to harvest. Wherever possible, plots were drilled before mid-October or crops with appropriate sowing dates were chosen for burning out; fungicide treatments were applied by a CO_2 knapsack sprayer in 240 l water/ha at 250 kPa. All other husbandry operations were according to usual farm practice. The first of the 1-, 2-, 3- and 4-spray programmes of prochloraz or flusilazole, and the single sprays, were applied at commercial rates; all other sprays were applied at half-rates. The design was modified in 1994 according to results in the first 2 years. In 1995 there were 10 treatments with a single untreated control (Table 6)

All plots were oversprayed with fenpropimorph and chlorothalonil at GS33-37 and GS49-55.

Records of eyespot were taken from untreated plots and from all sprayed plots prior to the first spray application and subsequently at 2-week intervals thereafter until GS75-81. The number of leaf sheath bases penetrated by the pathogen was recorded for each lesion found

in all plots on the sampling dates between *c.*12 February and *c.* 22 July (GS81). Untreated control plots were always assessed first and no further assessments were performed in sprayed plots if there were less than 10% plants affected by eyespot in the untreated plots. If there were less than 20% tillers affected by moderate or severe eyespot lesions in the untreated plots at GS75, then sprayed plots were not assessed until GS81.

In 1992 only, all samples containing suspect eyespot lesions were kept in a deep-freeze until the end of the experiment. The frozen material was sent to CSL Harpenden in September to determine the presence of R- & W-pathotypes, though many of the early tissue samples did not withstand freezing and thawing. Only the latest (GS75-85) of the tissue samples yielded eyespot isolates satisfactorily. During 1993 and 1994, all isolations were made on fresh tissue samples.

In 1995, DNA diagnostics, developed at the John Innes Centre for the W- and the R-pathotypes of *P.herpotrichoides*, were used to detect and quantify the two pathotypes in crop samples at four sites (Table 3). Eyespot was assessed visually at each application of fungicide and then DNA was extracted for analysis. The methodology for processing the samples is described in Section 1.2.

All plots were harvested and yield expressed at 85% dry matter. Additional data on grain quality were obtained for some sites. Routine soil analyses were performed and site husbandry details were recorded.

Table 3. Sites, cultivars, sowing dates, eyespot incidence and severity

1992	Cherhill Wilts	Lambourn Berks	Markle Mains East Lothian	Terrington Norfolk
Cultivar	Beaver (5)	Mercia (5)	Beaver	Pastiche (5)
Sowing date	24.9.91	11.10.91	14.10.91	11.10.91
Eyespot early (%)	87	21	60	69
late (EI)	48	15	73	82
1993	Avebury Wilts	Goathurst Somerset	Markle Mains East Lothian	Terrington Norfolk
Cultivar	Mercia	Beaver	Beaver	Pastiche
Sowing date	8.10.92	7.10.92	14.10.92	13.10.92
Eyespot early (%)	51	56	13	49
late (EI)	44	53	44	22
1994			Markle Mains East Lothian	Terrington Norfolk
Cultivar			Beaver	Pastiche
Sowing Date			14.10.93	4.11.93
Eyespot early (%)			10	32
late (EI)			27	22
1995	Morley Norfolk	Rosemaund Hereford	Markle Mains East Lothian	Stonham Norfolk
Cultivar	Beaver	Soissons (4)	Riband (6)	Beaver
Sowing Date	6.10.94	14.10.94	25.10.94	28.9.94
Eyespot early (%)	20	24	21	19
late (EI)	9	11	50	33

Table 4. Treatment numbers, target spray dates (week numbers) and treatment regimes, 1992 & 1993

Week No.	Spray date	Treatment regimes
1	12 Feb	p p f f
2	26 Feb	p p f f
3	11 Mar	p p p f f f
4	25 Mar	p p p f f f
5	08 Apr	p p p p f f f f
6	22 Apr	p p p f f f
7	06 May	p p p p f f f f
		1 2 3 4 5 6 7 8 9 10 11 12 13 2 3 4 5 6 7 8 9 10 11 12 13
		Treatment timings

Table 5. Treatment numbers, target spray dates (week numbers) and treatment regimes, 1994

Week No.	Spray date	Treatment regimes
1	08 Feb	p p p
2	22 Feb	p p p f c
3	08 Mar	p p p p p
4	22 Mar	p p p p p f c
5	05 Apr	p p p p p
6	19 Apr	p p p p p f c
7	03 May	p p p p
8	17 May	p p p p
		1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
		Treatment number

The experiment was simplified in 1995 (Fig. 3) mainly to reduce the number of treatments for which the DNA diagnostic would be used.

Table 6. Treatment numbers, target spray dates (week numbers) and treatment regimes 1995

Week No.	Spray date	Treatment regimes
1	05 Dec	p c
2	14 Feb	p c
3	14 Mar	p c
4	14 Apr	p c
5	01 May	p c
		1 2 3 4 5 6 2 3 4 5 6
		Treatment timing

In Tables 4-6, Treatment 1 was untreated and p=prochloraz; f=flusilazole; c=cyprodinil.

1.3.3 Results

The development, incidence and severity of eyespot, (expressed as eyespot index), varied markedly across the 14 site/year combinations as did the relationships between eyespot index, yield and the relative proportions of the R- and W- pathotypes identified in pathogen populations at each site. In the following accounts, a significant effect refers to $P < 0.05$ and yield differences are those compared to untreated, control plots.

1.3.3.1 Yield effects and eyespot control at individual sites

1992. At Cherhill and Lambourn there were no significant effects of fungicide treatments on yield despite differences between treatments of up to 0.87 t/ha and 0.92 t/ha, respectively. At Terrington and Edinburgh there were significant effects on yield. At Edinburgh, yield increases of up to 1.06 t/ha were associated mainly with application of prochloraz, although three or four applications of flusilazole had similar effects. Despite this variability, fungicide treatments significantly affected eyespot indices at all four sites, three of which had moderate to severe eyespot attacks. At Cherhill, only three flusilazole treatments (including the earliest spray timing) reduced disease levels. At Lambourn, the site with least eyespot, seven of the prochloraz and six of the flusilazole treatments significantly reduced eyespot; three or four applications gave the most consistent effects, although the single most important spray appeared to be one of the mid-range timings. At Edinburgh, eyespot was reduced by only four of the prochloraz and one of the flusilazole treatments, and at Terrington, all but three (single spray) treatments reduced eyespot. At the latter two sites it was not possible to identify a single spray timing which was likely to have contributed most to eyespot control.

1993. At Avebury, Goathurst and Edinburgh, fungicide treatments significantly promoted yields. Nine prochloraz and seven flusilazole treatments gave yield increases of up to 1.09 t/ha at Avebury, ten flusilazole and eight prochloraz treatments gave yield increases of up to 0.55 t/ha at Goathurst and five prochloraz and two flusilazole treatments gave yield increases of up to 1.92 t/ha at Edinburgh. At these sites only the later single, two-spray and multiple-spray programmes tended to give significant increases over the untreated controls. There were no significant yield effects due to fungicide application at Terrington. Goathurst was the only site where there were significant treatment effects on eyespot, despite all four sites having moderately severe eyespot attacks. There, all treatments reduced the eyespot index by up to 90%, with two or more sprays of prochloraz having the greatest effects.

1994. At Edinburgh and Terrington there were significant reductions in moderate eyespot and increases in yield.

1995. At Morley and Rosemaund, there were no significant effects of the fungicide treatments on either yield or eyespot. There was very little eyespot at both of these sites. At Edinburgh, all five single applications of prochloraz and two of the cyprodinil applications reduced eyespot but all treatments, except the earliest application of cyprodinil, resulted in significant yield increases. At Stonham, only the three later applications of cyprodinil reduced eyespot. Three cyprodinil and one prochloraz spray timings increased yield significantly, although inconsistently.

Because of changes in experimental design during the 4 years it was not possible to carry out a balanced analysis of all 14 experiments. Consequently, overall analyses for eight sites (1992-93), two sites (1994) and four sites (1995) are presented below.

1.3.3.2 Overall effects of fungicide on eyespot and yield

1992-1993. Both prochloraz and flusilazole gave significant average increases in yield over the untreated controls. Prochloraz gave a greater mean yield increase than flusilazole, though the difference between the two fungicides was marginal (Table 7). In terms of their timing, both fungicides had a similar effect, all timings resulting in significant yield increases. Differences between timings (single and multiple spray programmes) were less marked; the best application was made in early to mid-April, although this was not significantly better than other single sprays applied from late March to early May. Sprays applied between mid-February and mid-March were less effective than the best single spray timing which was significantly improved in effect only by one of the multiple-spray programmes, that of four applications from mid-February to early May. Results for eyespot control paralleled those for yield, although the most marked differences between spray timings were those of single sprays compared to those of three or four sprays. Within single-spray timings, no timing gave significantly greater control of eyespot compared to any other, and the relationship between yield and the associated eyespot index was relatively poor. The converse was true for the two to four-spray programmes..

1994. Little disease at both sites in 1994 meant that all fungicide effects were non-significant (Table 8).

1995. Both prochloraz and cyprodinil provided significant average reductions in eyespot and significant increases in yield, but there were no differences between the average effects of the two fungicides and their five single-application timings. Even average timings showed no significant differences (Table 9).

1.3.3.3 Eyespot development

There was a significant correlation (r=0.61) between the largest recorded incidence of eyespot (% plants or tillers affected at GS13-37), and the eyespot index in the untreated plots at GS75-85. However, at six of the 14 sites, fungicide application was justified, (based on the previously accepted threshold of >20% plants or tillers affected on one or more occasions during late winter-early spring up to GS33-37). This was in spite of the fact that eyespot failed to develop substantially at the grain filling stage. At most sites eyespot developed in a fairly typical pattern: lesions became less apparent during May/June but then became obvious, penetrating lesions, albeit not until late in the grain-filling period. The disease became sufficiently severe to cause lodging at only one site, Markle Mains, in 3 of the 4 years. This being so, it is of value to examine further the results from Markle Mains.

1.3.3.4 Effects of fungicides on eyespot, lodging and yield at Markle Mains, 1992-94

Multiple 3- or 4-spray programmes of prochloraz significantly reduced the eyespot index and the degree of lodging, and improved yield (Table 10). A 2-spray programme of the conventional stem extension timings (6 + 8) also gave significant control of eyespot and

lodging. The degree of control declined by GS 30/31 - 33/37 (timings 7 + 9). However, a similar programme applied at mid-tillering (timings 5 + 7), improved eyespot control and lodging. In comparison with prochloraz, flusilazole was less effective at controlling eyespot, although effects on lodging and yield did not always follow this trend.

For the multiple prochloraz sprays, eyespot and yield were negatively correlated ($r = -0.817$, $p \leq 0.001$), the correlation between eyespot and lodging was less evident ($r = 0.603$, $p \leq 0.005$) and lodging was not as closely associated with yield ($r = -0.448$, $p \leq 0.02$). The association between yield and eyespot was even stronger for the flusilazole treatments ($r = -0.978$, $p \leq 0.001$). Lodging with yield and eyespot with lodging were less closely associated ($r = -0.760$, $p \leq 0.01$ and 0.603, $p \leq 0.05$ respectively).

Table 7. Mean yield and Eyespot Index for 8 sites, 1992-93

	Yield t/ha			Eyespot Index		
	Prochloraz	Flusilazole	Mean	Prochloraz	Flusilazole	Mean
Untreated	7.82		7.82	48.62		48.62
+ + + +	8.50	8.42	8.46	26.61	32.03	29.32
+ + +	8.28	8.33	8.30	28.39	31.98	30.19
+ + +	8.25	8.27	8.26	29.70	34.73	32.22
+ +	8.29	8.18	8.23	30.48	35.30	32.89
+ +	8.27	8.20	8.24	34.57	37.21	35.89
+	7.98	7.88	7.93	38.79	38.86	38.82
+	7.95	7.95	7.95	41.26	41.94	41.60
+	7.96	8.02	7.99	40.67	41.30	40.98
+	8.14	8.04	8.09	35.71	39.11	37.41
+	8.23	8.22	8.22	40.31	42.20	41.25
+	8.16	7.95	8.06	41.62	42.35	41.99
+	8.22	8.04	8.13	34.8	44.93	39.86
Mean	8.19	8.12		35.24	38.29	

L.S.D.	Yield	EI
Untreated vs mean prochloraz (p) & flusilazole (f)	0.112	3.68
Mean p. vs mean f	0.059	1.97
Untreated vs mean timing	0.180	5.90
Mean timings	0.21	6.81

Table 8. Mean yield and Eyespot Index for 2 sites, 1994

	Yield t/ha			Eyespot Index		
	Prochloraz	Flusilazole	Cyprodinil	Prochloraz	Flusilazole	Cyprodinil
Untreated		9.24			23.33	
+ + + +	9.48			12.22		
+ + + +	9.59			14.00		
+ + +	9.37			11.56		
+ + +	9.69			16.44		
+ +	9.13			18.67		
+ +	9.34			14.89		
+ +	9.44			22.67		
+ +	9.25			14.00		
+ +	9.66			18.22		
+ +	9.27			18.89		
+	9.02			24.22		
+	9.14	9.21	9.38	18.00	25.78	24.00
+	9.32			26.67		
+	8.96	9.34	9.59	19.33	21.33	17.33
+	9.28			21.56		
+	9.46	9.42	9.07	19.56	20.22	18.89
+	9.36			18.22		
+	9.77			18.34		

LSD — There were no significant differences between any of the treatments.

Table 9. Mean yield and Eyespot Index for 4 sites, 1995

	Yield			Eyespot Index		
	Prochloraz	Cyprodinil	Mean	Prochloraz	Cyprodinil	Mean
Untreated	8.31		8.31	25.34		25.34
+	8.72	8.67	8.69	14.22	17.78	16.00
+	8.90	8.60	8.75	15.83	16.00	15.91
+	8.74	8.55	8.64	15.82	17.33	16.57
+	8.75	8.70	8.73	16.36	13.78	15.07
+	8.61	8.75	8.68	16.44	10.67	13.55
Mean	8.74	8.65		15.73	15.11	

L.S.D.	Yield	EI
Untreated vs mean prochloraz & cyprodinil	0.178	5.17
All other comparisons non-significant		

Table 10. Effects of multiple sprays on eyespot lodging and yield, Markle Mains, 1992-4

Timings	Eyespot index	Lodging %	Yield t/ha	Eyespot index	Lodging %	Yield t/ha
2 3 4 5 6 7 8 9		Prochloraz			Flusilazole	
untreated	47.77	34.85	9.36	47.77	34.85	9.36
+ + + +	10.12	0	10.38	28.04	29.1	9.92
+ + + +	24.26	16.8	10.09			
+ + +	8.26	0	10.23	31.53	2.5	10.14
+ + +	19.74	4.0	10.16	39.68	20.8	9.78
+ + +	27.37	16.8	10.55			
+ +	27.82	16.8	9.74			
+ +	24.26	16.8	9.98			
+ +	34.04	16.8	9.78			
+ +	15.79	5.0	10.02	33.38	8.8	9.91
+ +	27.50	6.1	9.98	46.33	33.3	9.69
+ +	29.15	16.8	9.81			
+ +	26.04	16.8	9.22			
+ +	31.37	15.8	10.03			
L.S.D.	9.478	15.58	0.344	9.478	15.58	0.344

All single-spray prochloraz timings gave significant reductions in eyespot, but no single timing was better than any other (Table 11). Early sprays of prochloraz controlled eyespot better than some of the flusilazole sprays, but there was no consistent trend. Lodging was also reduced by all timings, the more when prochloraz was applied at mid-tillering (timing 5). Sprays at the start of stem extension (timings 7 and 8) had no effect on lodging. Timing 9 (GS 33/37) was evaluated in year 3 only. In that year, split sprays (2+7 and 2+9, Table 8) were included to investigate the possibility of combining eyespot control at early tillering with improved lodging control at mid-tillering. However, these did not show a yield benefit over other split treatments (Table 12). Eyespot, lodging and yield were all unaffected by single sprays of flusilazole, although there was a tendency for lodging to be best controlled by the mid-tiller timings and to decline by stem-extension.

Table 11. Effect of single sprays on eyespot, lodging and yield, Markle Mains

Timings	Eyespot index	Lodging %	Yield t/ha	Eyespot index	Lodging %	Yield t/ha
1 2 3 4 5 6 7 8 9	Prochloraz			Flusilazole		
untreated	47.77	34.8	9.36	47.7	34.8	9.36
+	30.87	16.8	9.97			
+	30.5	16.1	9.79	42.49	38.3	9.03
+	32.68	16.1	9.62	46.46	26.1	9.62
+	41.03	21.6	9.76	42.57	21.6	9.57
+	31.10	4.5	9.82	37.87	20.0	9.70
+	32.53	17.0	10.13	42.04	20.0	9.76
+	33.74	20.9	10.96	39.64	21.1	9.68
+	34.01	18.7	9.84	39.96	34.1	9.60
+	31.82	16.8	10.43			
L.S.D.	9.478	15.58	0.344	9.478	15.58	0.344

For the single prochloraz and flusilazole sprays there was a strong negative correlation between eyespot and yield ($r = -0.907$, $p \leq 0.001$ and -0.944, $p \leq 0.0001$, respectively). That between eyespot and lodging was less marked, ($r = 0.603$, $p \leq 0.001$ for the prochloraz and $r = 0.603$, $p \leq 0.01$ for the flusilazole treatments). Lodging was less closely associated with yield ($r = -0.583$, $p \leq 0.001$) for prochloraz, although there was a slightly stronger association for the flusilazole treatments ($r = -0.722$, $p \leq 0.001$).

Similar correlations were apparent in the Markle Mains results for 1995.

Table 12. Effect of single sprays on eyespot, lodging and yield, 1993-94 season only

Treatments*	Yield t/ha	% lodging	Eyespot Index
	9.94	37.5	49.7
p	10.92	6.0	22.0
p	11.06	6.5	17.7
p	11.05	4.7	21.0
p	11.13	3.5	25.6
p	10.82	7.0	23.7
c	10.50	22.5	35.7
c	10.74	16.3	41.0
c	10.58	13.3	44.0
c	10.76	31.5	41.3
c	10.95	27.0	31.0
L.S.D.	0.589	16.84	11.11

*Single sprays on 5.12.94, 14.2.95, 14.3.95, 14.4.95, 1.5.95
p = prochloraz; c = cyprodinil

There was a reasonable correlation between eyespot levels at GS87 and crop yield, but in three of the four years, the level of eyespot at the start of the season was at or below 20%, and in many cases below 4%, until after the watery ripe growth stage (Table 13). The correlation between yield and lodging, also at GS87, was poor yet highly significant. This suggests that despite high levels of lodging, the plot harvester was able to retrieve the grain. The correlation between eyespot and lodging was strong, but fungicides, especially prochloraz, applied at tillering resulted in the least lodging, due possibly to a growth regulatory effect of the fungicide rather than a direct response to eyespot control.

Table 13. Overall correlation analysis, Markle Mains, 1992-95

Yield	1.0		
Eyespot	-0.507***	1.0	
Lodging	-0.474***	0.815***	1.0
	Yield	Eyespot	Lodging

1.3.3.5 Relationships between yield, eyespot and the occurrence of R- and W-pathotypes

Correlation analysis showed much variation across the site/year combinations in the relationships between crop loss, eyespot level the proportion of R- and W-isolates separately and together. There was also no consistent pattern, although when severe eyespot was controlled well by fungicide it sometimes caused significant yield reductions. The results (Table 14) indicated that yield was negatively correlated with the eyespot index at Cherhill (1992), Markle Mains (each year except 1994), and at Avebury and Goathurst (1993). Yield was similarly correlated with the percentage of R-type isolates at Markle Mains (1992) and the percentage of W-type isolates or DNA-based estimates of W-type at Markle Mains (1992, 1993 and 1995). The eyespot index was positively correlated with isolates or DNA-based estimates of the R-type at Markle Mains (all 4 years), Goathurst (1993), Morley and Stonham (1995). Similarly, eyespot correlated well with the incidence of isolates or DNA-based estimates of the W-type at Markle Mains in the 3 years, 1992-94. The incidence of R- and W-types was also correlated at three of the 14 sites and there was no real indication of a negative correlation between the two pathotypes. These significant effects were also apparent in cross-site and cross-year analyses (Table 15).

1.3.3.6 DNA Diagnostics

Estimates of W- and R-pathotypes of eyespot and their combination at each site is presented in Appendix 1.

Stonham. DNA diagnostics showed that the R-type predominated; the W-type was detected only at low levels in the final sample (20.7.95). The R-type was not detected in December but reached moderate levels in February and March. This level appeared to remain similar through April and May, before increasing again in July. The analyses of February samples indicated that applications of prochloraz and cyprodinil in December both reduced R-type eyespot, and similarly for the March analyses. However, samples in April, May and July showed no differences between treated and untreated plots. Plants from plots treated with cyprodinil in April and May appeared to contain less eyespot than those in untreated plots or plots treated at other times.

Morley. Diagnostics indicated that the level of eyespot was very low. Neither W- nor R-pathotypes were detected, except in samples taken in July when low levels of the R-type and very low levels of the W-type were detected in untreated plots and in most of the plots treated with cyprodinil.

Markle Mains. Both W- and R-types were detected. Similar levels of both types were detected in March-August samples, whereas in samples taken in May the R-type predominated. Only four samples were analysed. No eyespot was detected in December. Low levels of both pathotypes were detected in samples taken in March, although plots treated with prochloraz and cyprodinil in December appeared to have slightly lower levels than untreated plots. The level of both pathotypes was moderate in final samples from untreated plots where there was a high average level of eyespot. The level of the R-type in cyprodinil-treated plots was similar to that in untreated plots, irrespective of the time of application, whilst that of the W-type was variable but appeared to be less in later-treated plots (April and May). In contrast, the level of both pathotypes, in particular the W-type, was lower than that in untreated plots. The overall level of eyespot was most reduced where p[rochloraz was applied in either February, April or May.

Rosemaund. Only three samples were analysed. The final sample was not submitted for processing and so results couold not be related to yield effects. Rosemaund was the only site where eyespot (W-type) was detected in December, indicating that infection was intitiated earlier than at the other sites. Both pathotypes were detected later and neither was predominant. In March, moderate levels of both pathotypes were detected and the eyespot level appeared to be reduced where either prochloraz or cyprodinil had been used in December. The levels of both pathotypes was low and variable in the final samples (8 May) and no relationship between the eyespot estimates and treatment could be made.

Table 14. Correlation coefficients for yield, eyespot index and % R- and W-pathotypes for individual sites

1992	Yield	EI	%R	*1993*	Yield	EI	%R
Cherhill				**Avebury**			
EI	-0.41*			EI	-0.524**		
%R	-0.17	0.334		%R	0.025	0.027	
%W	-0.041	0.037	0.084	%W	-0.248	0.146	-0.257
Lambourn				**Goathurst**			
EI	0.068			EI	-0.570***		
%R	#	#		%R	-0.243	0.742***	
%W	#	#	#	%W	#	#	#
M a r k l e				**M a r k l e**			
Mains				**Mains**			
EI	-0.861***			EI	-0.670***		
%R	-0.829***	0.871***		%R	-0.331	0.584**	
%W	-0.619***	0.617***	0.655***	%W	-0.456*	0.460*	0.600**
Terrington				**Terrington**			
EI	-0.026			EI	-0.267		
%R	0.265	0.252		%R	0.297	0.010	
%W	0.138	-0.004	-0.093	%W	-0.034	-0.176	-0.135

1994				*1995*			
				Morley			
				EI	0.086		
				%R	0.295	0.675*	
				%W	-0.347	-0.263	-0.401
				Rosemaund			
				EI	-0.160		
				%R	-0.114	0.144	
				%W	-0.293	0.493	0.402
M a r k l e				**M a r k l e**			
Mains				**Mains**			
EI	-0.315			EI	-0.814**		
%R	-0.223	0.778***		%R	-0.532	0.783**	
%W	-0.154	0.717***	0.625***	%W	-0.729*	0.535	0.357
Terrington				**Stonham**			
EI	0.055			EI	-0.570		
%R	#	#		%R	-0.599	0.938***	
%W	0.035	0.002	#	%W	-0.234	0.093	0.255

- no isolates of the R- or W-pathotype made

Table 15. Correlation coefficients for yield, eyespot index and % R- and W-pathotypes for individual years and all years combined

		Yield	EI	R
1992	EI	0.183		
	%R	-0.110	0.209*	
	%W	0.009	0.240*	0.614***
1993	EI	-0.520***		
	%R	-0.009	0.398***	
	%W	-0.172	0.080	0.089
1994	EI	-0.472**		
	%R	0.317*	0.119	
	%W	0.451**	-0.080	0.84***
1995	EI	0.209		
	%R	0.133	0.830***	
	%W	0.097	0.517***	0.439**
1992-95	EI	-0.006		
	%R	0.067	0.517***	
	%W	-0.0068	-0.127	0.252*

1.3.4 Discussion

Results from these experiments suggest that, as a limiting factor in cereal production, eyespot still presents a variable, inconsistent and largely unpredictable risk. The emergence of the R-type as, on average, the predominant pathotype in field populations of *P. herpotrichoides,* compounds the problem further since there are no commercially available fungicides that give efficient and consistent control of eyespot caused by the R-type. Whereas prochloraz, flusilazole and cyprodinil appear able to give a good and relatively consistent degree of control of W-type eyspot, only prochloraz appears capable of giving good control of R-type eyespot. However, even prochloraz is erratic in performance against the R-type.

Among the prochloraz applications made at single timings, none could be identified as the best for controlling eyespot. However, there was a tendency for the earlier appliocations to perform better than the late ones, especially at Markle Mains where multiple sprays offlusilazole and cyprodinil appear able to give a good and relatively consistent degree of control of W-type eyespot, only prochloraz appears capable of giving good control of R-type eyespot. However, even prochloraz is erratic in its performance against the R-type. prochloraz and, to a much lesser extent, also flusilazole gave the greatest reductions in lodging. At other sites there was no such trend, and mid- or later season timings gave the greatest eyespot reductions. Despite strong correlations between eyespot, lodging and yield, exceptionally one or more of the single timings increased yield compared to all other single-sprays, multiple-sprays and the untreated control. The influence of these fungicide

treatments and other late sprays of all three fungicides on other leaf diseases cannot be discounted, despite attempts to eliminate effects of other diseases by over-spraying all experiments with broad-spectrum fungicides at GS33-37 and 49-55.

The results showed that eyespot incidence (percentage plants/tillers affected) between December and May cannot be relied upon to indicate the potential eyespot level at grain-filling. Even increasing the eyespot threshold from 20% to 40% tillers affected did not improve the success of predicting severe attacks, since sites where the thresholds were lower than 20% experienced moderate to severe eyespot during grain-fill. However, 14 sites is probably far too small a sample upon which to base any useful forecast of eyespot based on an early-season threshold. Establishing a relationship between early- and late-season disease levels may also be complicated by changes in the relative proportions of the R- and W-pathotypes during the different times of the season. Both of the pathotypes occur early in the season when the W-pathotype may predominate, but as the season progresses the R-pathotype becomes predominant. This trend was confirmed by both conventional isolation techniques and DNA diagnostics (Table 16).

Greater success in defining eyespot risk might occur from collecting data from a much larger number of sites than was used in this project. Visual and DNA eyespot diagnosis to establish the proportion of R- and W-types should then be performed on samples taken on four occasions each season, from untreated plots and those treated with fungicide (farm applied to reduce the work load). This investigation could be combined with the CSL/ADAS annual national survey of diseases in winter wheat and winter barley.

The levels of eyespot in samples subjected to visual and DNA diagnosis appeared to be comparable, although more rigorous tests are needed if the DNA diagnostic is to become a benchmark test for determining the accuracy of visual estimation, particularly by non-experts. It was apparent that the DNA diagnostic was able to detect both pathotypes before eyespot lesions became obvious, thus providing an earlier warning than that achieved by visual inspection. However the predictive value of being able to detect eyespot early needs further investigation, (see also Section 1.1).

The development of eyespot to damaging levels appeared to be associated with the predominance of the R-pathotype in the population. However, results of the DNA diagnostics suggested that this was less marked in that year than in the other years. Clearly, the R-pathotype is able to cause substantial yield reductions when eyespot becomes moderate to severe, and even more so when lodging occurs.

In terms of fungicide timing, results from the 14 experiments failed to reveal that any one timing of prochloraz, flusilazole or cyprodinil or any combination of timings of prochloraz and flusilazole as consistently the best treatment for controlling eyespot and lodging or maintaining yield potential. Overall, prochloraz was significantly superior to flusilazole and cyprodinil but there was considerable variation across sites often with flusilazole or cyprodanil performing better than prochloraz. Thus, on any one site prochloraz was considerably poorer in disease control than flusilazole. This could not be explained on the basis of the relative incidence of the R- and W-pathotypes. Similar results were obtained with cyprodinil both overall and on individual sites.

Table 16. Results of DNA diagnostic estimates of W- and R-pathotypes and their combination (B)

Treatment and Sample Date

Stonham	15.12.94			14.2.95			14.3.95			11.4.95			2.5.95			20.7.95		
	W	R	B	W	R	B	W	R	B	W	R	B	W	R	B	W	R	B
untreated																		
p				0	0	0	0	1	1	0	1	1	0	1.3	1.3	0	1.7	1.7
p				0	1.7	1.7	0	1	1	0	1	1	0	1	1	0	2	2
p							0	1.7	1.7	0	1.7	1.7	0	1.3	1.3	0	1.7	1.7
p										0	1.3	1.3	0	1	1	0	1.7	1.7
p													0	1	1	0.7	2	2.7
c				0	0	0	0	0.7	0.7	0	1	1	0	1.3	1.3	0.3	2	2
c				0	1.7	1.7	0	1	1	0	0.7	0.7	0	1.3	1.3	0.3	1.3	1.6
c							0	1.7	1.7	0	1	1	0	1	1	1	1.3	2.3
c										0	1.3	1.3	0	1	1	0	0.7	0.7
c													0	1.3	1.3	0	0.3	0.3

Morley	15.12.94			14.2.95			14.3.95			11.4.95			2.5.95			20.7.95		
	W	R	B	W	R	B	W	R	B	W	R	B	W	R	B	W	R	B
untreated	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3	0.8	1.1
p				0	0	0	0	0	0	0	0	0	0	0	0	0	1.3	1.3
p				0	0	0	0	0	0	0	0	0	0	0	0	0	1.8	1.8
p							0	0	0	0	0	0	0	0	0	0	1	1
p										0	0	0	0	0	0	0	1.3	1.3
p													0	0	0	0	1	1
c				0	0	0	0	0	0	0	0	0	0	0	0	0.5	1	1
c				0	0	0	0	0	0	0	0	0	0	0	0	0.5	1	1.5
c							0	0	0	0	0	0	0	0	0	0	0.8	1.3
c										0	0	0	0	0	0	0.5	0	0
c													0	0	0	0.5	0	0.5

Table 16 (continued)

Markle Mains	12.12.94			14.2.95			13.3.95			11.4.95			8.5.95			8-8-95		
	W	R	B	W	R	B	W	R	B	W	R	B	W	R	B	W	R	B
untreated	0	0	0				0.8	0.8	1.6				0.3	2	2.3	2	2.3	4.3
p	0	0	0				0	0	0				0	0	0	1.8	1	2.8
p							0	1	1				0	0.5	0.5	0	1.5	1.5
p							0	0.5	0.5				0	0.3	0.3	0.5	1	1.5
p													0	1	1	0	1	1
p													0	1	1	0.3	1	1.3
c	0	0	0				0.5	0	0.5				0	2.5	2.5	2	2.8	4.8
c							0.3	1.5	1.8				0	1.3	1.3	0.3	2.5	2.8
c							0.3	1	1.3				0	1.5	1.5	1.8	3	4.8
c													0	1.8	1.8	1	2.5	3.5
c													0	3	3	0	2.8	2.8

Rosemaund	12.12.94			14.2.95			13.3.95			11.4.95			8.5.95			8.8.95		
	W	R	B	W	R	B	W	R	B	W	R	B	W	R	B	W	R	B
untreated	0.3	0	0.3				1.3	1	2.3				1	1.5	2.5			
p	0	0	0				0.3	0.3	0.6				0	0.8	0.8			
p							1.5	0.5	2				0	0.8	0.8			
p							2.3	0	2.3				0.3	0.3	0.6			
p													0.3	0	0.3			
p													0.5	0.5	1			
c	0	0	0				1	0	1				0.5	0.5	1			
c							1.8	0	1.8				0	0.8	0.8			
c							0.8	0.8	1.6				0	0	0			
c													0	1	1			
c													0	0	0			

Greater knowledge of the activity of these fungicides against the R- and W-pathotypes, both separately and together, is needed if the efficiency of eyespot control is to be improved with such moderately effective chemicals. Probably, more effective fungicide products are needed for consistently reliable levels of eyespot control. Currently, eyespot appears to be over-rated for its importance as a disease of winter cereals. This may be simply because of the inability of current fungicides to control it effectively and therefore reveal true rather than perceived yield losses.

2. FUSARIUM DEVELOPMENT AND CROP LOSS

2.1 FIELD EXPERIMENTS

D.R. Jones (ADAS, Rosemaund), S. Oxley (SAC, Edinburgh), A.K. Lees, D.W. Parry (Harper Adams) and R.J. Cooke (Morley)

2.1.1 Introduction

A series of field experiments was initiated to determine the effect of fusarium on grain yield and quality, and to establish the timing of any suitable fungicide application for disease control.

2.1.2 Methods

In order to estimate the yield loss caused by fusarium, it was necessary to generate differing severities of disease and then to relate yield to disease severity. The method adopted in this part of the project was to attempt to control fusarium development at different times during the growth of the crop by means of fungicides. This approach had not been possible previously because of the lack of fungicides with consistent activity against *Fusarium* species. The present work was made possible when Ciba Agriculture agreed to provide two recently-introduced fungicides known to have activity against *Fusarium* spp. These were fenpiclonil, used as a seed treatment and fludioxonil (previously referred to as CGA 173506), used as a foliar spray, in order to manipulate the development of *Fusarium* species. (N.B. In this section, reference to fusarium and *Fusarium* species includes *Microdochium nivale*). Fenpiclonil was marketed as Beret seed treatment from 1993-1995, and fludioxonil as Beret Gold Seed treatment from 1995.

There were eleven field experiments, 7 funded by HGCA and 4 by MAFF, in the three years of the project, as the following sites:

SAC Edinburgh (HGCA)	1991/92, 1992/93, 1993/94
Morley Research Centre (HGCA)	1991/92, 1992/93, 1993/94
Harper Adams Agricultural College (HGCA)	1991/92
ADAS Rosemaund (MAFF)	1991/92, 1992/93
ADAS Terrington (MAFF)	1991/92, 1992/93

The basic principle of the design was that protection against fusarium started at a series of six dates from November to May, and that after each plot received its first spray, the protection was maintained by sprays at monthly intervals until May; there was also an untreated control. The fungicide was fludioxonil (as CGA 173506) at 1.0 l/ha. In 1992/93 and 1993/94, there were additional treatments designed to control each of fusarium, eyespot and sharp eyespot, and each combination. The fungicide for eyespot control was prochloraz (as Sportak 45 at 0.9 l/ha), and an experimental fungicide was used against sharp eyespot. All spray treatments were superimposed on each of two seed treatments in a two-way factorial layout. Seed treatments in were fenpiclonil in each year, and either organomercury (in 1991/92) or untreated (in 1992/93 and 1993/94). At Terrington in 1992/93, it was not

possible to drill the experiment as planned, due to very wet soil conditions in autumn 1992, and the spray treatments were superimposed on a crop grown from seed treated with carboxin plus thiabendazole. All experiments were on first wheats, to minimise the risk of other stem-base diseases, and all received overall fungicide applications at GS39 and GS59, to control foliar diseases.

For all experiments, stem-base diseases were assessed at GS31 and GS75. Nodal and internodal fusarium infections were clearly distinguishable symptoms at GS75, and were recorded separately. Results were expressed as indices on a 0 - 100 scale where 0 = no disease and 100 = all tillers severely affected. Trials were harvested, and yield, thousand-grain weight and specific weight recorded.

2.1.3 Results

Full results of the effects of treatments were presented in each Annual Interim Report. A summary is given in Table 17, which provides an indication of fusarium incidence at each site, the degree of control given by the full protection programme and the magnitude of yield increase from fungicide treatment.

Table 17. Disease indices and yields from untreated plots and from the full treatment of fenpiclonil from November to May (7 sprays); results for nil seed treatment (organomercury seed treatment in 1991/92 only).

Year	Site	Fusarium (nodal) index		Fusarium (internodal) index		Yield (t/ha)	
		Untreated	Full	Untreated	Full	Untreated	Full
1991/92	Edinburgh	35.6	25.3	27.1	38.2	7.85	8.88
1991/92	Morley	48.3	35.1	52.9	41.8	7.83	8.04
1991/92	Rosemaund	58.2	19.6	35.6	32.0	5.88	6.15
1991/92	Terrington	50.7	56.0	44.9	32.0	7.37	7.56
1991/92	Harper Adams	57.3	30.7	44.0	25.8	5.76	6.19
1992/93	Edinburgh	7.1	3.1	31.1	29.3	6.98	8.09
1992/93	Morley	21.3	7.1	10.0	10.2	7.51	8.03
1992/93	Rosemaund	57.8	18.2	27.1	12.9	5.39	5.68
1992/93	Terrington	4.0	1.9	32.3	34.3	9.95	10.33
1993/94	Edinburgh	6.2	1.8	15.1	8.0	7.99	8.52
1993/94	Morley	21.8	2.2	13.3	2.7	7.63	7.72

In each year, *F nivale* was the predominant species during the winter and spring, but *F culmorum* became prevalent by GS75 at most sites.

In two of the 1991/92 trials (Morley and Rosemaund), all programmes of fludioxonil sprays gave a reduction in fusarium infection of nodes, whereas only the programmes which started in November or January had any effect at Harper Adams and none of the programmes had any effect at the other two sites. Internodal infection was reduced to a small extent by the earliest spray at Terrington and by most programmes at Harper Adams and Morley. At Edinburgh, treatments gave a large reduction in fusarium incidence at GS31, but this effect had disappeared by GS75. In addition, sprays applied up to March

reduced late-season eyespot. There were statistically significant ($P<0.05$) yield increases from all fungicide programmes at Edinburgh and Rosemaund, and from most at Morley, but not at the other two sites. There were no consistent effects on grain quality.

In 1992/93, nodal fusarium levels at Edinburgh, were higher in plots which did not receive fludioxonil until May than in any other spray treatments. The incidence of internodal fusarium was higher than of nodal fusarium, but there were no significant differences in internodal fusarium. At Morley, all treatments reduced nodal infection, though the May spray was less effective than the earlier sprays. Effects on internodal infection were similar but smaller. At Rosemaund, the earlier applications gave the greatest reduction in nodal fusarium, but effects on internodal infection were less consistent. At Terrington, fusarium at GS31 was reduced by all fludioxonil treatments applied by that date, but these differences were not evident at GS75. As in 1991/92, there were large yield increases from all fungicide treatments at Edinburgh, and also increases in thousand-grain weight. There were also significant, though smaller, yield increases at Morley from all fungicide treatments, with the smallest increase from the May application. Yield increases were associated with increases in thousand-grain weight, but there were no effects on specific weight. There were no differences in yield or grain quality at Rosemaund or Terrington.

In 1993/94, stem-base disease levels were lower than in the first two years. At Morley, nodal fusarium was reduced by all fludioxonil and prochloraz treatments, and internodal fusarium showed a similar trend, but there were no significant differences in either nodal or internodal fusarium at Edinburgh. Eyespot indices were reduced by all prochloraz and fludioxonil treatments at both sites. All spray treatments gave significant increases in yield and thousand grain weight at Edinburgh, but no effect on specific weight. There was also an effect of seed treatment on thousand grain weight, which was significantly lower from fenpiclonil treatment than from untreated seed. At Morley, there were small but statistically significant yield increases from some of the fludioxonil treatments, but no significant effects of treatments on thousand grain weight or specific weight.

Selected results from the individual experiments, and the mean results, are presented in Appendix.

The only individual site where there was a significant regression of yield on any disease was Morley in 1992/93, where there was a significant regression of yield on nodal fusarium index:

$$Y = 8.39 - 0.028\ F_n;\ r^2=0.741,\ P<0.001$$

where Y = yield, F_n = nodal fusarium index.

When data for all 11 sites in the 3 years were aggregated, regressions of mean yield from each treatment on each of nodal and internodal fusarium at GS75 and a multiple regression on both categories of diesase were as follows:

$Y = 8.62 - 0.0372\ F_n$; $r^2 = 0.662$, $P<0.001$ (Figure 2.1.1);

$Y = 8.83 - 0.0411\ F_i$; $r^2 = 0.286$, $P=0.049$ (Figure 2.1.2);

$Y = 8.21 - 0.0490\ F_n + 0.0250\ F_i$; $r^2 = 0.646$, $P = 0.001$;

where Y = mean yield, F_n = mean nodal fusarium index, F_i = mean internodal fusarium index.

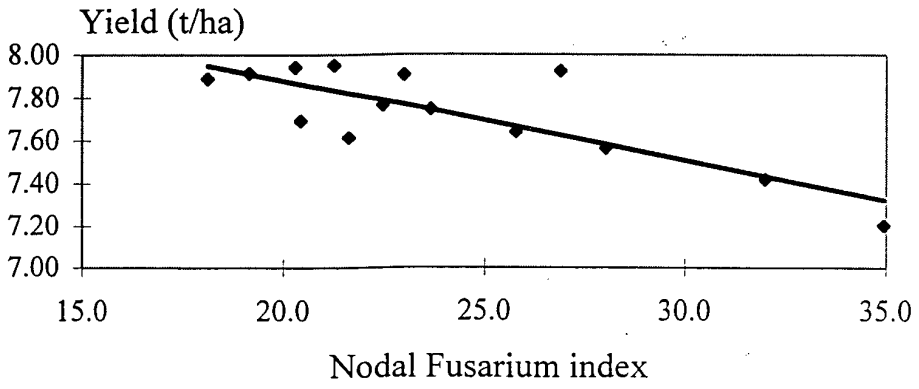

Fig. 16. Relationship between mean yield of each treatment and nodal fusarium index at GS75, according to equation (2)

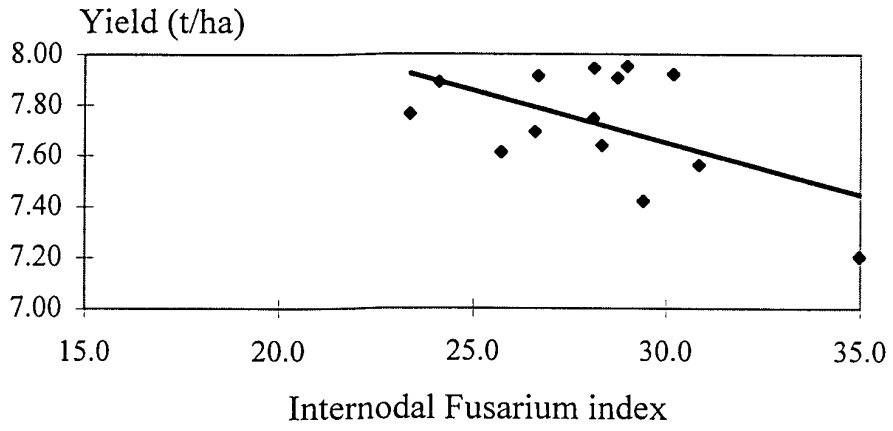

Fig. 17. Relationship between mean yield of each treatment and nodal fusarium index at GS75, according to equation (3)

2.1.4 Conclusions

In some experiments, fludioxonil spray programmes which started at any time up to April gave some control of nodal infection, whereas in others there was little, if any, effect of fludioxonil. Where there was an effect, the degree of control was very variable, the best being approximately 75% at Morley in 1992/93. Since fludioxonil is a protectant fungicide with no systemic activity, this suggests that the infections which cause nodal lesions may not occur until April at some sites. However, it is possible that, in some situations, the protectant fungicide prevented further development of lesions resulting from earlier infection. In contrast to nodal infection, fludioxonil had much less effect on internodal fusarium, with indications that the earlier spray timings were most effective (at Harper Adams and Terrington in 1991/92 and Rosemaund in 1992/93). The internodal lesions often appear to have developed from the crown, and it is not unexpected that a protectant fungicide had little effect on such deep-seated infection. The overall conclusion is that although the fungicide did give some control of stem-base fusarium, it did not give reliable control. In view of the long period during which infection occurred, it is probable that a fungicide with systemic activity would be required to give commercially acceptable control.

There were very few indications of any effects of fenpiclonil seed treatment on stem-base fusarium. There is evidence that fenpiclonil seed treatment gives good control of seed-borne fusarium, which can affect the incidence of stem-base fusarium later in the life of the crop. In the present series of experiments seed was not tested for fusarium but, in the absence of any recorded effects of seed treatment on the disease at either GS31 or GS75, it is probable that the main source of infection was soil-borne rather than seed-borne. It can be concluded that fenpiclonil, applied as a seed treatment, has little, if any, activity against soil-borne fusarium, probably due to its lack of systemic activity.

Considering data from individual sites, there was a relationship between crop yield and nodal fusarium only at Morley in 1992/93, where the fusarium index in untreated plots was only 28 and there were very few severe lesions. At some other sites treatments reduced fusarium to a similar degree (e.g. Harper Adams in 1991/92 and Rosemaund in 1992/93), but without any effect on yield. On the other hand, there were yield increases at Edinburgh in all 3 years and at Morley in 1991/92 which did not appear to be related to fusarium control. At Edinburgh, in 1991/92 there was some effect of fludioxonil on eyespot but there was not a significant relationship between eyespot and yield.

The overall regressions and the Morley experiment in 1992/93 indicate that there may be a relationship between stem-base fusarium, particularly nodal infection, and yield loss, but it does not prove a casual relationship. In order to establish the significance of stem-base fusarium on crop yield, sites with a higher incidence of severe lesions are required. However, for a disease which develops over a long period, it is probable that other factors affecting crop development will influence the effect of fusarium on crop yield, and the effects of the disease cannot be considered in isolation from crop physiology, nor from the interactions with other stem-base diseases.

Few conclusions can be drawn about interactions between stem-base diseases, since the only other disease to develop to appreciable levels was eyespot at Edinburgh. Prochloraz reduced eyespot, as expected. Fludioxonil also appeared to have some effect, but less than prochloraz.

Finally, the effect of stem-base fusarium on yield is not the only aspect of the disease which needs to be considered. Stem-base infection provides a source of inoculum for fusarium ear blight, which can affect both grain yield and grain quality. It is possible that control of stem-base fusarium may influence the risk of ear blight and, therefore, be of greater significance through its effect on ear blight than in direct effects on yield and grain quality.

2.2 EXPERIMENTS IN SEMI-CONTROLLED CONDITIONS

A.K. Lees, D.W. Parry (Harper Adams) and P. Nicholson (John Innes)

2.2.1 Introduction

The first year's work at Harper Adams aimed to create a fusarium disease gradient in field experiments by sequential applications of fungicide, and to monitor the effects of disease at GS31 and GS75 on yield (*see Section 2.1*). However, because the fungicides employed proved to be less effective than expected, attention became focused on studying the relationship between infection by *F. culmorum* and *M. nivale*, disease severity and yield in a partially-controllable experimental system in which disease severity could be varied mainly by artificial inoculation. In 1992/93, the first year of using this approach, disease was also varied by fungicide treatment. In this system, wheat was grown in rows in large plastic containers to simulate a crop grown outdoors. Disease severity gradients were generated by inoculation with different quantities and timings of the desired pathogen. Molecular techniques were employed to monitor infection.

2.2.2 Materials and Methods

1993 experiments. Seed of winter wheat, cv Mercia, treated with the fungicide Cerevax, was sown in potting compost in large plastic containers and grown outdoors under semi-natural conditions with drip-irrigation. Treatments consisted of artificial inoculation with *F. culmorum* and/or *M. nivale* with various applications of the fungicides fludioxonil (1l/ha) and Sportak Delta (1.25l/ha). Inoculum of *M. nivale* and *F. culmorum* (10,000 spores/g soil) was applied as a spore suspension to each container separately. The fungicides were applied at regular intervals throughout the season. At GS31 and GS75, plants were assessed for symptom severity (Scott & Hollins, 1974). A disease severity index was calculated for each treatment. At GS75, plants were recorded for presence of *Fusarium* species by making conventional isolations on potato dextrose agar from sections of stem base taken from the main tillers The pathogens were identified from spore and colony morphology. All treatments were harvested and the yield and 1,000- grain weight of each sample calculated to 85% dry matter.

1994 and 1995 experiments. These studied the effect of inoculum density and time of inoculation with *F. culmorum* and *M. nivale* on disease severity and yield. Mercia was again grown in the plastic containers. The treatments were inoculations with *F. culmorum, M. nivale* or an equal mixture of the two species, each inoculated at three different times: March, May and June in 1994, and February, April and May in 1995. At each time and with each species different inoculum concentrations were applied to containers in a plant row in order to create disease severity gradients. The concentrations were 0, 1,000, 5,000 and 10,000 spores/g soil in 1994, and 0, 500, 1,000 and 5,000 spores/g soil in 1995. Each concentration was replicated three times. At GS31 and GS75, plants were taken from each of the plots and assessed for disease, as in 1993. Stem base sections were also assessed for *F. culmorum*, *M. nivale* and other stem-base pathogens at the John Innes Centre, using

DNA-based diagnostic techniques as described in Section 1.2. Again, more precise quantification is possible using competitive PCR and will be carried out on these samples at a later date when competitor templates have been refined for the two pathogens. At harvest, yields and thousand grain weights at 85% dry matter were recorded.

2.2.3 Results

2.2.3.1 ***1993 experiment.*** At GS31, disease severity was greater in the treatments inoculated with *M. nivale* than in those inoculated with *F. culmorum* or uninoculated. However, by GS75, disease severity was similarly high in all treatments, except for those which had received six applications of fludioxonil where disease was significantly less than those receiving fewer fungicide applications.

Control treatments were infected with both *F. culmorum* and *M. nivale*. Of the uninoculated treatments, 7/10 stem bases which had received no fungicide application, and 9/10 which had received 6 fungicide applications were infected with *F. culmorum*. Fungicide application did not therefore appear to have an effect on the incidence of these species. In the inoculated treatments, the species isolated were predominantly those that had been applied. Average yields ranged from 4.9-7.9t/ha but it was difficult to establish any relationship between fungicide and inoculation treatments and yield. There was no significant difference in 1,000-grain weight between any treatments.

2.2.3.2 ***1994 and 1995 experiments.*** At GS31, there was more disease in those treatments which had been inoculated with the three highest spore concentrations each year than in the uninoculated treatments (Fig. 18(a), Table 18). Symptom severity also appeared to be related to inoculum density, being greatest in the treatments inoculated with 10,000 spores/g than in those receiving less inoculum. Similarly, at GS75, disease severity corresponded to inoculum density (Fig. 18(b), Table 18). There was more disease at GS75 on plants inoculated early in the season than later, at all inoculum concentrations. Disease was also greater with 5,000 spores/g compared with 1,000/g but not with 10,000/g compared with 5,000/g.

The increase in disease severity with increasing *F. culmorum* inoculum appeared to be related to a decrease in the average yield of the treatments, in both years (Table 19). In 1994, the treatments inoculated in March gave more disease at GS31 and GS75 than those inoculated later. This early inoculation was associated with striking reductions in grain yield. Among treatments inoculated in May 1994, a significant yield reduction was only evident at the highest inoculum concentration, and inoculations made in June resulted in no significant effects on yield.

Of the three inocula examined, *F. culmorum* appeared to have the greatest effect on yield. Treatments inoculated with mixed *F. culmorum* and *M. nivale* gave similar results to *F. culmorum* alone. However, average yields were generally less influenced by the mixed inoculum than by *F. culmorum* alone. Similar results were noted in those treatments inoculated with *M. nivale* alone in 1994. When applied in March, inoculation with *M. nivale* at all concentrations caused a significant reduction in average yield. However,

applied in May, only the highest inoculum concentration was associated with a yield reduction, and the June inoculation had no effect on yield (Table 19). There were no differences in yield between the controls of each treatment.

In general, the results in 1994 were supported by those in the 1995 experiment. However, in 1995, time of inoculation appeared to be less related to grain yield in all treatments than in 1994 (Table 19). The relationship between yield loss and inoculum concentration was strong for *F. culmorum*, *M. nivale* and the mixed inoculum. Yield reductions in the range of up to 25-30% were observed in some treatments which had received early, high-concentration applications. Yield reduction was related to inoculum concentration in every case, with increasing concentration giving increasing disease severity at both GS31 and GS75, and concomitant reductions in yield.

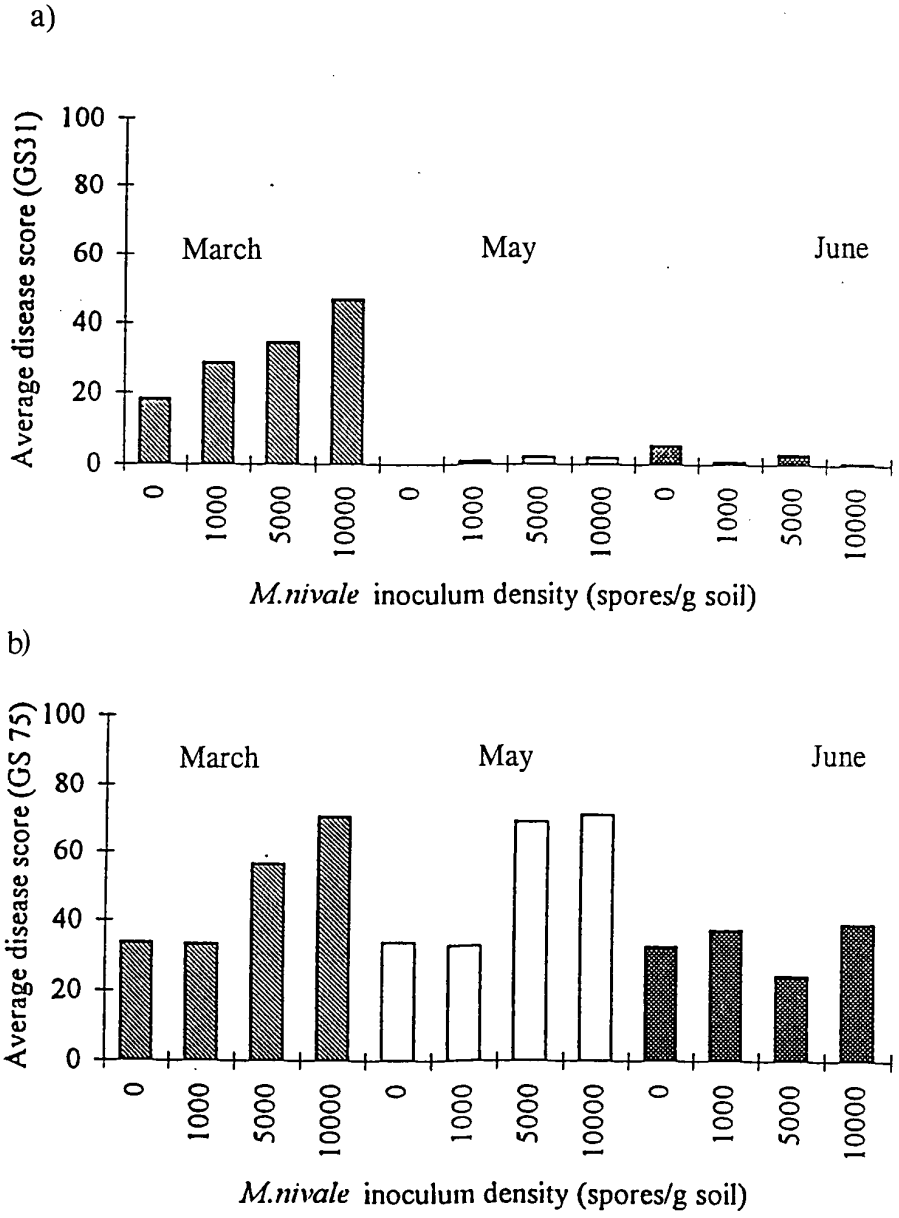

Fig. 18. Effect of inoculation by *M. nivale* at four inoculum concentrations in March, May and June on the average disease severity index of plants sampled at GS31 and GS75 in 1994

Table 18. Effect of inoculum concentration and timing on disease severity at GS31 and GS75 in 1995

Inoculum (conidia/g soil)	GS31	GS75		
	Inoculated Feb.	Inoculated Feb.	Inoculated April	Inoculated May
F. culmorum				
0	2.87	22.67	14.20	14.13
500	6.73	24.50	39.00	15.50
1,000	16.17	18.50	27.00	36.60
5,000	15.80	43.20	28.00	37.30
F.culmorum + M.nivale				
0	14.67	14.17	29.86	26.00
500	10.78	29.70	23.50	33.50
1,000	19.70	29.00	23.33	34.83
5,000	16.10	34.00	33.67	38.67
M.nivale				
0	9.40	23.50	17.00	37.60
500	18.33	25.80	28.17	25.83
1,000	25.70	29.17	26.45	28.50
5,000	22.50	32.17	36.50	37.17

Table 20 shows the results of DNA analysis for samples at GS31 and 75 in 1995. At GS31, plants which had received inoculum in February were assessed for the presence of *F. culmorum* and *M. nivale*. The amount of *F. culmorum* was low in uninoculated plants but increased with increasing inoculum load for plants which had been inoculated with either *F. culmorum* alone or with a mixture of *F. culmorum* and *M. nivale*. Colonisation of uninoculated plants by *M. nivale* was greater than by *F. culmorum* in the same plants. However, the amount of *M. nivale* was greater in plants inoculated with *M. nivale* or a mixture of *M. nivale* and *F. culmorum*, although the effect of conidia/g was not as distinct as that observed for *F. culmorum*. At GS75, the amount of *F. culmorum* detected in inoculated plants still appeared to be related to the inoculum load. In addition, *F. culmorum* was detected at higher levels in plants inoculated either in February or April than in plants inoculated in May. The amount of *M. nivale* at this time was generally less than that of *F. culmorum* and there was no obvious relationship between the amount of *M. nivale* detected and the inoculum density or the timing of inoculation.

Table 19. Average yield of winter wheat following inoculation with different concentrations of *F. culmorum, M. nivale* and a mixture of the two pathogens, on three occasions in 1994 and 1995.

Species applied and concentration (spores/g soil in 1994 and (1995)	Yield (t/ha)					
	Inoculation:					
	March, 1994	Feb., 1995	May, 1994	April, 1995	June, 1994	May, 1995
F.culmorum:						
0 (0)	8.13	7.79	7.38	8.09	8.27	8.57
1,000 (500)	5.58	7.48	8.61	7.07	8.95	7.58
5,000 (1000)	4.84	6.45	6.68	6.64	7.65	6.67
10,000 (5000)	5.24	4.93	5.59	4.94	7.36	4.76
F.culmorum + M.nivale:						
0 (0)	8.27	7.68	7.86	7.54	6.96	7.32
1,000 (500)	8.14	6.34	7.11	5.96	7.63	6.96
5,000 (1000)	5.98	5.67	6.72	5.73	7.83	5.63
10,000 (5000)	6.17	4.88	6.78	4.37	7.01	4.94
M.nivale:						
0 (0)	8.92	7.57	7.72	7.12	7.42	7.49
1,000 (500)	7.09	6.46	7.17	6.32	7.19	7.41
5,000	7.44	5.70	8.50	6.54	6.42	6.48
10,000	7.11	5.05	6.65	5.13	6.95	5.77

Table 20. **Estimated amount (0-3 scale, based on PCR band density) of *F.culmorum* and *M.nivale* in samples taken at GS31 and GS75 from plants inoculated with *F.culmorum*, *M.nivale* and a mixture of the two pathogens in 1995**

Inoculum (conidia/g soil)	GS31		GS75					
	Inoculated Feb.		Inoculated Feb.		Inoculated April		Inoculated May	
	F.culmorum	*M.nivale*	*F.culmorum*	*M.nivale*	*F.culmorum*	*M.nivale*	*F.culmorum*	*M.nivale*
F.culmorum								
0	0.4	0.6	1.7	1	1	1	0.7	1
500	1	0	2	1.7	2	2	1	1
1,000	1.7	0.3	1.7	0.7	2	1.7	1	1.7
5,000	2.7	0.7	3	2.7	2.3	1	0.7	1.7
F.culmorum + N.nivale								
0	0.2	0.7	0	1.3	1	0.7	2	0.7
500	1	1	2.3	1.3	2.3	0.7	0.3	0.3
1,000	1.3	1.3	1	2	3	1.7	1	0.3
5,000	1.3	1	1.3	1.3	3	0.7	0.7	0.3
M.nivale								
0	0	0.9	0.7	0	1.3	0.3	2	1
500	0.3	1.3	0.7	1.3	0.3	1.3	0.7	0.7
1,000	0	1.3	0	1.3	0.3	0.7	1.3	1.7
5,000	0.7	1	0.3	1.7	0.3	1	1	1.3

2.2.4 Conclusions

In contrast to experiments in the field, the container system, which allowed wheat plants to be grown outdoors in a manner similar to natural conditions, allowed for the provision of relatively uniform pathogen inoculum and for examination of the effects of fusarium in the absence of other pathogens such as *P. herpotrichoides*. In this system, the plants grew well and produced realistic yields. The conclusions can therefore be regarded as meaningful and applicable to natural field conditions.

In the initial experiment, in 1993, all the imposed treatments gave high disease severities at GS75. Infection also occurred in the control treatments, probably because of cross-contamination by splash dispersal of spores during irrigation. In 1994, the fungicide was only able to reduce disease severity when applied repeatedly and the only effect on yield was a reduction in those treatments in which the highest number of fungicide applications also reduced disease severity. It is likely that the fungicide, whilst controlling disease, also had a phytotoxic effect due to its repeated application.

In the following experiments, in 1994 and 1995, where disease was successfully varied by the timing and concentration of applied inoculum, clear evidence for reductions in yield caused by *M. nivale* and *F. culmorum* was obtained. Artificial inoculation by both *M. nivale* and *F. culmorum* gave reliable disease symptoms by GS31, in both years. Disease levels were low in the uninoculated plots at GS31, although some symptoms were observed. Despite the precautions taken to minimise cross contamination between trays, some disease in the control plots occurred. Seed, soil-borne inoculum and air-dispersed ascospore inoculum (*M. nivale*) could not be excluded. However, the aim of the experiment was to create a disease differential between control and inoculated plots, so low disease amounts in the control treatments were not a serious problem. Disease severity, at GS31, increased with increasing inoculum load. This effect appeared to become less evident at the highest concentration applied, suggesting that a maximum inoculum threshold had been reached.

Early inoculation had the greatest effect on final disease severity (at GS75), in 1994, independently of the species applied, and there was also a relationship with the inoculum load. At GS75, symptoms were evident on plants which had received a late (June) inoculation, although the level of disease was generally less, and not so much affected by inoculum concentration. In 1995, however, the time when inoculum was applied appeared to be less important, with inoculum load having the greatest effect on disease severity. These results suggest that, although late infection by *M. nivale* and *F. culmorum* is possible under these conditions and can contribute to disease severity, it is the early infections which are responsible for greatest disease levels.

Results of the DNA analyses confirmed that there had been contamination of treatments by both pathogens. The problem appeared greatest with *M. nivale,* probably because of its ability to produce air-dispersed ascospores. In addition, it is possible that the sensitivity of the primers used to detect this pathogen were higher than those used to detect *F. culmorum.* There is evidence, however, that colonisation by *F. culmorum* was greatest in plants inoculated early in the season and in those receiving the highest inoculum load. Thus, early

infection and exposure to high inoculum concentrations were reflected in the amount of *F. culmorum* detected late in the season, suggesting that these may be important factors in the development of this pathogen. The moderate background contamination by *M. nivale* obscured any similar relationship between time of inoculation and inoculum load for this pathogen. Application of a mixed inoculum with an equal number of conidia of *M. nivale* did not appear to affect the amount of *F. culmorum* detected, suggesting that any competition between the isolates of the two species did not result in a notable reduction in degree of colonisation by *F. culmorum* in plants later in the season.

Yield was reduced in treatments inoculated with *M. nivale* and *F. culmorum* according to inoculum density; there was no difference in the reduction in yield caused by the two different pathogens, or by the mixed inoculum. This suggests that despite the predominance of infections by *M. nivale* early in the season, followed by an increase in *F. culmorum* later, it is possible for both pathogens to infect winter wheat at all stages of the growing season, providing that the inoculum density is sufficient and the environmental conditions are favourable for growth. Those treatments which had received the highest density of inoculum of *M. nivale* and *F. culmorum*, at the earliest inoculation date in 1994, and had the highest disease scores at GS75, also had the lowest yields. In 1995, yield appeared to be related to inoculum concentration rather than time of application. These results therefore imply that early detection and eradication of the pathogens could be more effective as a control measure than later fungicide applications administered when disease symptoms were clearly visible.

It is difficult to relate the results from DNA analysis in 1995 to the yields obtained. A major limitation here is the absence of supportive analysis taken at other growth stages throughout the season. Hence all that is provided are 'snapshots' of disease at two key growth stages without any indication of disease progress between.

3. REFERENCES

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APPENDIX

FUSARIUM FIELD EXPERIMENTS, 1993

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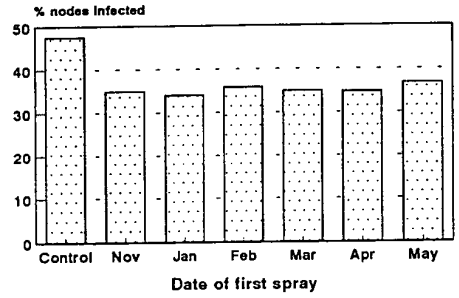
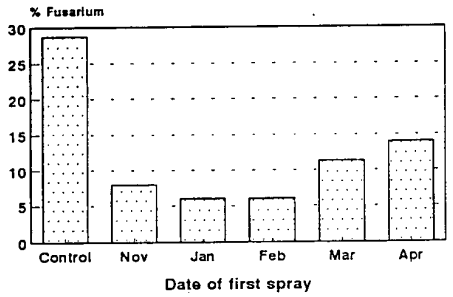
Nodal Fusarium GS75



Edinburgh
Fusarium at GS31



Morley
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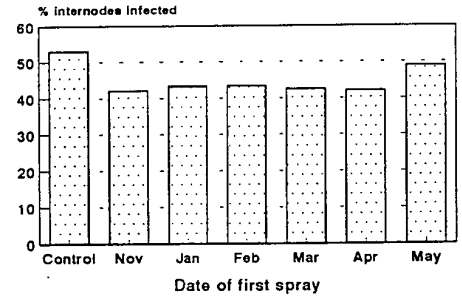
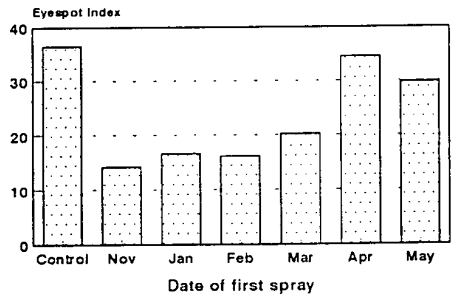
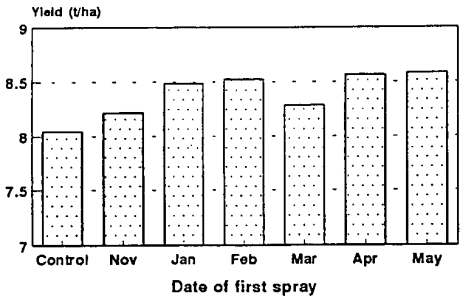


Edinburgh
Eyespot GS75



Morley
Yield



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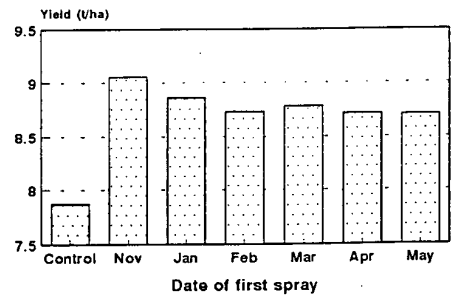
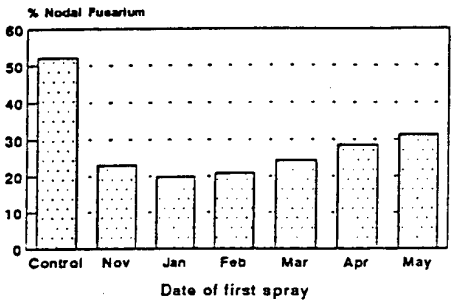
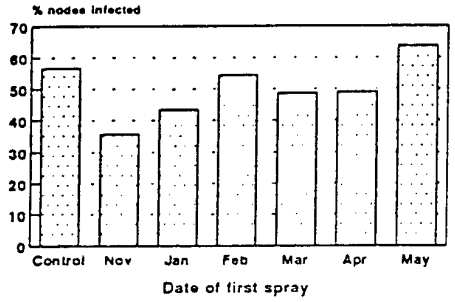
Yield



Rosemaund
Nodal Fusarium GS75



Harper Adams
Nodal Fusarium GS75



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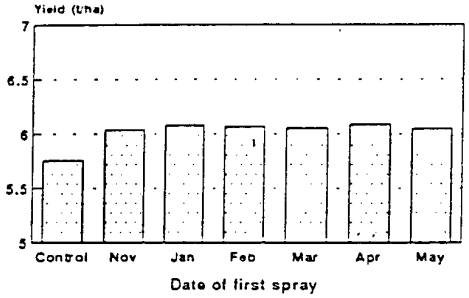

Harper Adams
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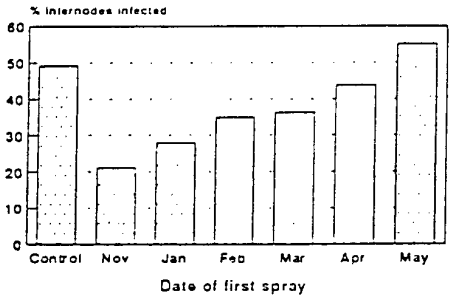
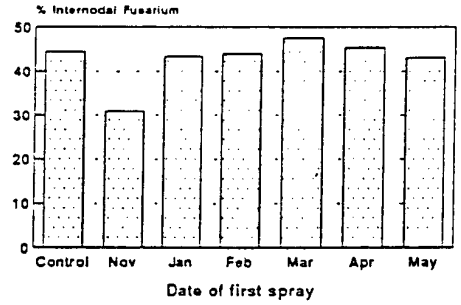
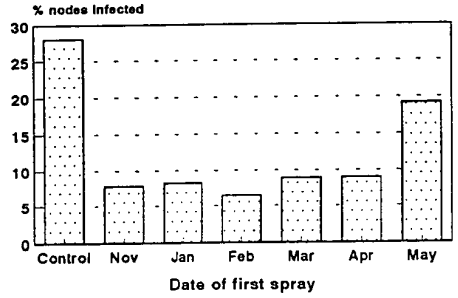


Terrington
Internodal Fusarium GS75

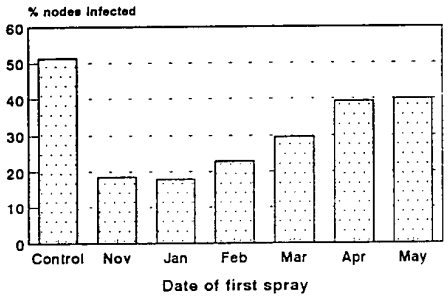


FUSARIUM FIELD EXPERIMENTS, 1994

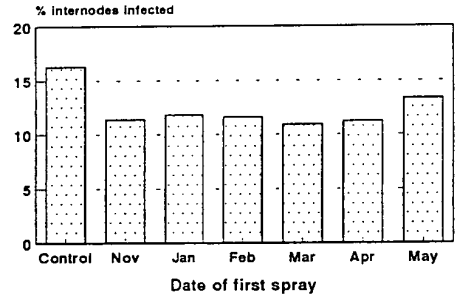
Morley
Nodal Fusarium GS75



Rosemaund
Nodal Fusarium GS75



Morley
Internodal Fusarium GS75



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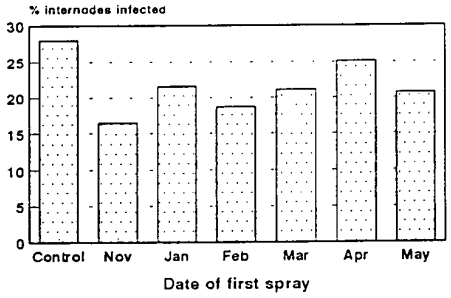
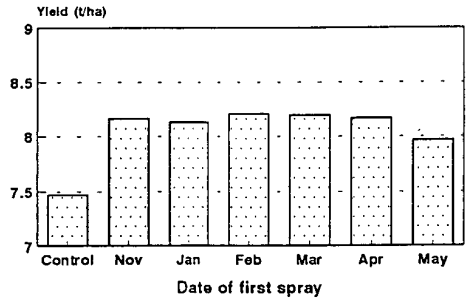
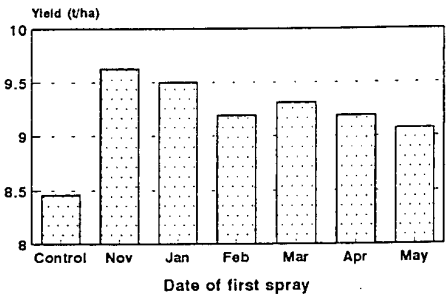
Internodal Fusarium GS75



Morley
Yield



Edinburgh
Yield



FUSARIUM FIELD EXPERIMENTS, 1995

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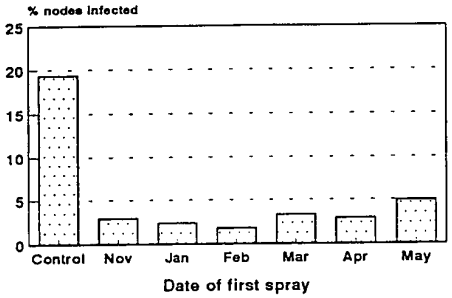

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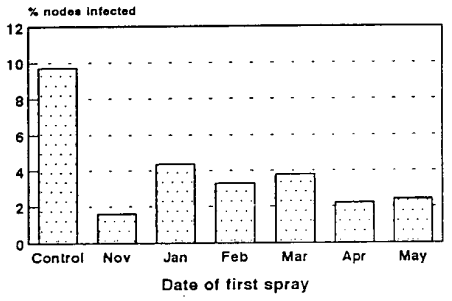

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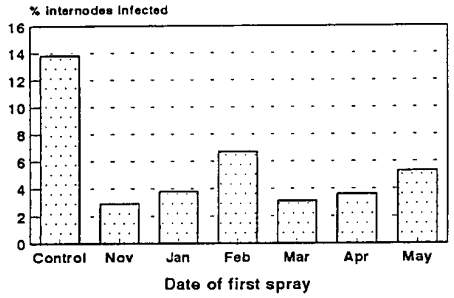

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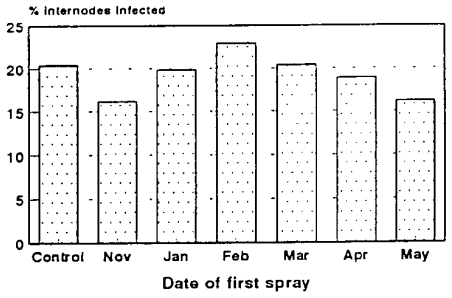

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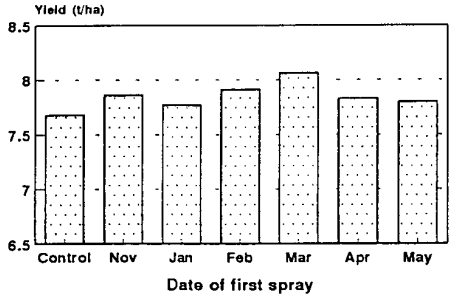
Yield



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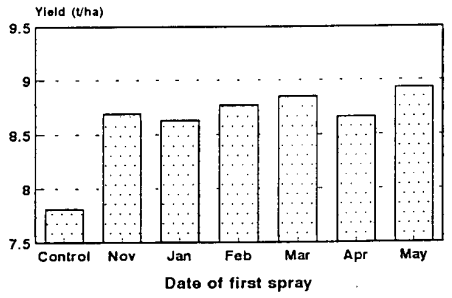
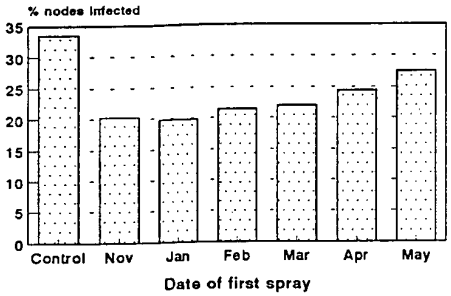


Mean results 1993-95

Nodal Fusarium GS75



Mean results 1993-95

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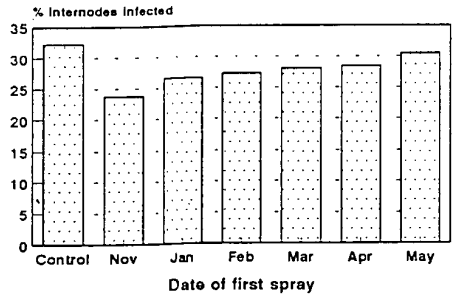

Mean results 1993-95

Yield