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# PROJECT REPORT No. 261

## UNDERSTANDING EFFECTS OF NEW WHEAT FUNGICIDES ON DISEASE DEVELOPMENT, CROP GROWTH AND YIELD

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# UNDERSTANDING EFFECTS OF NEW WHEAT FUNGICIDES ON DISEASE DEVELOPMENT, CROP GROWTH AND YIELD

by

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**CONTENTS**

## ABSTRACT

Field experiments were undertaken for three years at two sites to investigate whether strobilurin fungicides have physiological effects on winter wheat that could be detected and exploited in the field, and to test the effects of mixing strobilurins with azole fungicides.

On a resistant cultivar at a low disease site, there were consistent yield increases resulting from strobilurin application, although yield responses were smaller than at the site with severe disease. Dose-response curves for strobilurins were mostly similar to those for azole fungicides but, in one instance, azoxystrobin showed a steeper curve with low curvature, indicating that there would have been increases in yield at doses above the maximum tested (the full commercial rate of 1.0 litre/ha). Experiments on the interaction between fungicides and nitrogen assimilation indicated that strobilurin fungicides did not affect nitrogen uptake, but there was an indication (not statistically significant) that the optimum nitrogen rate was slightly higher for azoxystrobin and kresoxim-methyl, compared with epoxiconazole or an untreated control. There were no differences between strobilurins and epoxiconazole in maximum green area index, and no evidence of any effect of strobilurins on radiation use efficiency either pre-anthesis or post-anthesis.

Under conditions of severe foliar disease, mixtures of a strobilurin (azoxystrobin or kresoxim-methyl) with epoxiconazole gave greater persistence of disease control than epoxiconazole alone, resulting in greater green canopy duration. Canopy size showed a close inverse relationship with disease, and yield was closely correlated with canopy size. In an experiment on interaction with seed rate, there were effects of fungicides on disease, and of both fungicides and seed rate on canopy size, crop biomass, grain yield and harvest index. However, there were no statistically significant interactions between fungicide and seed rate.

There were no clear indications that yield increases resulted from physiological effects on the crop. However, it is possible that conventional assessments of canopy and crop growth are not sufficiently sensitive to detect small differences that may result from physiological effects. The possibility that strobilurins do affect crop physiology cannot be discounted but, since any such effect is likely to be small, it would need extensive and sensitive experimentation to identify and characterise it. Under conditions of severe disease, yield was strongly correlated with increase in canopy duration, and there was no evidence of any physiological effects of strobilurins. Overall, these results show that the value of physiological effects to growers under normal conditions of moderate or high disease risk would be small in relation to the large fungicidal effects that occur consistently.

It can be concluded that strobilurins are a valuable addition to the fungicide armoury available to growers, providing long-lasting protectant activity to complement the eradicant activity of azole fungicides. Mixtures were generally efficacious for disease control. There were no antagonistic effects between azoxystrobin and epoxiconazole, and indications that mixtures showed synergism. If there are additional physiological effects of strobilurins on wheat, they should be regarded as an added bonus from use of these fungicides, rather than a core feature of their activity. The experiments on interaction of strobilurin with seed rate, cultivar and nitrogen showed that there is no need to alter the basic agronomy of the crop in order to gain maximum benefit from strobilurins, except for a small increase in nitrogen rates to exploit the greater yield potential.

## INTRODUCTION

The strobilurin fungicides were introduced into the UK cereal market in 1997, and soon became a key component of disease control strategies on wheat because of their breadth of disease spectrum and long persistence of activity. Data from the ADAS/CSL winter wheat disease surveys show that the proportion of winter wheat crops treated at least once with a strobilurin rose from 5% in 1997 to 47% in 1998, 79% in 1999 and 84% in 2000 (N V Hardwick, personal communication).

Early field experiments with strobilurin fungicides showed increases in green canopy and yield which could not be related directly to improvements in disease control. This was observed particularly with kresoxim-methyl plus epoxiconazole, compared with epoxiconazole alone, but also with azoxystrobin plus epoxiconazole (Jones and Bryson, 1998; Jones, 2000). This raised interest in whether the strobilurins have direct physiological effects on plants which contribute to green canopy duration, light interception or radiation use efficiency and thereby to yield.

There is evidence from glasshouse, growth room or detached leaf studies that kresoxim-methyl can affect various physiological processes in plants, including a reduction in dark inactivation of nitrate reductase, reduction in ethylene biosynthesis and increased abscisic acid levels. However, it is important to know whether these and other physiological effects can be shown to be of benefit in the field, and how they are affected by the way in which fungicides are used in practice, often at reduced doses and in mixtures.

## OBJECTIVES

The basic objectives of the project were to investigate whether strobilurin fungicides have physiological effects on winter wheat that could be detected and exploited in the field, and to show whether benefits from strobilurins were affected by their use in mixtures with azole fungicides. Within this overall objective, the specific objectives of the 6 experiments within the project were as follows:

1. To determine the dose-response to strobilurin fungicides in the absence of disease
2. To determine whether there is any interaction between response to strobilurin fungicides and cultivar susceptibility to disease
3. To investigate whether there is an interaction between strobilurin fungicides and seed rate
4. To investigate the interaction between strobilurin fungicides and nitrogen utilisation
5. To determine dose-response relationships for azole fungicides in mixture with strobilurins
6. To test whether there is synergy or antagonism between azoxystrobin and epoxiconazole

Reports on each of these 6 experiments are given in Appendices to this report.

## MATERIALS AND METHODS

Field experiments were completed in each of the 1998, 1999 and 2000 harvest years at ADAS Rosemaund, Herefordshire and ADAS Boxworth, Cambridgeshire. Details of sites and years of each experiment are in Table 1 (sites coded RM and BX respectively).

Table 1. Experiment sites

| Experiment | 1997/98 | 1998/99 | 1999/2000 |
|---|---|---|---|
| 1. Dose-response in absence of disease | BX | BX | BX |
| 2. Strobilurin x cultivar interaction | BX, RM | | |
| 3. Strobilurin x seed rate interaction | | | RM |
| 4. Strobilurin x nitrogen interaction | | BX | BX |
| 5. Azole dose-response in strobilurin mixture | RM | RM | RM |
| 6. Synergy or antagonism | RM | RM | RM |

The design for each experiment was a randomised block with three replicates. Experiments which included destructive sampling for growth analysis (Experiments 1-4) had duplicate plots of each treatment, one of which was used for growth analysis and the other for disease assessment and yield determination. Fungicides were applied in two-spray programmes, at GS 31-32 and GS 39 (Tottman and Broad, 1987); details of fungicide treatments are given in the summary of results from each experiment. Other agronomic inputs followed good commercial practice, except in Experiments 3 and 4 where seed rate and nitrogen respectively were treatment factors.

Growth analysis was done at 4 or 5 critical stages from GS 33 to GS 87 in each of Experiments 1-3 and weekly from GS 31 in Experiment 4 to determine shoot numbers and total biomass and, in some instances, water-soluble carbohydrate levels and canopy size. These experiments were also assessed at 7 or 10 day intervals for disease and green leaf area. To show the effect of disease integrated over time, disease was expressed as area under the disease progress curve (AUDPC). Canopy size was determined on each of these dates from measurement of leaf width and length, and the integral of canopy area over time was expressed as healthy area duration from GS 39 (HAD39; Bryson *et al.*, 1997). A pre-harvest biomass assessment, to determine harvest index and components of yield, was undertaken in Experiments 3 and 4, and one site in Experiment 2. Experiments 5 and 6 were assessed twice for disease. All experiments were harvested and yields expressed at 85% dry matter.

### Results and Discussion

*Experiment 1*

Dose-responses were determined in experiments on the disease-resistant cultivar Spark which had only low levels of foliar disease (primarily *Septoria tritici*). In the third year, overall protectant fungicides were applied, which reduced disease in comparison with a totally untreated control, but did not prevent disease altogether. The fungicides used in the first two years are detailed in the dose-response curves, shown in Figure 1, and azoxystrobin and kresoxim-methyl (without an azole) were used in the third year.

In each experiment, there was a clear trend of lower disease with increasing fungicide dose, and increases in green canopy appeared to correspond with disease severity, even though only in one year did a regression of canopy (expressed as HAD39) on disease (AUDPC for leaves 1-4) account for more than 50% of the variance. There were clear dose-responses for yield for all fungicides, except for epoxiconazole in 1997/98. The shapes of the curves for all fungicides in 1998/99 and 1999/2000 (not shown) were broadly similar, and kresoxim-methyl plus epoxiconazole in 1997/98 also showed this pattern, which is consistent with most of the HGCA-funded work on Appropriate Fungicide Doses (Paveley, 2000). However, azoxystrobin in 1997/98 had a much flatter curve. There was a strong indication that yield would have continued to rise at doses above the highest dose tested, the maximum recommended rate of 1.00 litre/ha. In Experiment 6 within this project, azoxystrobin also gave a curve of this type in 1997/98, under high disease conditions, in contrast with the results from epoxiconazole at the same site, and with both fungicides in the other two years of that experiment.

No statistically significant effects of fungicides on crop biomass were recorded in any year, even though there were differences in yield. This suggests that measurement of biomass in this way may not be sufficiently sensitive to account for the relatively small effects of fungicide on yield that were recorded in this experiment.

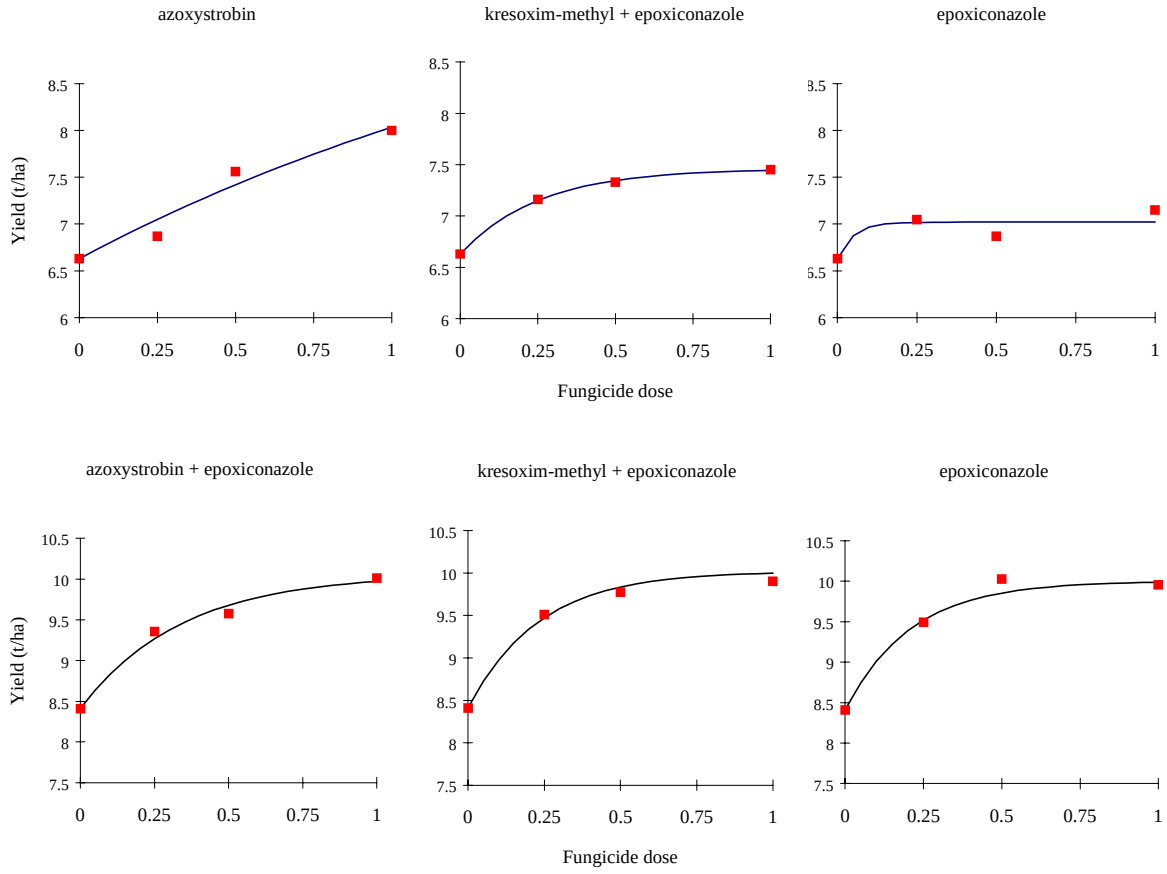


Figure 1. Relationship between yield and dose, 1997/98 (above) and 1998/99 (below). Solid lines are fitted curves, and actual data points are also shown.

*Experiment 2.*

Azoxystrobin, kresoxim-methyl plus epoxiconazole and epoxiconazole alone, all at full rates, were compared with an untreated control on three cultivars differing in susceptibility to disease, Riband (susceptible), Charger (intermediate) and Spark (resistant), at two sites, one where severe disease is common (Rosemaund) and one where it is uncommon (Boxworth).

The two experiments provided a marked contrast in terms of disease severity, and in effects of fungicides on disease control, crop growth and yield. There was a severe *Septoria tritici* epidemic at Rosemaund, with marked differences in disease between the three cultivars and large yield increases from fungicide (up to 5.03 t/ha on Riband, 3.28 t/ha on Charger and 3.55 t/ha on Spark). In contrast, at Boxworth, there were moderately severe epidemics of *S. tritici* and brown rust on Riband, but little disease on Charger and Spark. Yield increases were large on Riband (up to 3.96 t/ha), but smaller on the other cultivars (1.93 and 1.13 t/ha on Charger and Spark respectively).

There were benefits from using a strobilurin fungicide. At Rosemaund, the mean yield advantage of the kresoxim-methyl mixture over epoxiconazole alone was 0.54 t/ha, and the difference was statistically significant on each of the three cultivars, despite the contrasting disease severities. There were also indications, though not statistically significant, that the treatment with kresoxim-methyl had less disease on each of the top three leaves. Canopy size was greater where the mixture was used, although there was no measurable effect on total biomass, grain number per ear, or harvest index. At Boxworth, there was yield advantage from the kresoxim-methyl mixture over epoxiconazole on each cultivar (mean 0.35 t/ha), though this was not statistically significant. There was no clear evidence of improved control of the low levels of disease from the mixture, but the mixture gave larger green canopies from mid-June until the end of green canopy life.

The performance of azoxystrobin was markedly inferior to that from kresoxim-methyl plus epoxiconazole or epoxiconazole alone, and shows the need for a triazole fungicide in mixture with a strobilurin under conditions of severe disease. At Rosemaund, azoxystrobin had a smaller canopy from mid-June onwards, which resulted in a lower yield than from kresoxim-methyl plus epoxiconazole or from epoxiconazole alone, with a lower grain number per ear and harvest index. At Boxworth, azoxystrobin also gave a lower yield than kresoxim-methyl plus epoxiconazole, although no differences were detected in canopy or crop biomass.

Although there were clear differences between cultivars and fungicides, there was only one significant cultivar x fungicide interaction in the experiment. This was in the yields at Rosemaund, where Riband gave a lower untreated yield but higher treated yields than other cultivars. Although yield increases from the strobilurins were smaller at Boxworth, there were clear indications of a yield benefit from strobilurins on all cultivars under conditions of low disease.

The relationship between yield and green canopy from GS 39 (HAD39) was good, except for the cultivar with least disease, Spark, at Boxworth. This suggests that, where disease caused substantial loss of green canopy, yield was strongly related to the increases in green canopy resulting from fungicide use, and that there was no evidence that radiation use efficiency was altered. The relationship between disease and HAD39 was also good at Rosemaund and, on Riband and Charger, at Boxworth. This suggests that loss of canopy was largely associated with disease, and that the effect of fungicides on canopy survival was primarily associated with disease control. Under low disease conditions, on Spark at Boxworth, relationships were poorer, particularly between yield and canopy. However, no clear evidence was found in the experiment to account for any other factors, such as physiological effects, which may have affected the response of the cultivar to the fungicides.

*Experiment 3*

Four fungicide programmes and an untreated control were evaluated on cv. Consort sown on 16 October 1999 at two seed rates, a low rate of 100/m$^2$ and a 'conventional' rate of 350/m$^2$. Comparing fungicides, few differences were evident in terms of control of *Septoria tritici*, but there were some small effects. On leaf 4, where eradicant activity would have been tested most severely, kresoxim-methyl plus epoxiconazole was less effective than epoxiconazole alone or in mixture with either of the other strobilurins. On each of the top three leaves, epoxiconazole in mixture with trifloxystrobin or azoxystrobin gave greater persistence of disease control than epoxiconazole alone or in mixture with kresoxim-methyl. This resulted in higher green leaf area indices in the last three weeks of green canopy life. There was a reasonably close relationship between green canopy integrated over time (HAD39) and disease, but a very close relationship between yield and HAD39 (Figure 2). This suggests that the increased yield which resulted from the fungicide treatments was directly related to the increase in green canopy duration and, therefore, it is unlikely that more efficient function of the canopy (perhaps through increased radiation use efficiency) was a significant factor.

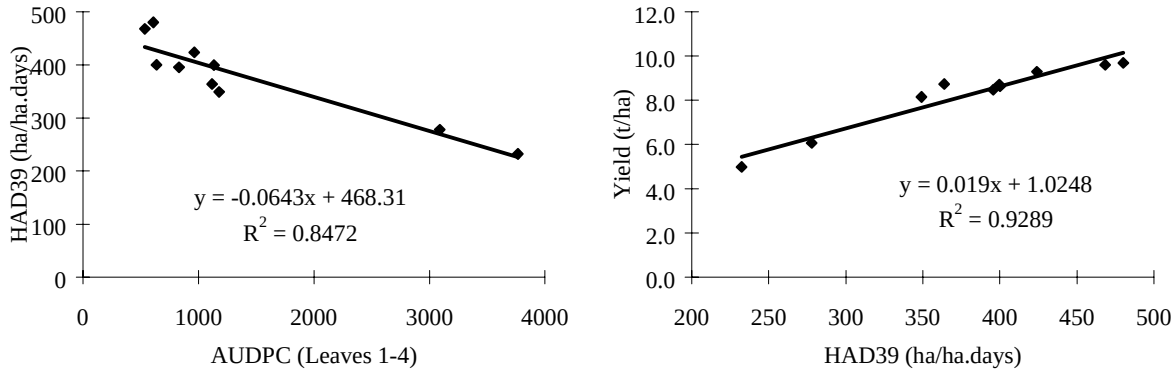


Figure 2. Relationships between canopy size integrated over time (healthy area duration from GS 39; HAD 39) and disease (area under disease progress curve for the upper 4 leaves) (left), and between yield and HAD39 (right).

A consistent feature throughout this experiment was the lack of statistical interactions between seed rate and fungicide. Effects of seed rate and fungicide were generally in line with what would be expected, but there was no evidence that the crop established from a low seed rate showed a different response to fungicides to the crop from the 'conventional' seed rate. Overall, this experiment did not provide any evidence to support the hypothesis that there may be a greater benefit from strobilurins in thin crops, where the lower stem leaves make a greater contribution to yield, than in thick crops. The actual crop canopy which resulted from sowing 100 seeds/m$^2$ in mid-October was not quite as small as some commercial crop canopies which result from late sowing or adverse conditions (e.g. following severe slug damage), but was sufficiently small to indicate that the benefits from strobilurins will not be fundamentally different on a thin crop.

*Experiment 4*

Four fungicides treatments (untreated, azoxystrobin, kresoxim-methyl and epoxiconazole) were superimposed on each of five nitrogen rates from 0 to 320 kg/ha on the disease-resistant cultivar Spark. Protectant fungicides were applied to the whole experiment to minimise foliar disease.

Disease levels were low, but *Septoria tritici* was present on the upper leaves from mid-June. Fungicides reduced disease, but had no effect on senescence which was not associated with disease. Increasing nitrogen rates increased disease, consistent with earlier work, but also delayed senescence, as determined by the calculated mid-point of senescence. There was an effect of nitrogen on total biomass, but not of fungicide. There was no difference between strobilurins and epoxiconazole in maximum green area index. It would appear, therefore, that the strobilurins had no effect on N uptake and canopy expansion. There was also no evidence of any effect on radiation use efficiency either pre-anthesis or post-anthesis. Differences between treatments in the rate of senescence appeared to be related to the level of disease in each treatment. Since no increase in nitrogen uptake was observed associated with the strobilurins, it was not possible to determine whether there were any physiological effects of differences in uptake on senescence.

Radiation use efficiency was higher with 160 kg/ha nitrogen than in nil nitrogen plots, but was not affected by fungicide. Fungicides increased yield at the higher levels of nitrogen (160 kg/ha or higher). Nitrogen effects on yield are shown in Figure 3. The strobilurin treatments had slightly higher nitrogen optima than epoxiconazole and the untreated, but this difference was not statistically significant.

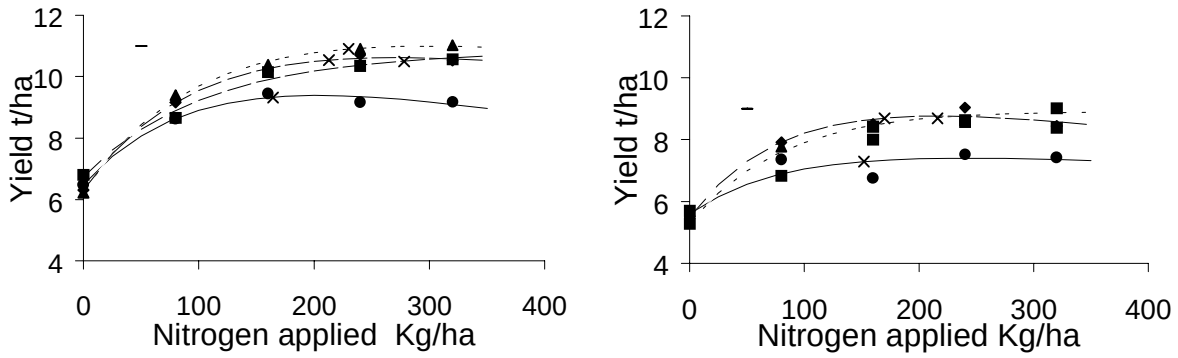


Figure 3. Fitted curves for combine yield with increasing N in 1999 (left) and 2000 (right). X denotes Nitrogen optima. No Strobilurin (—▲—), Azoxystrobin (– –■– –), Kresoxim-methyl (----◆----), Epoxiconazole (—●—). Error bar is s.e.d. with 38 df.

*Experiment 5*

In the first two years, azoxystrobin at half rate (0.50 litre/ha) was mixed with azole fungicides at zero, quarter, half and full rates. In 1999/2000, a smaller range of azoles was used, but each was also tested at one eighth rate. Trifloxystrobin at 1.00 litre/ha (half rate) was also included with four rates of epoxiconazole. *Septoria tritici* was the main disease in each year.

In 1997/98, all azoles gave marked reductions in disease compared with azoxystrobin alone, with little difference between fungicides at full rate (Figure 4). Differences between azoles became clearer at lower doses. Azoxystrobin alone increased yield by 1.86 t/ha, and there were substantial additional increases from mixture with each of the azole fungicides. There

was a greater difference between epoxiconazole and other azoles in yield than in disease control, with a higher yield from quarter rate epoxiconazole than from full rate of any other fungicide. In 1998/99, azoxystrobin alone gave a greater reduction in disease than in 1997/98. Epoxiconazole was the most effective fungicide for control of *S. tritici*, followed by fluquinconazole. The yield increase from azoxystrobin alone was 2.81 t/ha, larger than in 1997/98. Yield increases from addition of an azole fungicide were lower than in 1997/98. Fluquinconazole gave the next highest yields, but there was little benefit in yield from the other three azoles compared with azoxystrobin alone. All fungicide gave good *S. tritici* control in 1999/2000, but epoxiconazole was slightly more effective than the other azoles. Trifloxystrobin alone was more effective than azoxystrobin alone but, in mixture with epoxiconazole, there was no difference between these two strobilurins. As in the previous two years, epoxiconazole plus azoxystrobin gave higher yields than any other treatment. Trifloxystrobin alone gave a higher yield increase (2.89 t/ha) than azoxystrobin alone (2.65 t/ha), but this advantage was not apparent in mixture with epoxiconazole.

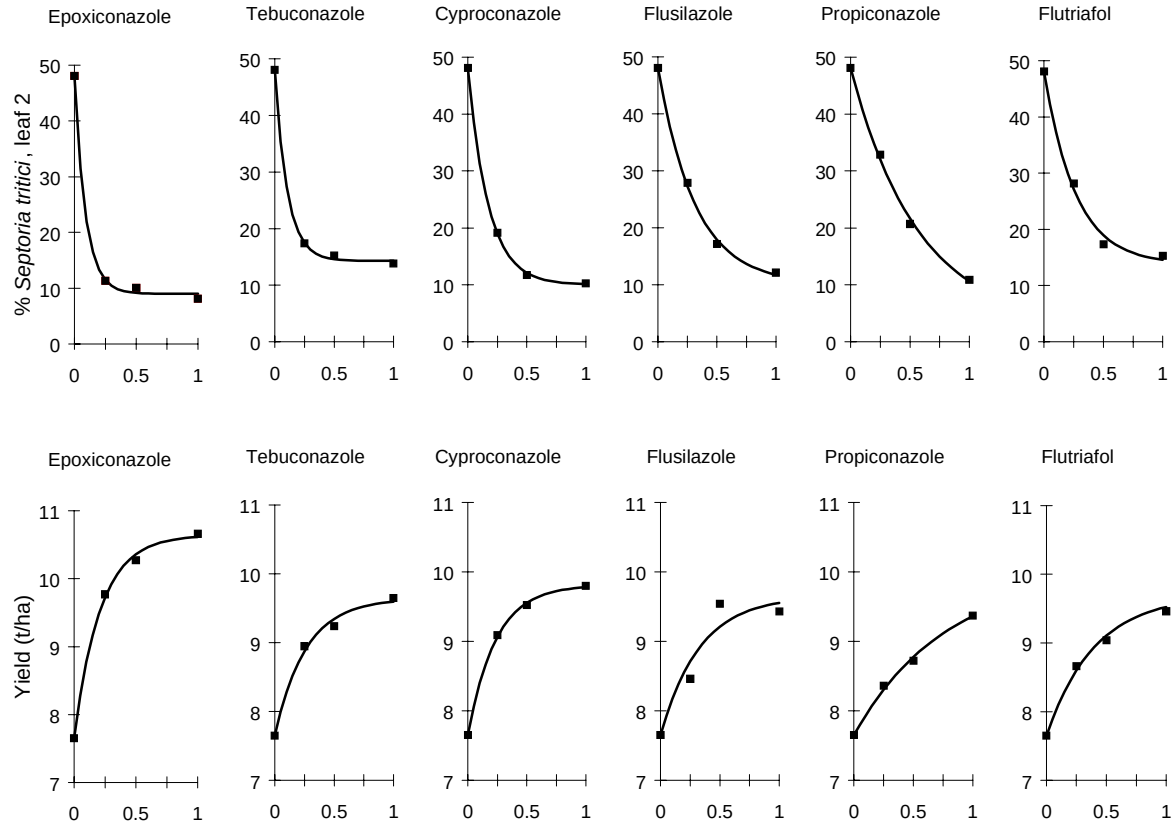


Figure 4. Dose-response curves for disease control on leaf 2 (above) and yield (below) from triazole fungicides in mixture with azoxystrobin, two-spray programme (GS 31/32 + GS 39), 1997/98. Untreated disease severity 79.0%, untreated yield 5.79 t/ha.

Dose-response curves for azoles in mixture with a strobilurin were generally similar to those for azoles when used alone (Paveley, 2000). This indicates that the basic properties of azole fungicides were not altered when in mixture with a strobilurin fungicide. Epoxiconazole was the most effective azole against *S. tritici* in the earlier HGCA-funded work on appropriate fungicide doses, and this superiority was evident in mixture with azoxystrobin, even though the strobilurin fungicide made a substantial contribution to both disease control and yield. Under the conditions of most severe disease and largest yield response to fungicide, in 1997/98, epoxiconazole gave a greater yield when used at one quarter of the recommended rate than any other azole fungicide at full rate. The azole which came closest to

epoxiconazole in disease control and effect on yield was fluquinconazole, but this was always second best. It can be concluded that, under high risk of *S. tritici*, epoxiconazole is likely to remain the most effective azole fungicide even when used in mixture with a robust dose of a strobilurin.

*Experiment 6*

Dose-responses were calculated for azoxystrobin and epoxiconazole alone, and for various mixtures with the two fungicides in 1:1, 2:1 or 1:2 ratio. From these, the additive dose model was used to calculate whether the performance of mixtures was better or worse than would be expected from the performance of the individual fungicides. In 1997/98, the results for each of the mixtures were below the line which represents equivalent performance of a mixture compared with its components, which suggested that there may have been synergistic effects between the fungicides (Figure 5). However, in the other years, results were less conclusive, and did not show any clear evidence of synergy.

This suggests that the result in 1997/98 may be a reflection of the beneficial effect given by mixture of the strobilurin with a triazole for control of severe disease, where the protectant activity of the strobilurin was complemented by the eradicant activity of the triazole. If mixture with a triazole compromised any non-fungicidal benefits from Amistar, this was more than outweighed by the superior disease control and the yield increase which resulted from this. In the later two years, each fungicide alone gave good disease control, so it is not surprising that it was not possible to show any specific benefit from mixtures.

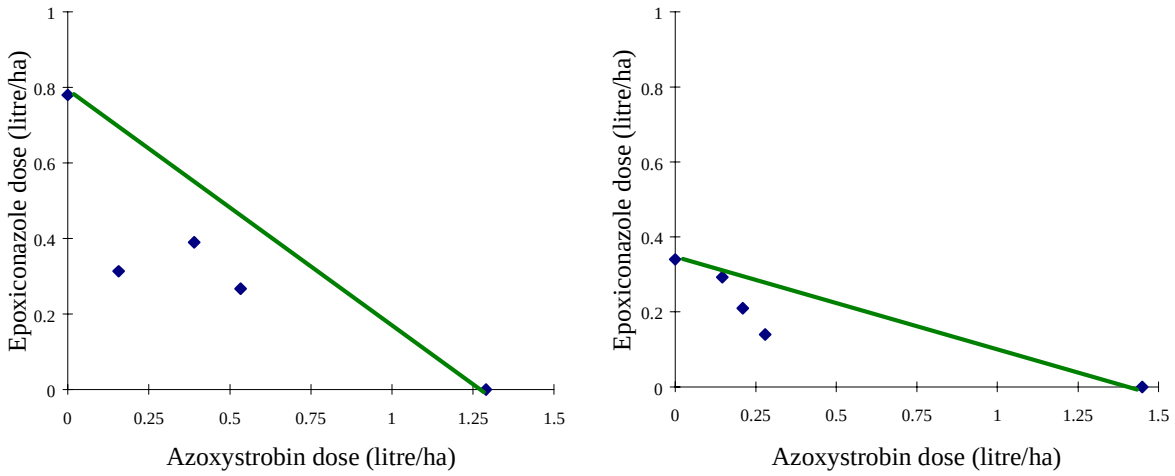


Figure 5. Test for synergy or antagonism between Amistar and Opus for yield (left) and *Septoria tritici* control on leaf 2 (right), 1997/98. The solid lines represent the dose of any single active or mixture required to give a yield increase of 5.5 t/ha or a reduction in disease of 80%, assuming no synergy or antagonism between the fungicides.

## OVERALL CONCLUSIONS

Under conditions of severe foliar disease, the strobilurins gave greater persistence of disease control than epoxiconazole, resulting in greater green canopy duration. Yield was closely correlated with canopy size. Mixtures of a strobilurin with an azole were generally efficacious for disease control under high disease pressure. There was some evidence of synergy between epoxiconazole and azoxystrobin, and no antagonistic effects were detected between azoxystrobin and any of the azoles included in the project. The ranking of azole fungicides for control of *Septoria tritici* was similar when used in mixture with azoxystrobin to the results from previous work with azoles tested alone; epoxiconazole maintained its superiority over other azoles when used in mixture with azoxystrobin. Where fungicidal activity was of prime importance, there was no evidence of any physiological effects of strobilurins.

On the resistant cultivar at the low disease site, yield increases were modest compared with those at the site with severe disease, However, there were consistent yield increases resulting from strobilurin application, and dose-response relationships for yield which corresponded with differences in disease control and canopy retention. There were no clear indications that yield increases resulted from physiological effects on the crop. However, it is possible that conventional assessments of canopy and crop growth are not sufficiently sensitive to detect small differences that may result from physiological effects. The possibility that strobilurins do affect crop physiology cannot be discounted but, since any such effect is likely to be small, it would need extensive and sensitive experimentation to identify and characterise it with certainty. Under conditions of severe disease, yield was strongly correlated with increase in canopy duration, and there was no evidence of any physiological effects of strobilurins. Overall, these results show that the value of physiological effects to growers under normal conditions of moderate or high disease risk would be small in relation to the large fungicidal effects that occur consistently.

It can be concluded that strobilurins are a valuable addition to the fungicide armoury available to growers, providing long-lasting protectant activity to complement the eradicant activity of azole fungicides. If there are additional physiological effects of strobilurins on wheat, they should be regarded as an added bonus from use of these fungicides, rather than a core feature of their activity. The experiments on interaction of strobilurin with seed rate, cultivar and nitrogen showed that there is no need to alter the basic agronomy of the crop in order to gain maximum benefit from strobilurins, except for a small increase in nitrogen rates to exploit the greater yield potential.

## APPENDIX 1

### Experiment 1: Dose-response to strobilurin fungicides in the absence of disease

### Introduction

Early field experiments with strobilurin fungicides showed increases in green canopy and yield which could not be related directly to improvements in disease control. This was observed particularly with kresoxim-methyl plus epoxiconazole, compared with epoxiconazole alone, but also with azoxystrobin plus epoxiconazole (Jones, 2000). This raised interest in whether the strobilurins have direct physiological effects on plants which contribute to green canopy duration and thereby to yield.

There is evidence from glasshouse, growth room or detached leaf studies that kresoxim-methyl can affect various physiological processes in plants. Dark inactivation of nitrate reductase was reduced (Köhle *et al.*, 1997), and degradation of nitrate reductase inhibited (Glaab and Kaiser, 1999) which may increase nitrogen uptake and lead to a larger canopy size and, possibly, increased radiation use efficiency. Grossman and Retzlaff (1997) and Grossman *et al.* (1999) showed that kresoxim-methyl inhibited ethylene biosynthesis and increased abscisic acid levels, which may delay senescence. They also showed that stomatal aperture was reduced, which could reduce transpiration and, consequently, water stress on the plants.

In addition to the possibility of direct physiological effects on the crop, it has also been suggested by Bertlesen *et al*. (2001) that the high frequency of defence reactions against attempted fungal infection could result in the associated energy expenditure adversely affecting yield. They hypothesised that the yield advantage observed in field experiments for azoxystrobin treated crops could be due to the initiation of fewer defence reactions by the plants, particularly when compared with treatment with epoxiconazole.

These findings raise the possibility that some of the beneficial effects of strobilurins observed in the field are physiological rather than pathological. This experiment was designed to test whether such effects could be observed in the absence of disease, and to determine the dose-response for any such effects.

### Methods

One experiment was established each year at ADAS Boxworth, Cambridgeshire, in 1997/98, 1998/99 and 1999/2000, on a clay loam of the Hanslope series. In 1997/98, the strobilurin products, azoxystrobin and kresoxim-methyl plus epoxiconazole, and also epoxiconazole alone, were included at quarter, half and full recommended rates. The design was similar in 1998/99, except that azoxystrobin was mixed with epoxiconazole, to provide comparability with the kresoxim-methyl treatment. In 1999/2000, azoxystrobin and kresoxim-methyl were tested at quarter, half, full and 2x recommended rates, and protectant fungicides were applied to minimise the risk of foliar disease, so that non-pathogenic effects of fungicides could be observed. Fungicides are detailed in Tables 1.1 - 1.3. Fungicides were applied in 200-250 litre/ha water, using an MDM Oxford Precision Sprayer. The experiments were on cv. Spark, which has a good profile of resistance to the main foliar pathogens of wheat (Anonymous, 1998). The experimental design was a randomised block with three replicates. Each experimental plot consisted of two adjacent sub-plots, sizes in the range 36-60 $m^2$, one of which was used for disease assessment and yield estimation and the other for destructive sampling for growth analysis.

Table 1.1. Fungicides in 1997/98

| Treat-ment | Active ingredient | Product | Application rate of product/ha |
|---|---|---|---|
| 1 | --- | Untreated | --- |
| 2 | Azoxystrobin | Amistar | 0.25 litre |
| 3 | Azoxystrobin | Amistar | 0.50 litre |
| 4 | Azoxystrobin | Amistar | 1.00 litre |
| 5 | Kresoxim-methyl + epoxiconazole | Landmark | 0.25 litre |
| 6 | Kresoxim-methyl + epoxiconazole | Landmark | 0.50 litre |
| 7 | Kresoxim-methyl + epoxiconazole | Landmark | 1.00 litre |
| 8 | Epoxiconazole | Opus | 0.25 litre |
| 9 | Epoxiconazole | Opus | 0.50 litre |
| 10 | Epoxiconazole | Opus | 1.00 litre |

Table 1.2. Fungicides in 1998/99

| Treat-ment | Active ingredient | Product | Application rate of product/ha |
|---|---|---|---|
| 1 | --- | Untreated | --- |
| 2 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.25 + 0.25 litre |
| 3 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 0.50 litre |
| 4 | Azoxystrobin + epoxiconazole | Amistar + Opus | 1.00 + 1.00 litre |
| 5 | Kresoxim-methyl + epoxiconazole | Landmark | 0.25 litre |
| 6 | Kresoxim-methyl + epoxiconazole | Landmark | 0.50 litre |
| 7 | Kresoxim-methyl + epoxiconazole | Landmark | 1.00 litre |
| 8 | Epoxiconazole | Opus | 0.25 litre |
| 9 | Epoxiconazole | Opus | 0.50 litre |
| 10 | Epoxiconazole | Opus | 1.00 litre |

Table 1.3. Fungicides in 1999/2000

| Treat-ment | Active ingredient | Product | Application rate of product/ha |
|---|---|---|---|
| 1 | --- | Untreated | --- |
| 2 | ---* | Untreated | --- |
| 3 | Azoxystrobin* | Amistar | 0.25 litre |
| 4 | Azoxystrobin* | Amistar | 0.50 litre |
| 5 | Azoxystrobin* | Amistar | 1.00 litre |
| 6 | Azoxystrobin* | Amistar | 2.00 litre |
| 7 | Kresoxim-methyl* | Stroby | 0.0625 kg |
| 8 | Kresoxim-methyl* | Stroby | 0.125 kg |
| 9 | Kresoxim-methyl* | Stroby | 0.25 kg |
| 10 | Kresoxim-methyl* | Stroby | 0.50 kg |

*Treatments 2-10 received the following overall fungicide applications to minimise the risk of foliar disease: Chlorothalonil as Bravo at 1.0 l/ha plus quinoxyfen as Fortress at 0.15 l/ha on 27 April (GS 31), followed by chlorothalonil as Bravo at 1.0 l/ha on 31 May (GS 45).

In each year, each treatment was applied on two occasions, at GS 31-32 and GS 39 (Tottman & Broad, 1987). Other agronomic inputs followed good commercial practice.

Foliar diseases were assessed as visual estimates of the percentage leaf area infected by each disease on each leaf layer, on 10 tillers taken at random from each sub plot at approximately 10 day intervals from the date of the first fungicide application until all leaves were senescent. Percentage green leaf area was also estimated. To provide a cumulative measure of the effect of disease during the life of the stem leaves, the area under disease progress curve (AUDPC) was calculated for each treatment. This can be visualised on a graph showing disease progress over time, as the area under the line showing disease development for that treatment.

For each experiment, fitted curves were calculated for the dose-response for yield, using the exponential function $y = a + be^k$. An example is shown in Figure 1.1. Within this model, parameter a is the lower asymptote which represents the lowest level of disease achievable, and is a measure of the efficacy of the fungicide. Parameter b is the difference between the untreated AUDPC and the lower asymptote, which represents the range of disease control which could potentially be achieved by the fungicide. Parameter k is a measure of the curvature of the line.

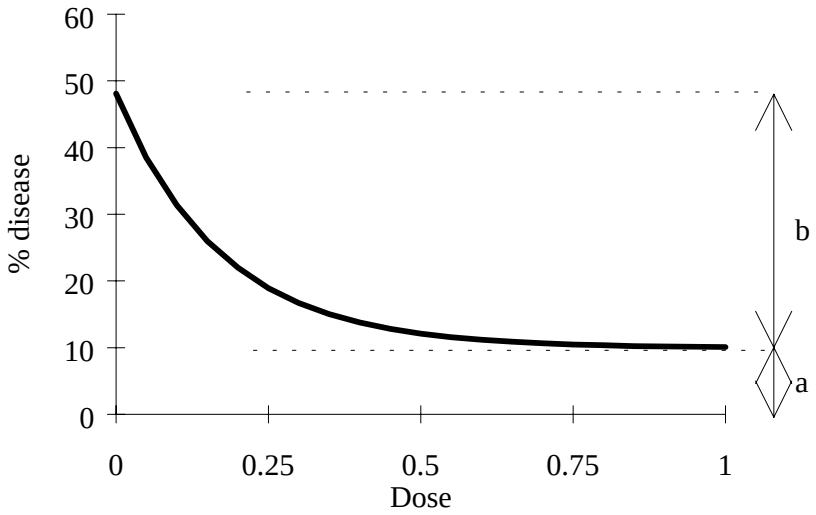


Figure 1.1. Example dose - response curve, showing parameters a and b.

In the figures below, actual data points are shown, and the solid line is the fitted curve.

Growth analysis was done at flag leaf emergence (GS 39), when 50% of all shoots reached mid-anthesis (GS 65), at the milky-ripe stage (GS 75) in 1998/99 and 1999/2000 only, and at mid-senescence (GS 87), calculated at 750 ºCd after GS 39 (Foulkes & Scott, 1998). On each occasion, an area of 0.50 $m^2$ was sampled, leaving at least 0.5 m between samples and at least three rows from plot edges to avoid edge effects (Austin & Blackwell, 1980). The plants were cut at ground level and taken to the laboratory for analysis. The number and dry weight of shoots were assessed separately for potentially fertile, dead and dying shoots. A shoot was classified as dying when no further leaf was emerging and the most recently emerged leaf was yellowing (Thorne & Wood, 1987).

Green area index (GAI) was calculated by determining actual leaf size on each occasion that diseases were assessed, using two of the ten leaves taken from each plot. Leaf length and width were measured , to the nearest 0.1 mm, and leaf area determined using a form factor of 0.83 (Bryson *et al.*, 1997). GAI was calculated using the mean number of shoots per plot

from assessments between mid-anthesis and mid-senescence. The leaf areas were then integrated over time from GS 39 until the end of all green canopy to give healthy area duration from GS 39 (HAD39; Bryson *et al*., 1997). This provides a measure of green canopy size during the period in which photosynthesis is contributing primarily to grain filling rather than to canopy structure.

At harvest in 1999/2000, a further 0.5 $m^2$ quadrat was taken for estimation of yield components. Final crop dry weight and ear number were recorded. The ears were then threshed, grain dry weight measured and harvest index calculated. Grain yield was measured from the 2 m x 24 m strip allocated within each sub plot using a plot combine harvester. Grain was analysed for moisture content and specific weight using GAC 2000 grain analysis computer (Dickey-John Corporation). The thousand grain weight was determined on grain samples taken from the combine. The number of grains per ear was then calculated using the combine grain yield, the thousand grain weight and the number of ears/$m^2$.

## Results

### 1997/98

The only foliar disease recorded was *Septoria tritici*, but levels were very low, with less than 1% leaf area affected on either of the top two leaves on 30 June, rising to 9.7% and 4.2% on leaves 1 and 2 respectively at the final assessment on 20 July. The severity on leaf 3 was 3.1% at the penultimate assessment on 20 June, rising to 11.7% on 30 June.

Disease severity in untreated plots was very low and differences between fungicides and rates were not statistically significant (Table 1.4).

Differences between treatments in green leaf area index were statistically significant only at the final two assessments, on days 189 and 201 (8 and 20 July; Figure 1.2). On 9 July, azoxystrobin at full rate, half and full rates of epoxiconazole and all rates of kresoxim-methyl plus epoxiconazole had larger canopies than the untreated control, as did all of these treatments, plus quarter rate epoxiconazole, on 20 July.

Canopy size and duration from GS 39, expressed as HAD39, showed that kresoxim-methyl plus epoxiconazole at full rate and epoxiconazole at half rate were the treatments with greatest HAD39 values ($P$=0.056) (Table 1.5).

There were significant differences between treatments in shoot numbers at GS 75 only, when kresoxim-methyl plus epoxiconazole at full rate and epoxiconazole at half rate had significantly higher shoot numbers than the untreated control (Table 1.6).

There were no significant differences between treatments in total crop dry matter although there was an indication, at GS 75, that kresoxim-methyl plus epoxiconazole at full rate and epoxiconazole at half rate had larger canopies than other treatments (Table 1.5).

There were significant effects of treatments on yield, but not on specific weight (Table 1.6). The dose-response curves for yield show that, with azoxystrobin, there was an increase in yield up to the highest dose (1.0 litre/ha), and no indication that the curve had become asymptotic whereas, for the other fungicides, the curves were asymptotic at this dose (Figure 1.3). There were poor relationships between HAD39 and disease (44.3% variance accounted for), and between yield and HAD39 (19.5% of variance accounted for) (Figures 1.4 and 1.5).

Table 1.4. Area under *Septoria tritici* disease progress curve for each fungicide on leaves 1-4, 1997/98.

| Fungicide | Rate (proportion of recommended rate) | Area under disease progress curve (AUDPC) | | | |
|---|---|---|---|---|---|
| | | leaf 1 | leaf 2 | leaf 3 | leaf 4 |
| Untreated | ---- | 170.2 | 117.3 | 233.0 | 124.8 |
| Azoxystrobin | 0.25 | 92.9 | 81.0 | 122.3 | 42.8 |
| Azoxystrobin | 0.50 | 37.2 | 23.6 | 181.0 | 75.6 |
| Azoxystrobin | 1.00 | 56.5 | 34.0 | 139.4 | 50.4 |
| Kresoxim-methyl + epoxiconazole | 0.25 | 24.7 | 17.6 | 81.1 | 157.9 |
| Kresoxim-methyl + epoxiconazole | 0.50 | 22.1 | 14.3 | 69.4 | 46.6 |
| Kresoxim-methyl + epoxiconazole | 1.00 | 34.2 | 22.7 | 52.7 | 51.5 |
| Epoxiconazole | 0.25 | 25.1 | 31.2 | 121.5 | 83.2 |
| Epoxiconazole | 0.50 | 38.0 | 11.9 | 99.4 | 46.0 |
| Epoxiconazole | 1.00 | 22.2 | 22.9 | 97.9 | 38.8 |
| SED (18 df) | | 36.93 | 32.05 | 47.90 | 102.2 |
| *P* | | 0.017 | 0.066 | 0.043 | 0.548 |

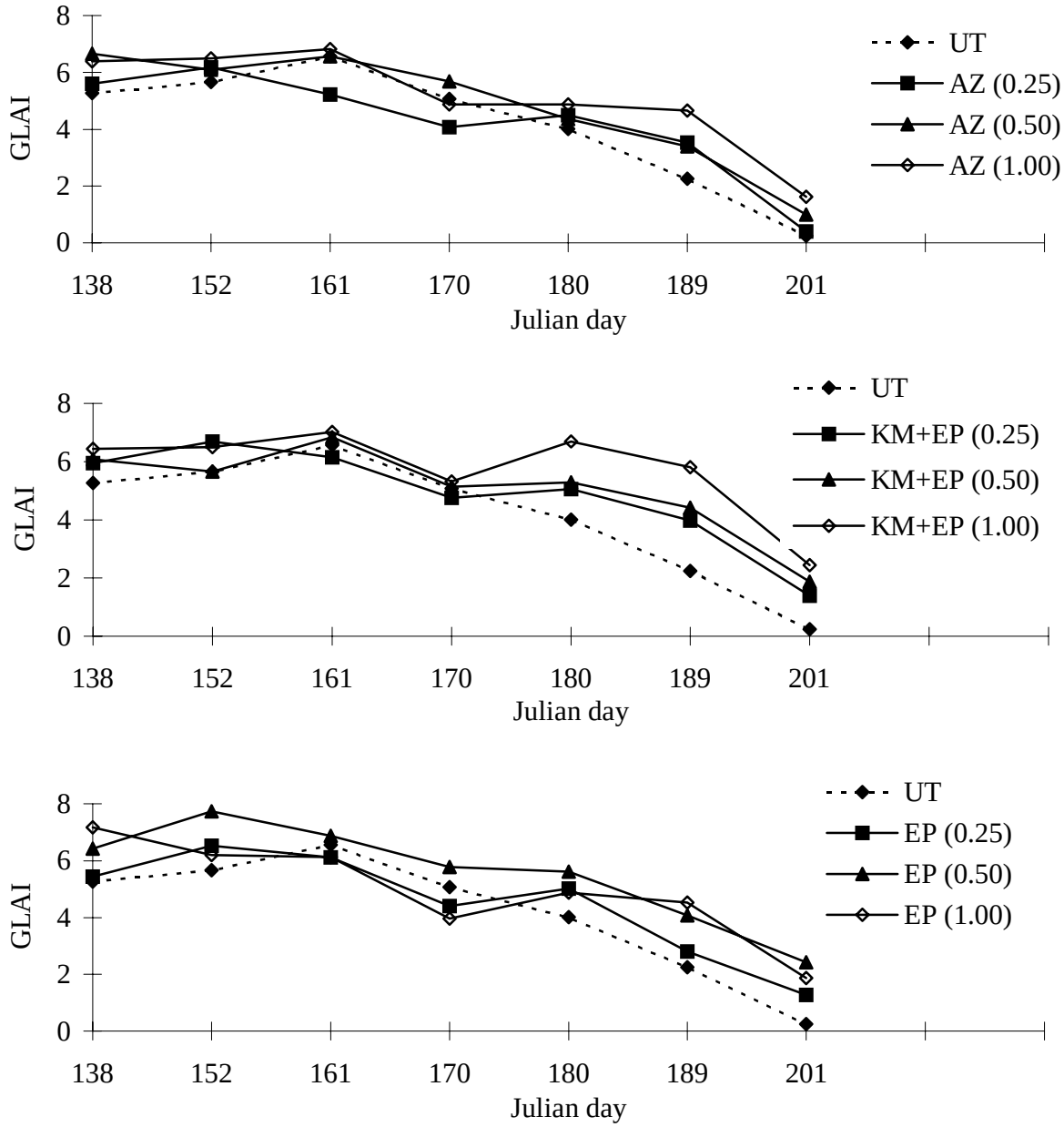


Figure 1.2. Green leaf area index (GLAI), 1997/98. AZ = azoxystrobin, KM = kresoxim-methyl, EP = epoxiconazole, UT = untreated control.

Table 1.5. Healthy Area Duration (HAD39) and total crop dry matter, 1997/98.

| Fungicide | Rate (proportion of recommended rate) | HAD39 (ha/ha days) | Total dry matter (t/ha) | | |
|---|---|---|---|---|---|
| | | | GS 39 (19 May) | GS 65 (8 June) | GS 75 (14 July) |
| Untreated | ---- | 272.4 | 8.39 | 10.55 | 14.55 |
| Azoxystrobin | 0.25 | 277.8 | 9.12 | 9.69 | 14.36 |
| Azoxystrobin | 0.50 | 313.0 | 8.71 | 10.53 | 13.94 |
| Azoxystrobin | 1.00 | 332.0 | 9.34 | 11.18 | 15.71 |
| Kresoxim-methyl + epoxiconazole | 0.25 | 317.2 | 8.61 | 10.60 | 15.13 |
| Kresoxim-methyl + epoxiconazole | 0.50 | 325.8 | 9.03 | 10.77 | 14.99 |
| Kresoxim-methyl + epoxiconazole | 1.00 | 372.5 | 9.44 | 10.99 | 19.91 |
| Epoxiconazole | 0.25 | 275.3 | 9.44 | 10.58 | 13.70 |
| Epoxiconazole | 0.50 | 361.2 | 9.06 | 10.82 | 20.56 |
| Epoxiconazole | 1.00 | 319.0 | 8.63 | 10.24 | 15.97 |
| SED (18 df) | | 31.09 | 0.420 | 0.664 | 2.335 |
| *P* | | 0.056 | 0.191 | 0.644 | 0.091 |

Table 1.6. Shoot numbers, yield, specific weight and harvest index, 1997/98.

| Fungicide | Rate* | Shoots/m GS 65 | Shoots/$m^2$ GS 75 | Yield (t/ha) | Specific weight (kg/hl) |
|---|---|---|---|---|---|
| Untreated | ---- | 692 | 669 | 6.62 | 78.7 |
| Azoxystrobin | 0.25 | 606 | 717 | 6.87 | 78.9 |
| Azoxystrobin | 0.50 | 750 | 718 | 7.56 | 80.3 |
| Azoxystrobin | 1.00 | 803 | 696 | 8.09 | 80.1 |
| Kresoxim-methyl + epoxiconazole | 0.25 | 670 | 689 | 7.16 | 79.1 |
| Kresoxim-methyl + epoxiconazole | 0.50 | 718 | 637 | 7.33 | 80.0 |
| Kresoxim-methyl + epoxiconazole | 1.00 | 795 | 828 | 7.45 | 79.5 |
| Epoxiconazole | 0.25 | 713 | 678 | 7.05 | 79.2 |
| Epoxiconazole | 0.50 | 752 | 882 | 6.87 | 79.8 |
| Epoxiconazole | 1.00 | 682 | 704 | 7.15 | 79.0 |
| SED (18 df) | | 66.2 | 55.6 | 0.278 | 0.55 |
| *P* | | 0.179 | 0.009 | 0.003 | 0.076 |

* rate expressed as a proportion of the recommended rate

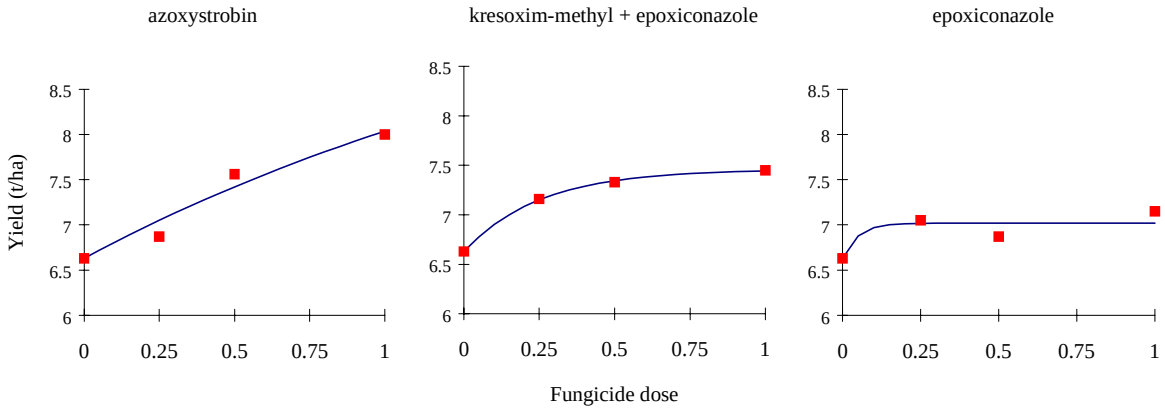


Figure 1.3. Relationship between yield and dose, 1997/98. Solid lines are fitted curves, and actual data points are also shown.

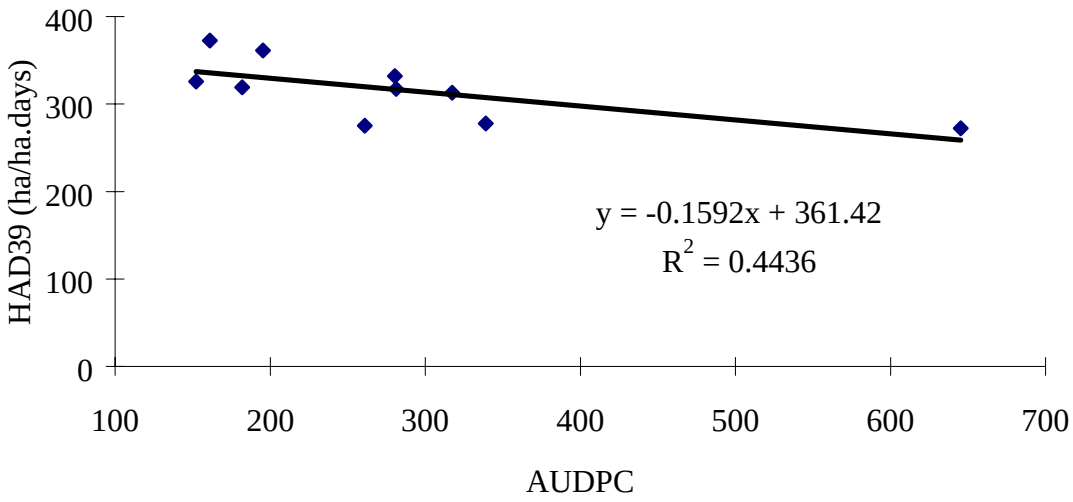


Figure 1.4. Relationship between canopy size integrated over time (HAD39) and disease (sum of AUDPC, leaves 1-4), 1997/98.

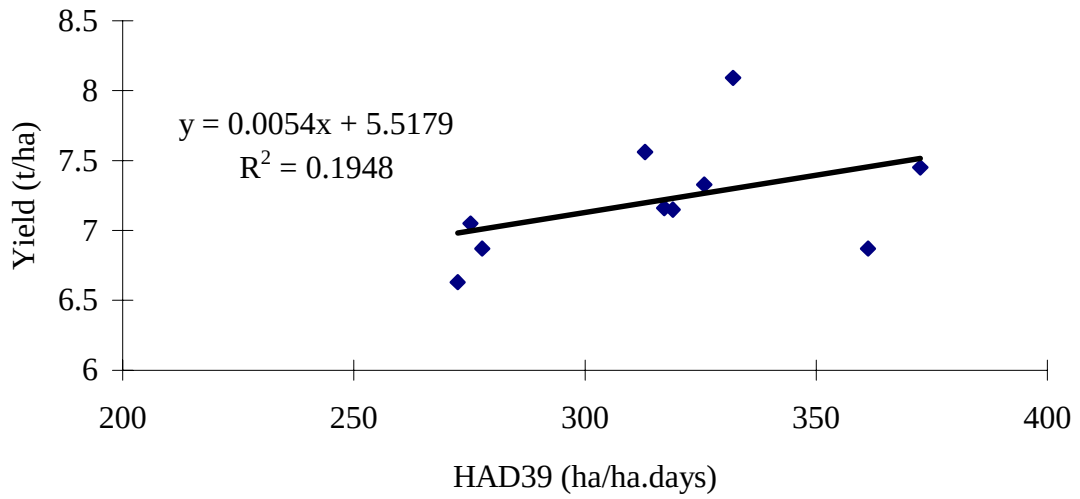


Figure 1.5. Relationship between yield and canopy size integrated over time (HAD39), 1997/98.

**1998/99**

Foliar disease levels on the top two leaves were very low (less than 1% leaf area affected) until July, when *Septoria tritici* increased rapidly, particularly in untreated controls, reaching a maximum severity of 41.7% on the flag leaves and 51.7% on leaf 2 by 13 July. Leaf 3 had low levels of *S. tritici* in June (10.7% in untreated plots on 19 June), which rose to 16.2% by the final assessment on 13 July.

Total disease over time, expressed as AUDPC, was greater in untreated plots than in 1997/98 (Table 1.7). Compared with the untreated control, all fungicides reduced AUDPC on the top two leaves, with the exception of quarter rate epoxiconazole on leaf 2. On leaves 3 and 4, quarter and half rate azoxystrobin plus epoxiconazole did not reduce AUDPC.

There were significant effects of fungicides on green leaf area index on day 194 (13 July) only (Figure 1.6). On this date, all fungicides and doses had larger green canopies than the untreated control, but there were no significant differences between treatments. There were no significant differences between treatments in canopy integrated over time (HAD39; Table 1.9).

There were no significant differences between treatments in shoot numbers in any of the assessments, nor were there any differences in total crop dry matter (Table 1.8).

All fungicides increased yield, but there were no significant differences in specific weight (Table 1.9). Full rates of each fungicide gave yield increases in the range 1.55-1.62 t/ha. The dose-response curves for yield show similar curves for each fungicide, approaching the asymptote at the highest dose (Figure 1.7).

The regression HAD39 on disease (sum of AUDPC for leaves 1-4 accounted for 61.4% of the total variance, and the regression of yield on HAD39 accounted for 59.5% of the variance (Figures 1.8 and 1.9).

Table 1.7. Area under *Septoria tritici* disease progress curve for each fungicide on leaves 1-4 in 1998/99.

| Fungicide | Rate (proportion of recommended rate) | Area under disease progress curve (AUDPC) | | | |
|---|---|---|---|---|---|
| | | leaf 1 | leaf 2 | leaf 3 | leaf 4 |
| Untreated | ---- | 416.3 | 567.5 | 469.0 | 805.7 |
| Azoxystrobin + epoxiconazole | 0.25 | 164.0 | 311.6 | 412.5 | 627.2 |
| Azoxystrobin + epoxiconazole | 0.50 | 37.3 | 307.6 | 327.3 | 499.3 |
| Azoxystrobin + epoxiconazole | 1.00 | 28.4 | 235.5 | 126.3 | 484.9 |
| Kresoxim-methyl + epoxiconazole | 0.25 | 22.9 | 198.1 | 148.2 | 395.1 |
| Kresoxim-methyl + epoxiconazole | 0.50 | 7.5 | 70.1 | 145.0 | 189.9 |
| Kresoxim-methyl + epoxiconazole | 1.00 | 2.0 | 63.4 | 71.5 | 175.6 |
| Epoxiconazole | 0.25 | 109.6 | 408.0 | 255.5 | 271.3 |
| Epoxiconazole | 0.50 | 51.4 | 211.4 | 202.0 | 277.8 |
| Epoxiconazole | 1.00 | 6.8 | 54.2 | 213.9 | 111.2 |
| SED (18 df) | | 63.70 | 92.30 | 93.10 | 146.60 |
| *P* | | <0.001 | <0.001 | 0.007 | 0.003 |

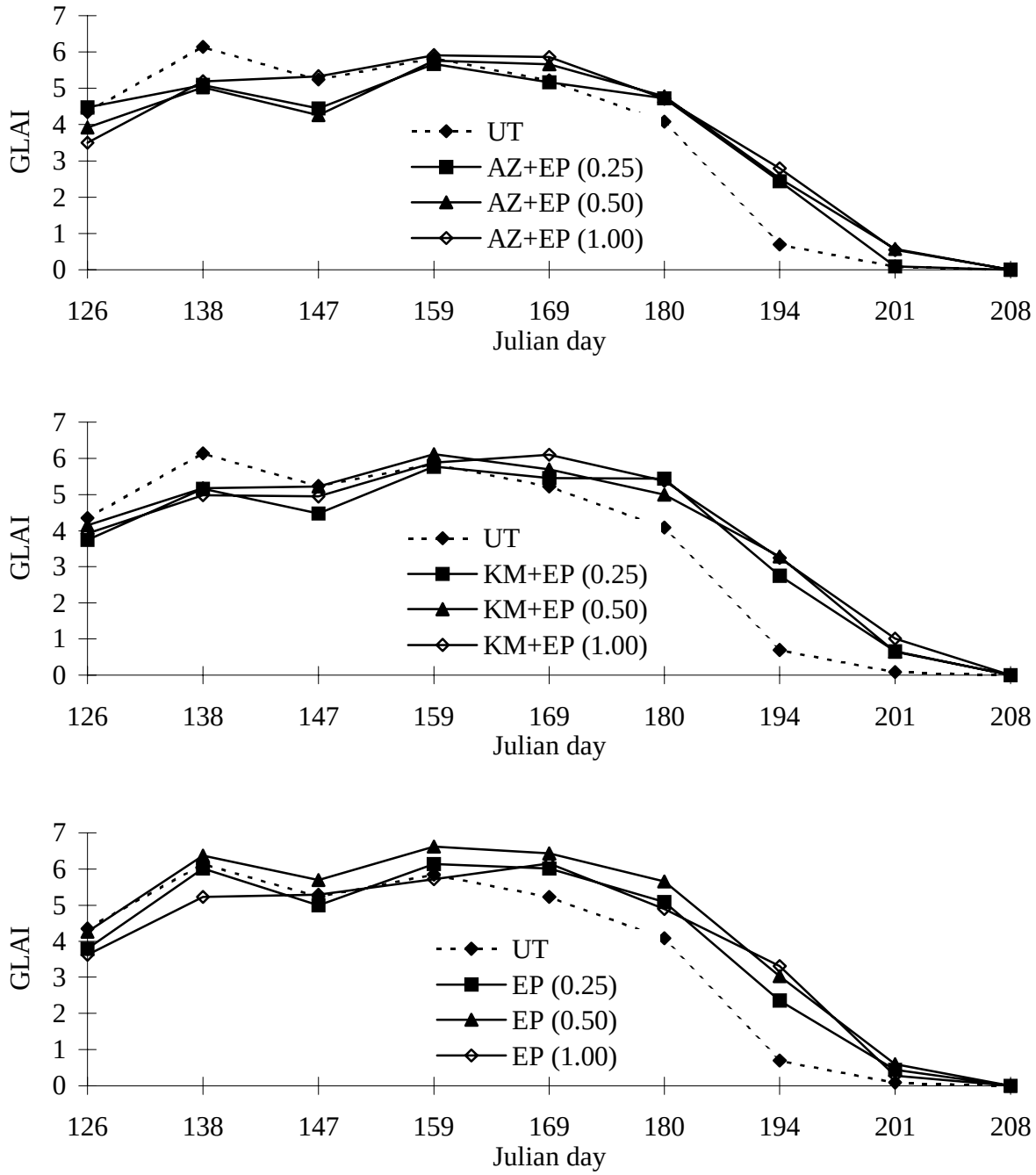


Figure 1.6. Green leaf area index (GLAI) in 1998/99. AZ = azoxystrobin, KM = kresoxim-methyl, EP = epoxiconazole, UT = untreated control.

Table 1.8. Total crop dry matter, 1998/99.

| Fungicide | Rate* | Total dry matter (t/ha) | | | |
|---|---|---|---|---|---|
| | | GS 39 (25 May) | GS 65 (14 June) | GS 75 (5 July) | GS 87 (19 July) |
| Untreated | ---- | 7.55 | 12.18 | 16.28 | 19.18 |
| Azoxystrobin + epoxiconazole | 0.25 | 7.59 | 11.74 | 16.69 | 18.85 |
| Azoxystrobin + epoxiconazole | 0.50 | 7.37 | 11.80 | 15.84 | 20.33 |
| Azoxystrobin + epoxiconazole | 1.00 | 6.90 | 12.43 | 16.10 | 19.03 |
| Kresoxim-methyl + epoxiconazole | 0.25 | 7.35 | 11.87 | 16.38 | 19.46 |
| Kresoxim-methyl + epoxiconazole | 0.50 | 6.96 | 11.71 | 17.47 | 19.04 |
| Kresoxim-methyl + epoxiconazole | 1.00 | 7.84 | 13.27 | 16.10 | 19.67 |
| Epoxiconazole | 0.25 | 7.96 | 11.49 | 17.07 | 20.58 |
| Epoxiconazole | 0.50 | 8.05 | 13.17 | 15.97 | 20.69 |
| Epoxiconazole | 1.00 | 7.88 | 12.24 | 16.99 | 18.06 |
| SED (18 df) | | 0.736 | 0.818 | 1.062 | 1.757 |
| *P* | | 0.883 | 0.399 | 0.840 | 0.884 |

* rate expressed as a proportion of the recommended rate

Table 1.9. Healthy Area Duration (HAD39), yield and specific weight, 1998/99.

| Fungicide | Rate* | HAD39 (ha/ha days) | Yield (t/ha) | Specific weight (kg/hl) |
|---|---|---|---|---|
| Untreated | ---- | 260.4 | 8.41 | 73.8 |
| Azoxystrobin + epoxiconazole | 0.25 | 271.4 | 9.35 | 73.9 |
| Azoxystrobin + epoxiconazole | 0.50 | 278.2 | 9.57 | 74.4 |
| Azoxystrobin + epoxiconazole | 1.00 | 296.1 | 10.01 | 74.3 |
| Kresoxim-methyl + epoxiconazole | 0.25 | 290.3 | 9.51 | 74.2 |
| Kresoxim-methyl + epoxiconazole | 0.50 | 304.4 | 9.77 | 74.6 |
| Kresoxim-methyl + epoxiconazole | 1.00 | 308.0 | 10.03 | 74.7 |
| Epoxiconazole | 0.25 | 299.8 | 9.49 | 74.3 |
| Epoxiconazole | 0.50 | 333.4 | 9.90 | 74.3 |
| Epoxiconazole | 1.00 | 303.4 | 9.96 | 73.2 |
| SED (18 df) | | 22.77 | 0.229 | 0.56 |
| *P* | | 0.171 | <0.001 | 0.409 |

* rate expressed as a proportion of the recommended rate

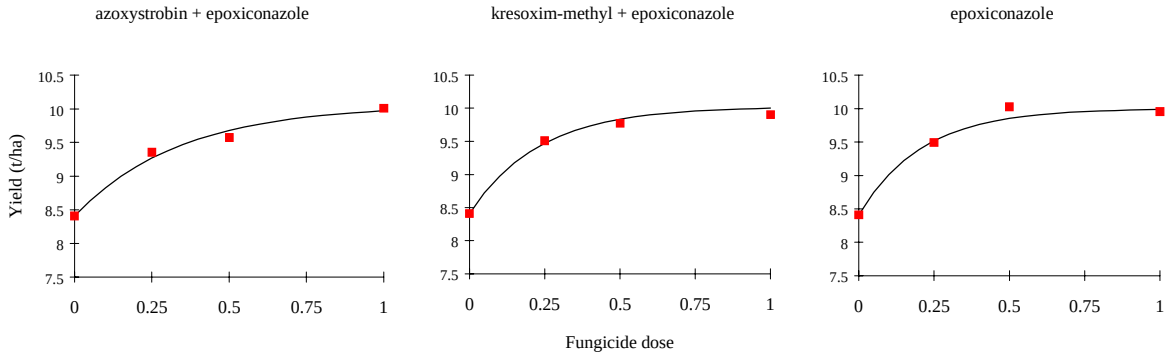


Figure 1.7. Relationship between yield and dose, 1998/99. Solid lines are fitted curves, and actual data points are also shown.

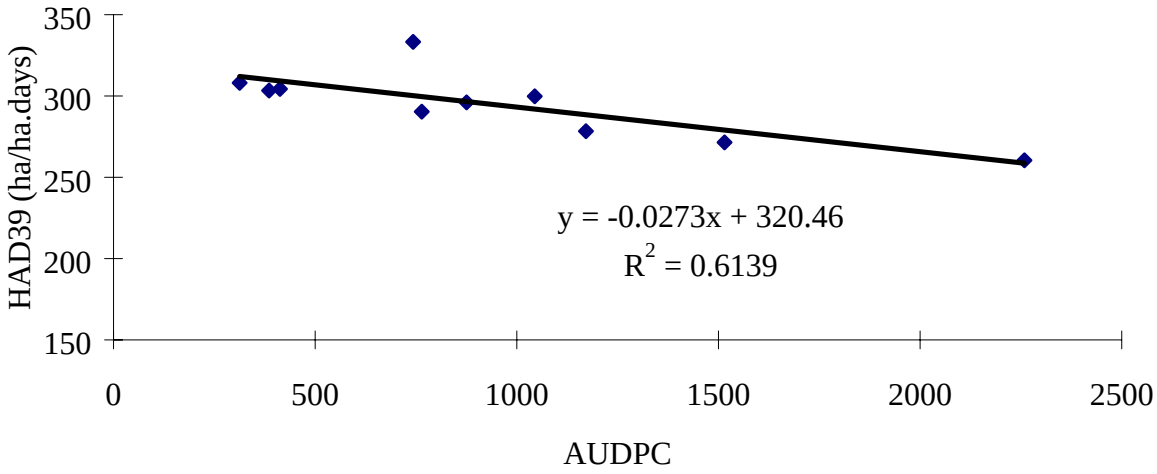


Figure 1.8. Relationship between canopy size integrated over time (HAD39) and disease (sum of AUDPC, leaves 1-4), 1998/99.

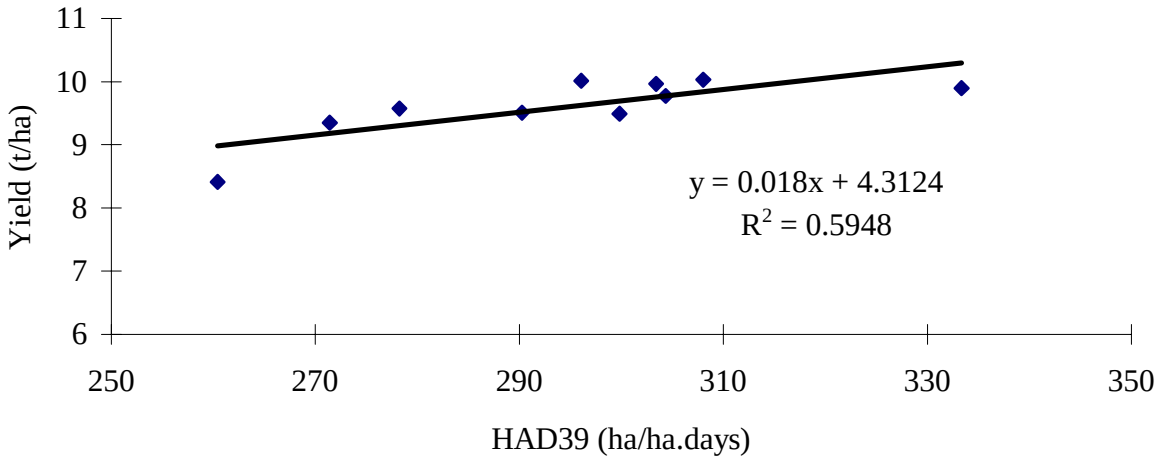


Figure 1.9. Relationship between yield and canopy size integrated over time (HAD39), 1998/99.

**1999/2000**

Foliar disease levels on the flag leaves were very low (below 1% in totally untreated plots on 8 July and below 3% on 16 July), until the final assessment on 21 July, when 18.7% leaf area was affected, compared with 6.2% in the no strobilurin treatment (No. 2). On leaf 2, 5.9% leaf area was affected in totally untreated plots on 30 June, which rose to 17.8% by 21 July, compared with 20.3% in no strobilurin plots. On leaf 3, disease increased steadily in totally untreated plots in June, reaching 45.8% by 8 July. The no strobilurin treatment had 32.5% *S. tritici* on this date.

The no strobilurin treatment reduced AUDPC on leaves 1, 3 and 4 compared with the totally untreated plots, with only traces of disease remaining on the flag leaves.(Table 1.10). Both strobilurins gave good disease control on all leaves, with evidence of a dose-response on leaves 2 and 3 but not on leaf 4.

Differences between treatments in green leaf area index were significant only on days 196 and 201 (15 and 20 July) (Figure 1.10). There was no difference between the no strobilurin and totally untreated plots, but all doses of both fungicides increased green leaf area index, except for quarter and half doses of kresoxim-methyl on day 201.

There were no significant differences between treatments in shoot numbers in any of the assessments, nor were there any significant differences in total crop biomass (Table 1.12).

There was not a statistically significant effect on yield, specific weight or thousand grain weight from the no strobilurin treatment (Table 1.12). All doses of each strobilurin increased yield, with maximum yield increases of 1.29 t/ha from azoxystrobin at 2.0 litre/ha, compared with 1.19 t/ha from the corresponding dose of kresoxim-methyl. Dose-response curves for the two fungicides were very similar, and were approaching the asymptote at doses below 1.0 l/ha (Figure 1.11).

The regression of HAD39 on disease (sum of AUDPC for leaves 1-4) accounted for 35.3% of variance (Figure 1.12). However, Treatment 10 had a strong weighting effect on the regression and, when this datum was removed, the regression for the remaining data accounted for 80.4% of variance. The regression of yield on HAD39 accounted for only 26.8% of the variance in yield (Figure 1.13).

Table 1.10. Area under *Septoria tritici* disease progress curve for each fungicide on leaves 1-4 in 1999/2000.

| Fungicide | Rate (proportion of recommended rate) | Area under disease progress curve (AUDPC) | | | |
|---|---|---|---|---|---|
| | | leaf 1 | leaf 2 | leaf 3 | leaf 4 |
| --- | ---- | 67.7 | 376.8 | 625.7 | 521.4 |
| ---* | ---- | 20.7 | 341.8 | 290.6 | 325.6 |
| Azoxystrobin* | 0.25 | 2.8 | 135.1 | 153.4 | 146.6 |
| Azoxystrobin* | 0.50 | 3.2 | 105.2 | 79.3 | 190.9 |
| Azoxystrobin* | 1.00 | 3.1 | 41.3 | 41.6 | 141.5 |
| Azoxystrobin* | 2.00 | 0.5 | 49.0 | 42.4 | 125.3 |
| Kresoxim-methyl* | 0.25 | 6.0 | 106.0 | 79.7 | 194.1 |
| Kresoxim-methyl* | 0.50 | 10.6 | 128.4 | 91.4 | 175.3 |
| Kresoxim-methyl* | 1.00 | 6.1 | 56.9 | 121.4 | 173.9 |
| Kresoxim-methyl* | 2.00 | 1.1 | 19.8 | 37.7 | 131.4 |
| SED (18 df) | | 12.03 | 50.20 | 53.30 | 61.40 |
| *P* | | <0.001 | <0.001 | <0.001 | <0.001 |

*Overall protectant fungicides applied at GS 31 and GS 45

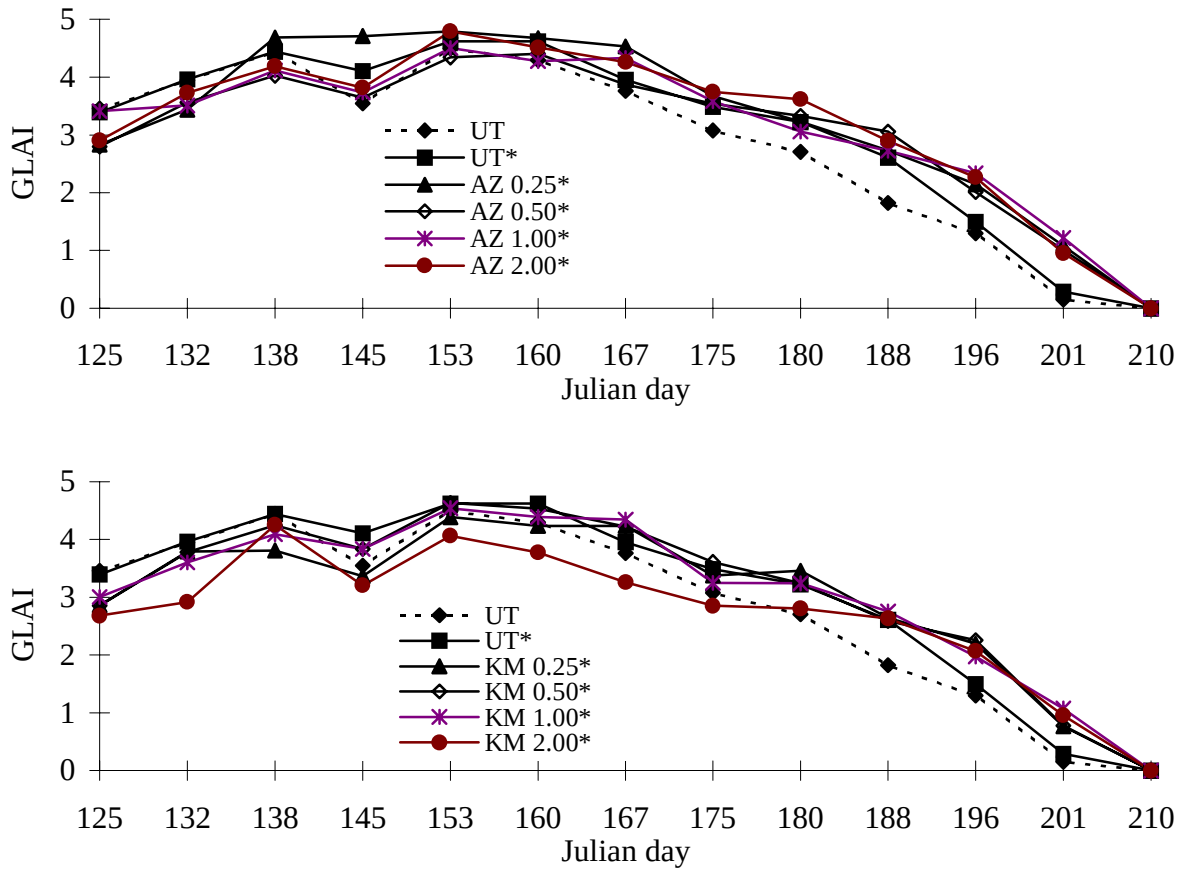


Figure 1.10. Green leaf area index (GLAI) in 1999/2000. AZ = azoxystrobin, KM = kresoxim-methyl, UT = untreated. * indicates overall protectant fungicides applied

Table 1.11. Healthy Area Duration (HAD39) and total crop dry matter, 1999/2000.

| Fungicide | Rate** | HAD39 (ha/ha days) | Total dry matter (t/ha) | | | |
|---|---|---|---|---|---|---|
| | | | GS 39 | GS 65 | GS 75 | GS 87 |
| --- | ---- | 169.8 | 6.34 | 10.72 | 16.31 | 17.34 |
| ---* | ---- | 191.3 | 6.88 | 12.07 | 15.47 | 17.47 |
| Azoxystrobin* | 0.25 | 212.4 | 6.60 | 11.99 | 16.60 | 17.90 |
| Azoxystrobin* | 0.50 | 198.8 | 6.89 | 11.00 | 16.50 | 17.70 |
| Azoxystrobin* | 1.00 | 202.7 | 7.16 | 11.05 | 15.80 | 16.79 |
| Azoxystrobin* | 2.00 | 210.2 | 7.01 | 10.72 | 15.49 | 17.28 |
| Kresoxim-methyl* | 0.25 | 195.9 | 7.06 | 10.85 | 16.10 | 17.83 |
| Kresoxim-methyl* | 0.50 | 201.8 | 7.73 | 11.85 | 16.50 | 17.20 |
| Kresoxim-methyl* | 1.00 | 199.6 | 7.10 | 11.54 | 14.63 | 19.31 |
| Kresoxim-methyl* | 2.00 | 174.8 | 6.64 | 10.53 | 17.15 | 17.75 |
| SED (18 df) | | 21.09 | 0.481 | 0.819 | 0.940 | 1.375 |
| *P* | | 0.581 | 0.335 | 0.478 | 0.361 | 0.869 |

*Overall protectant fungicides applied at GS 31 and GS 45
**Proportion of recommended rate

Table 1.12. Yield, grain quality and harvest index, 1999/2000.

| Fungicide | Rate** | Yield (t/ha) | Specific weight (kg/hl) | Thousand grain weight (g) | Harvest index | Grains per ear |
|---|---|---|---|---|---|---|
| --- | ---- | 7.24 | 76.8 | 39.6 | 39.8 | 30.9 |
| ---* | ---- | 7.26 | 77.1 | 41.9 | 41.5 | 32.2 |
| Azoxystrobin* | 0.25 | 8.15 | 77.5 | 43.8 | 47.2 | 29.8 |
| Azoxystrobin* | 0.50 | 8.42 | 77.3 | 44.5 | 41.4 | 30.0 |
| Azoxystrobin* | 1.00 | 8.54 | 78.0 | 45.6 | 43.2 | 31.6 |
| Azoxystrobin* | 2.00 | 8.55 | 77.7 | 45.5 | 42.7 | 31.2 |
| Kresoxim-methyl* | 0.25 | 8.02 | 77.1 | 43.6 | 39.1 | 31.9 |
| Kresoxim-methyl* | 0.50 | 8.25 | 78.0 | 43.7 | 40.5 | 30.2 |
| Kresoxim-methyl* | 1.00 | 8.19 | 77.0 | 43.7 | 42.0 | 33.0 |
| Kresoxim-methyl* | 2.00 | 8.45 | 77.8 | 44.8 | 42.4 | 32.1 |
| SED (18 df) | | 0.335 | 0.36 | 1.38 | 3.03 | 2.05 |
| *P* | | 0.005 | 0.005 | 0.014 | 0.409 | 0.825 |

*Overall protectant fungicides applied at GS 31 and GS 45
**Proportion of recommended rate

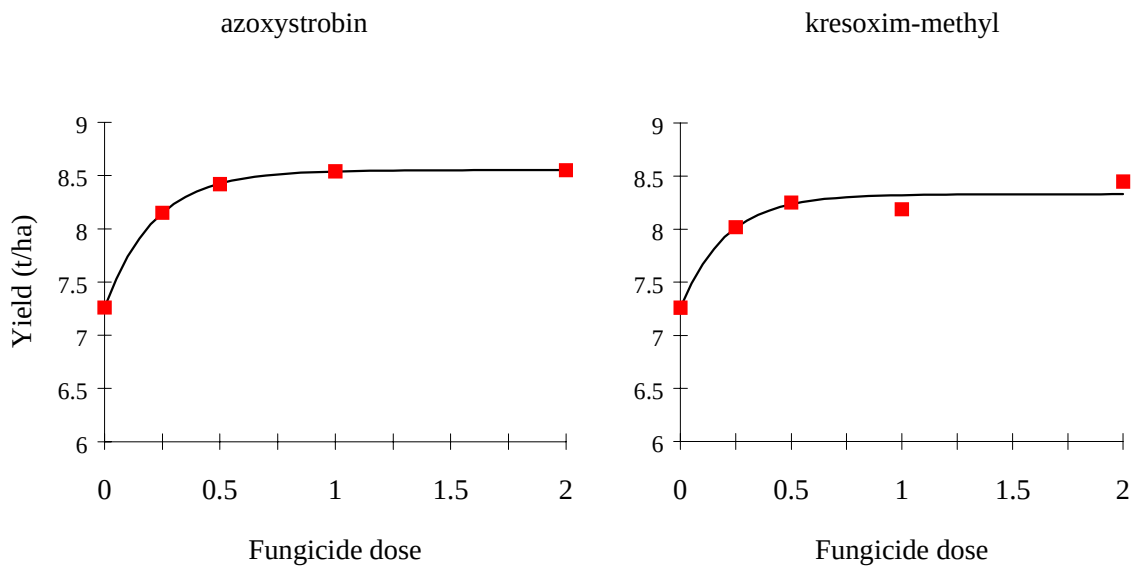


Figure 1.11. Relationship between yield and dose, 1999/2000. Solid lines are fitted curves, and actual data points are also shown.

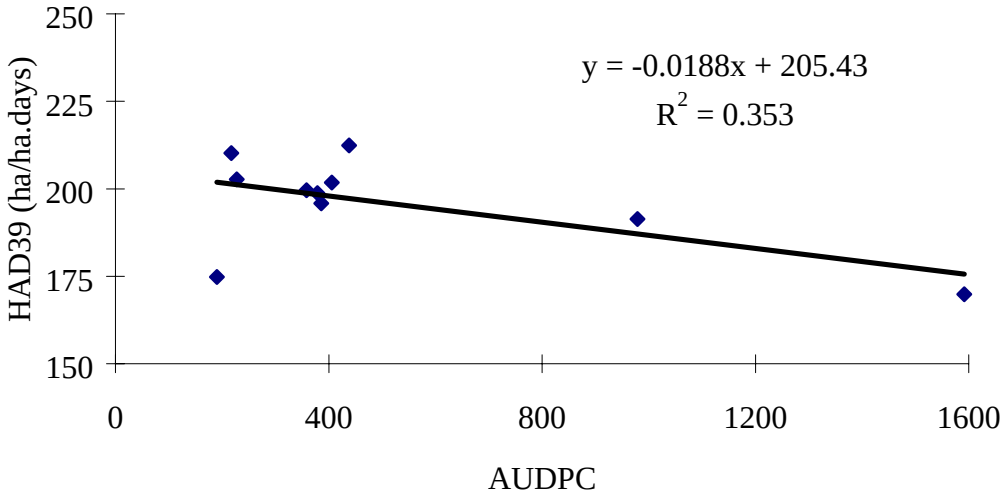


Figure 1.12. Relationship between canopy size integrated over time (HAD39) and disease (sum of AUDPC, leaves 1-4), 1999/2000.

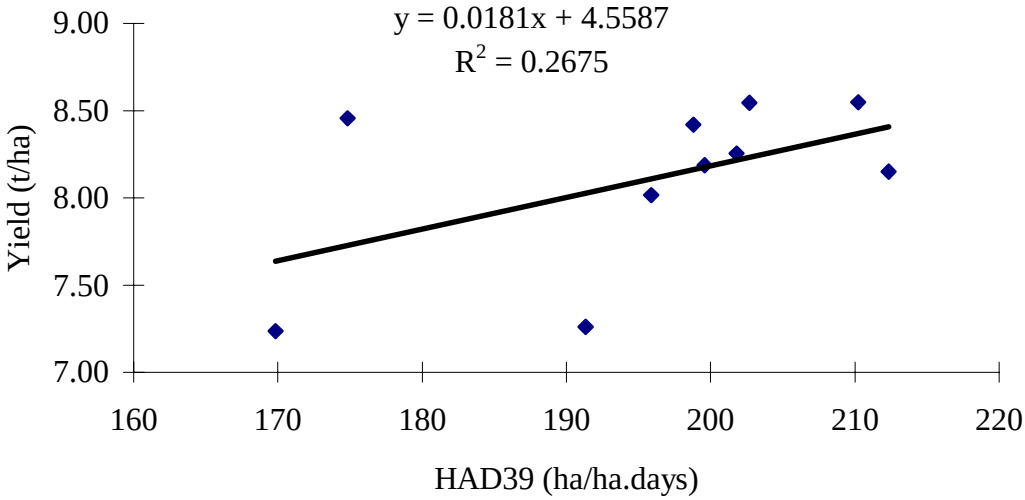


Figure 1.13. Relationship between yield and canopy size integrated over time (HAD39), 1999/2000.

**Discussion**

This experiment was located at ADAS Boxworth, a site at which severe foliar disease is uncommon. The cultivar selected, Spark, has good foliar disease resistance, but was affected to some extent by *Septoria tritici* in each of the three years of the experiment. In each year, disease severity was low until the end of June, but increased during July, particularly in the second and third years. Although disease severity was considerably lower than at the other site used within this project (ADAS Rosemaund), the experiments did not achieve the freedom from disease which had been hoped for. The overall protectant fungicides applied in 1999/2000 did reduce disease in comparison with the totally untreated control, but did not give a disease-free crop on which to study non-pathogenic effects of the fungicides.

Although disease severity was low, there was a clear effect of fungicide dose on *S. tritici* in the second and third years of the experiment. Differences in canopy size between treatments also became evident at around the same time that disease increased, although the relationships between canopy and disease were not particularly close. The effect of fungicide dose on green leaf area index appeared to be strongest in 1997/98, even though this was the year with least disease.

There were clear dose-responses for yield for all fungicides, except for epoxiconazole in 1997/98. The shapes of the curves for all fungicides in 1998/99 and 1999/2000 were broadly similar, and kresoxim-methyl plus epoxiconazole in 1997/98 also showed this pattern, which is consistent with most of the HGCA-funded work on Appropriate Fungicide Doses (Paveley, 2000). However, azoxystrobin in 1997/98 showed less curvature, with a strong indication that yield would have continued to rise at doses above the highest dose tested on 1.00 litre/ha. In Experiment 6 within this project, azoxystrobin also gave a curve of this type in 1997/98, under high disease conditions, in contrast with the results from epoxiconazole at the same site, and with both fungicides in the other two years of that experiment. The reason why azoxystrobin showed a different response in two individual experiments in 1997/98 is not clear, since they were different sites, cultivars and disease severities.

No statistically significant effects of fungicides on crop biomass were recorded in any year, even though there were differences in yield. This suggests that measurement of biomass in

this way may not be sufficiently sensitive to account for the relatively small effects of fungicide on yield that were recorded in this experiment.

Relationships between yield and canopy were poor in the first and third years and the best relationship, in 1998/99, accounted for only 59.5% of the total variance.

Overall, few conclusions can be drawn from this experiment regarding whether or not there are physiological effects of strobilurins which contribute significantly to crop yield. Although disease levels were relatively low, and yield increases modest compared with sites with severe disease, there were consistent dose-response relationships for yield which corresponded with differences in disease control and canopy retention. There were no clear indications that yield increases resulted from physiological effects on the crop, which is consistent with other Experiments within this Project. Because the differences were small, it may be that conventional assessments of canopy and crop growth are not sufficiently sensitive to detect small differences that may occur. The possibility of such differences resulting from physiological effects cannot be discounted but, since they are likely to be small, it would take very extensive and sensitive experimentation to detect them. These results show that their value to growers under normal conditions of moderate or high disease risk would be small in relation to the large fungicidal effects that occur consistently.

## APPENDIX 2

### Experiment 2: Interaction between strobilurin fungicides and cultivar susceptibility to disease

### Introduction

Early experimental work with strobilurin fungicides showed that, in some instances, strobilurin application to winter wheat during stem elongation resulted in greater duration of green canopy than resulted from application of triazole fungicides on the same dates, although there was little difference between fungicides in disease control (Jones, 2000). During the development of both kresoxim-methyl and azoxystrobin, it was shown that the yield benefits from a strobilurin programme compared with a 'conventional' programme based on triazole fungicides was evident across all wheat cultivars, not just those which were most susceptible to disease.

The aim of this experiment was to investigate differences in response to strobilurins between susceptible and resistant cultivars, at sites with differing risk of severe foliar disease.

### Methods

Experiments were established in 1997/98 on two sites, one with a low risk of foliar disease at ADAS Boxworth, Cambridgeshire, on a clay loam of the Hanslope series, and one on a site with high disease risk at ADAS Rosemaund, Herefordshire, on a silty clay loam of the Bromyard series.

Three cultivars were selected which differed in susceptibility to the major foliar diseases. Riband is very susceptible to both *Septoria tritici* and brown rust (rating of 3 for each disease), Charger is moderately susceptible to *S. tritici* (rating 5), and Spark has good resistance (rating 7 or above ) to all major foliar pathogens (Anonymous, 1998). Cultivars and fungicides are detailed in Table 2.1. Fungicides were applied in 200-250 litre/ha water, using an MDM Oxford Precision Sprayer. The experimental design at was a two-factor randomised block with three replicates. Each experimental plot consisted of two adjacent duplicate sub-plots, sizes in the range 36-60 $m^2$, one of which was used for disease assessment and yield estimation and the other for destructive sampling for growth analysis.

Table 2.1. Cultivars and fungicides

| Treat-ment | Cultivar | Fungicide | Product and application rate of product/ha | |
|---|---|---|---|---|
| 1 | Riband | Untreated | --- | --- |
| 2 | Riband | Azoxystrobin | Amistar | 1.00 litre/ha |
| 3 | Riband | Kresoxim-methyl + epoxiconazole | Landmark | 1.00 litre/ha |
| 4 | Riband | Epoxiconazole | Opus | 1.00 litre/ha |
| 5 | Charger | Untreated | --- | --- |
| 6 | Charger | Azoxystrobin | Amistar | 1.00 litre/ha |
| 7 | Charger | Kresoxim-methyl + epoxiconazole | Landmark | 1.00 litre/ha |
| 8 | Charger | Epoxiconazole | Opus | 1.00 litre/ha |
| 9 | Spark | Untreated | --- | --- |
| 10 | Spark | Azoxystrobin | Amistar | 1.00 litre/ha |
| 11 | Spark | Kresoxim-methyl + epoxiconazole | Landmark | 1.00 litre/ha |
| 12 | Spark | Epoxiconazole | Opus | 1.00 litre/ha |

Each treatment was applied on two occasions, at GS 31-32 and GS 39 (Tottman & Broad, 1987). Other agronomic inputs followed good commercial practice.

Foliar diseases were assessed as visual estimates of the percentage leaf area infected by each disease on each leaf layer, on 10 tillers taken at random from each sub plot at approximately 10 day intervals from the date of the first fungicide application until all leaves were senescent. Percentage green leaf area was also estimated. To provide a cumulative measure of the effect of disease during the life of the stem leaves, the area under disease progress curve (AUDPC) was calculated for each treatment. This can be visualised on a graph showing disease progress over time, as the area under the line showing disease development for that treatment.

Growth analysis was done at the third node stage, flag leaf emergence (GS 39), when 50% of all shoots reached mid-anthesis (GS 65), at the milky-ripe stage (GS 75) and at mid-senescence (GS 87), calculated at 750 ºCd after GS 39 (Foulkes & Scott, 1998). On each occasion, an area of either 0.50 $m^2$ (at Boxworth) or 0.81 $m^2$ (at Rosemaund) was sampled, leaving at least 0.5 m between samples and at least three rows from plot edges to avoid edge effects (Austin & Blackwell, 1980). The plants were cut at ground level and taken to the laboratory for analysis. The number and dry weight of shoots were assessed separately for potentially fertile, dead and dying shoots. A shoot was classified as dying when no further leaf was emerging and the most recently emerged leaf was yellowing (Thorne & Wood, 1987).

Green area index (GAI) was calculated by determining actual leaf size on each occasion that diseases were assessed, using two of the ten leaves taken from each plot. Leaf length and width were measured , to the nearest 0.1 mm, and leaf area determined using a form factor of 0.83 (Bryson *et al*., 1997). GAI was calculated using the mean number of shoots per plot from assessments between mid-anthesis and mid-senescence. The leaf areas were then integrated over time from GS 39 until the end of all green canopy to give healthy area duration from GS 39 (HAD39; Bryson *et al.*, 1997). This provides a measure of green canopy size during the period in which photosynthesis is contributing primarily to grain filling rather than to canopy structure.

The water soluble carbohydrate (WSC) content of the stems was determined, for the experiment at Rosemaund, at mid-anthesis, the development stage at it is near to its maximum (Foulkes *et al*., 1998), and at mid-senescence, for the experiments at Rosemaund

only. This was determined on 8 shoots randomly selected from each plot and flash dried at 105ºC for 2 hours. The WSC content was then assessed using the spectrophotometery method described by Thomas (1977).

At harvest, a further 0.81 $m^2$ quadrat was removed from each sub plot at Rosemaund only, for estimation of yield components. Final crop dry weight and ear number were recorded. The ears were then threshed, grain dry weight measured and harvest index calculated. Grain yield was measured from the 2 m x 24 m strip allocated within each sub plot using a plot combine harvester. Grain was analysed for moisture content and specific weight using GAC 2000 grain analysis computer (Dickey-John Corporation). The thousand grain weight was determined on grain samples taken from the combine. The number of grains per ear was then calculated using the combine grain yield, the thousand grain weight and the number of ears/$m^2$.

**Results - Boxworth**

Disease severity on the top two leaves remained very low through until late June, but *Septoria tritici* and brown rust both developed during the last three weeks of green leaf life. Both were most severe on Riband and least severe on Spark (Figures 2.1 and 2.2). Differences between cultivars in *S. tritici* severity, measured as area under disease progress curve (AUDPC) were significant on leaf 3 only, where Riband had most and Spark least disease, although there were indications of similar ranking for disease severity on leaves 2 and 4 (Table 2.2). For brown rust, Riband was affected more severely than the other two cultivars on leaves 1 and 2 (Table 2.3). All fungicides gave good control of both diseases on the top two leaves and, to a lesser extent, leaf 3, although differences for *S. tritici* on the flag leaves were not statistically significant. For both diseases, there were indications that kresoxim-methyl plus epoxiconazole gave the greatest reduction in disease, and azoxystrobin the smallest, although this also was not statistically significant.

Effects of fungicides on green canopy size were evident from mid-June on Riband and Charger, and also in July on Spark (Figure 2.4; Table 2.4). On Charger and Spark, green area was lost more quickly in untreated plots than in those treated with fungicide, but there were no significant differences between fungicides. On Riband, kresoxim-methyl plus epoxiconazole gave a greater canopy size than the other fungicides from day 162 until senescence.

Spark had higher shoot numbers than the other cultivars in each of the growth analyses, but there were no significant effects of fungicides on shoot number.

Growth analysis showed significant differences between cultivars at GS 39 only, when Charger had higher total dry matter than Spark (Table 2.4). Differences between fungicide treatments emerged only at the final assessment at GS 75, when all fungicide treatments had higher total dry matter than the untreated control, although differences between fungicides were not significant. There were no statistically significant interactions between cultivar and fungicide.

Yield differences were associated with both cultivar and fungicide (Table 2.5). Charger and Riband both gave higher mean yields than Spark. All fungicides increased yield, and the yield from kresoxim-methyl plus epoxiconazole was significantly higher than that from azoxystrobin. Cultivar x fungicide interactions were not statistically significant, but there was an indication of a greater yield response to fungicide on Riband (mean increase for the three fungicides 3.55 t/ha), compared with Charger (1.64 t/ha) or Spark (0.49 t/ha). Spark had the

highest specific weight and Riband the lowest. All fungicides increased specific weight, and the effect of fungicides on specific weight was greatest on Riband.

There were significant regressions on each cultivar of canopy duration (expressed as HAD39) on disease (expressed as total AUDPC for *S. tritici* and brown rust on the top four leaves) (Figure 2.5). The strongest relationship was on Charger ($R^2$ = 0.946), compared with Riband (0.889) and Spark (0.744). Relationships between yield and canopy size showed a similar pattern ($R^2$ = 0.931 for Charger, 0.878 for Riband and 0.582 for Spark; Figure 2.6).

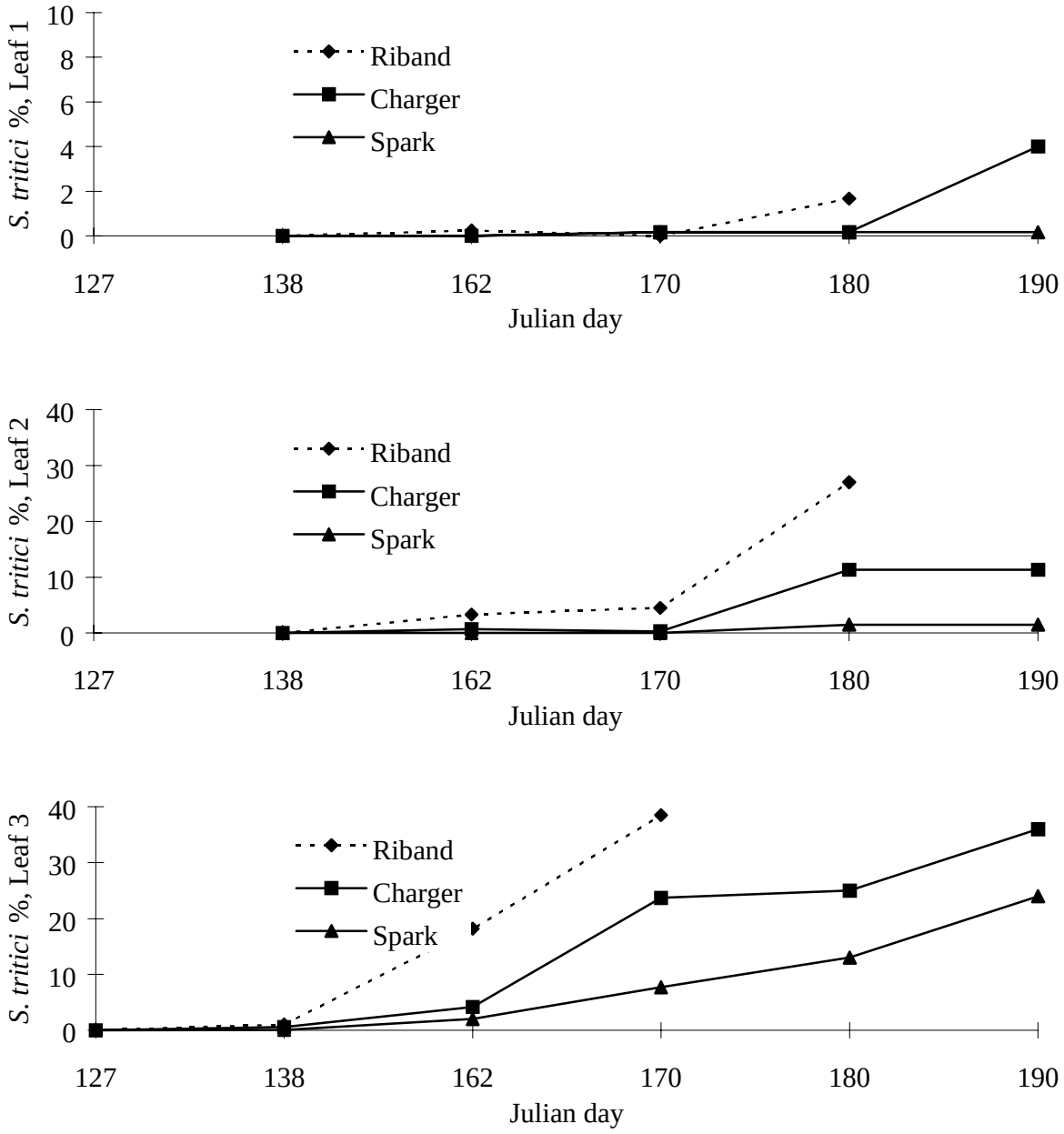


Figure 2.1. *Septoria tritici* development in untreated plots on leaves 1-3, Boxworth.

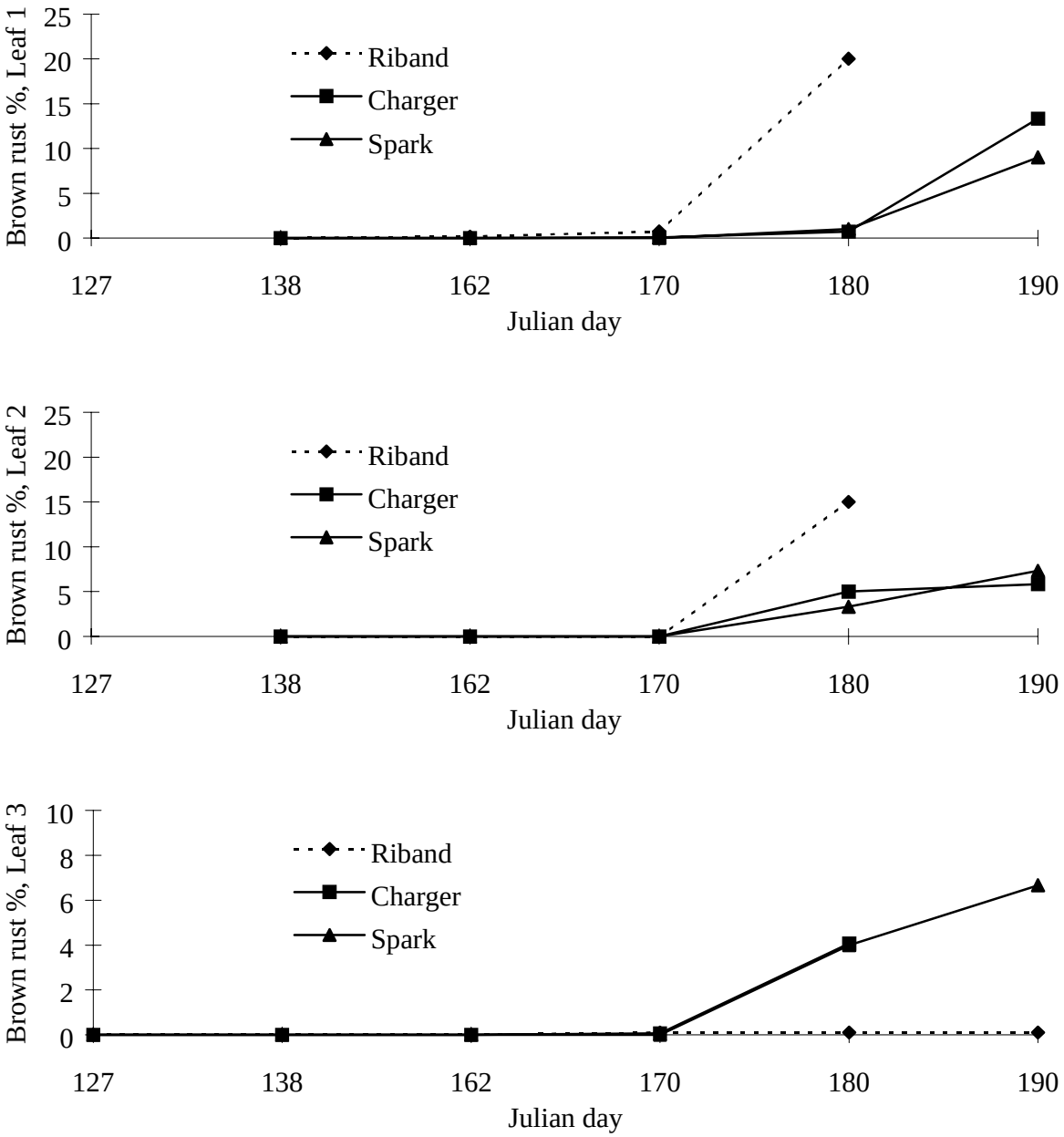


Figure 2.2. Brown rust development in untreated plots on leaves 1-3, Boxworth.

Table 2.2. Area under *Septoria tritici* disease progress curve for each fungicide on leaves 1-4, Boxworth.

| Cultivar | Fungicide | Area under disease progress curve (AUDPC) | | | |
|---|---|---|---|---|---|
| | | Leaf 1 | Leaf 2 | Leaf 3 | Leaf 4 |
| Riband | Untreated | 47.1 | 795.8 | 950.8 | 690.0 |
| Riband | Azoxystrobin | 1.3 | 48.7 | 483.0 | 689.3 |
| Riband | Kresoxim-methyl + epoxiconazole | 0.7 | 28.8 | 131.4 | 316.0 |
| Riband | Epoxiconazole | 21.6 | 19.2 | 226.0 | 410.0 |
| Charger | Untreated | 67.2 | 260.6 | 716.1 | 678.0 |
| Charger | Azoxystrobin | 27.8 | 39.0 | 198.5 | 375.3 |
| Charger | Kresoxim-methyl + epoxiconazole | 1.5 | 4.0 | 42.9 | 183.0 |
| Charger | Epoxiconazole | 1.7 | 19.6 | 138.5 | 294.1 |
| Spark | Untreated | 2.2 | 28.3 | 351.8 | 477.3 |
| Spark | Azoxystrobin | 8.3 | 20.2 | 84.9 | 218.7 |
| Spark | Kresoxim-methyl + epoxiconazole | 2.4 | 10.6 | 67.6 | 197.5 |
| Spark | Epoxiconazole | 1.3 | 15.6 | 58.3 | 168.1 |
| Riband | Mean | 17.7 | 223.1 | 447.8 | 526.3 |
| Charger | Mean | 24.5 | 80.8 | 274.0 | 382.6 |
| Spark | Mean | 3.6 | 18.7 | 140.7 | 265.4 |
| Mean | Untreated | 38.8 | 361.6 | 672.9 | 615.1 |
| Mean | Azoxystrobin | 12.4 | 36.0 | 255.5 | 427.8 |
| Mean | Kresoxim-methyl + epoxiconazole | 1.5 | 14.5 | 80.6 | 232.2 |
| Mean | Epoxiconazole | 8.2 | 18.1 | 140.9 | 290.7 |
| SED (22 df) | | | | | |
| Cultivar | | 11.74 | 84.9 | 89.1 | 100.6 |
| Fungicide | | 13.56 | 98.1 | 102.9 | 116.1 |
| Interaction | | 23.48 | 169.9 | 178.3 | 201.1 |
| *P* | | | | | |
| Cultivar | | 0.213 | 0.068 | 0.008 | 0.053 |
| Fungicide | | 0.058 | 0.004 | <0.001 | 0.016 |
| Interaction | | 0.354 | 0.048 | 0.454 | 0.881 |

Table 2.3. Area under brown rust disease progress curve for each fungicide on leaves 1-4, Boxworth.

| Cultivar | Fungicide | Area under disease progress curve (AUDPC) | | | |
|---|---|---|---|---|---|
| | | Leaf 1 | Leaf 2 | Leaf 3 | Leaf 4 |
| Riband | Untreated | 419.6 | 390.0 | 2.4 | 0.0 |
| Riband | Azoxystrobin | 122.8 | 164.5 | 39.9 | 0.0 |
| Riband | Kresoxim-methyl + epoxiconazole | 96.8 | 86.9 | 33.0 | 0.0 |
| Riband | Epoxiconazole | 111.3 | 112.5 | 40.7 | 0.0 |
| Charger | Untreated | 147.9 | 143.3 | 61.6 | 0.0 |
| Charger | Azoxystrobin | 77.3 | 77.3 | 10.2 | 0.0 |
| Charger | Kresoxim-methyl + epoxiconazole | 4.9 | 10.5 | 9.2 | 0.0 |
| Charger | Epoxiconazole | 13.7 | 11.6 | 39.8 | 0.0 |
| Spark | Untreated | 214.5 | 150.7 | 73.3 | 0.0 |
| Spark | Azoxystrobin | 47.1 | 35.7 | 5.7 | 0.0 |
| Spark | Kresoxim-methyl + epoxiconazole | 12.2 | 25.0 | 2.1 | 0.0 |
| Spark | Epoxiconazole | 26.5 | 16.9 | 5.8 | 0.0 |
| Riband | Mean | 187.6 | 188.5 | 29.0 | 0.0 |
| Charger | Mean | 61.0 | 60.7 | 30.2 | 0.0 |
| Spark | Mean | 75.1 | 57.1 | 21.8 | 0.0 |
| Mean | Untreated | 260.7 | 228.0 | 45.8 | 0.0 |
| Mean | Azoxystrobin | 82.4 | 92.5 | 18.6 | 0.0 |
| Mean | Kresoxim-methyl + epoxiconazole | 38.0 | 40.8 | 14.7 | 0.0 |
| Mean | Epoxiconazole | 50.5 | 47.0 | 28.8 | 0.0 |
| SED (22 df) | | | | | |
| Cultivar | | 40.40 | 45.30 | 10.64 | --- |
| Fungicide | | 46.60 | 52.30 | 12.28 | --- |
| Interaction | | 80.80 | 90.50 | 21.27 | --- |
| *P* | | | | | |
| Cultivar | | 0.009 | 0.012 | 0.696 | --- |
| Fungicide | | <0.001 | 0.005 | 0.082 | --- |
| Interaction | | 0.593 | 0.782 | 0.014 | --- |

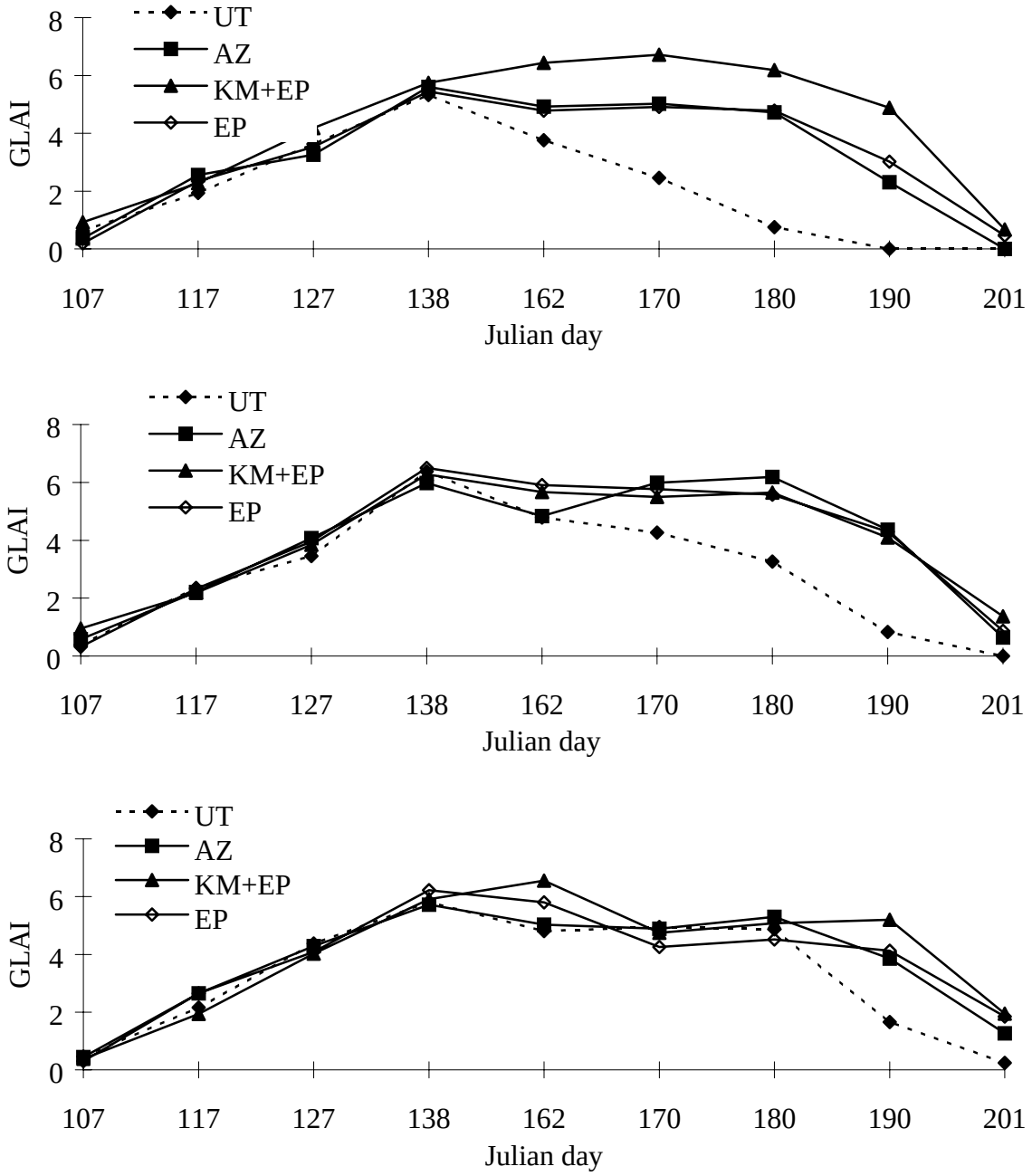


Figure 2.3. Green leaf area index (GLAI), Boxworth; Riband (upper), Charger (middle), Spark (lower).

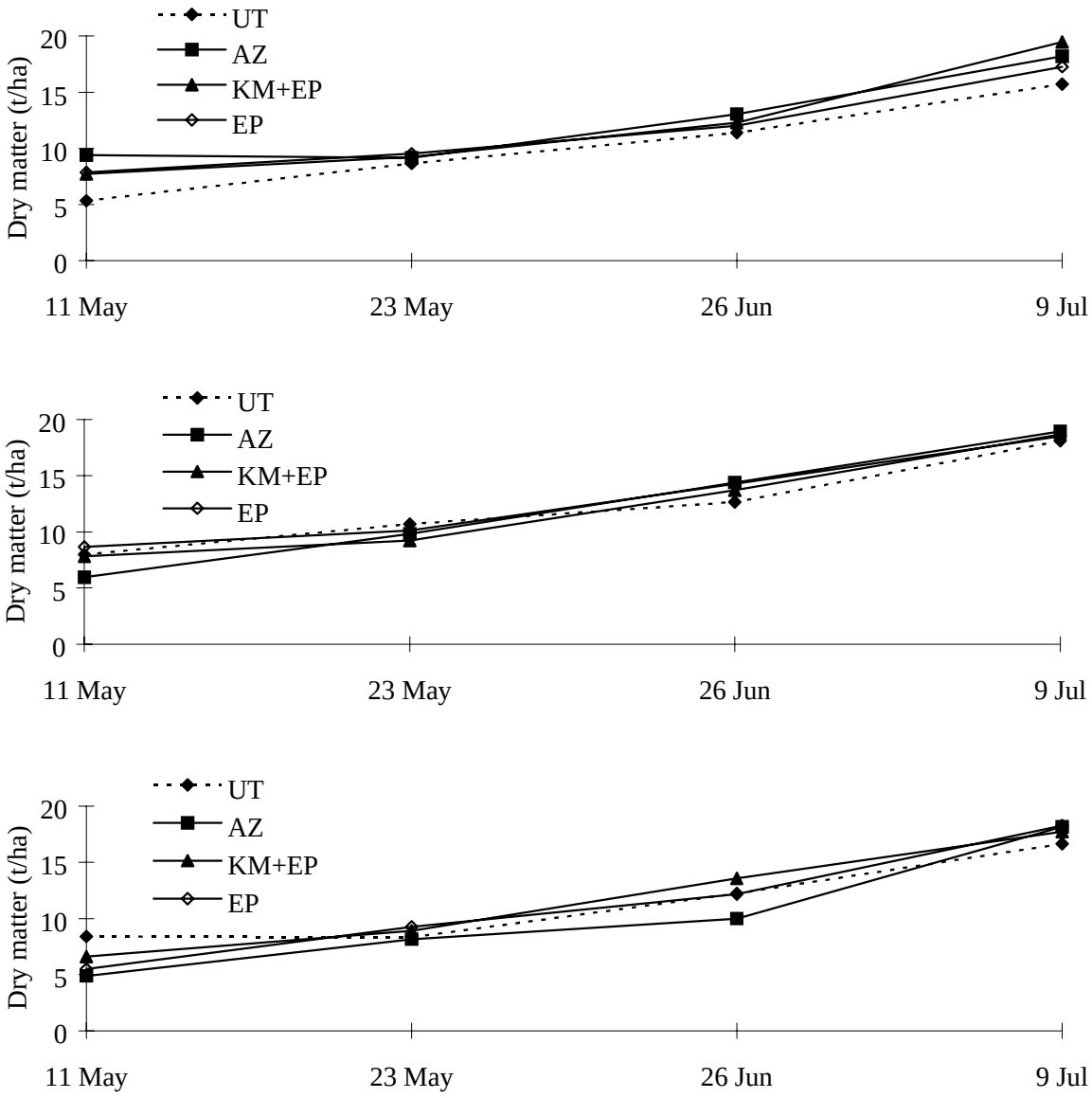


Figure 2.4. Total crop dry matter, Boxworth.

Table 2.4. Total crop dry matter, Boxworth.

| Cultivar | Fungicide | Total dry matter (t/ha) | | | |
|---|---|---|---|---|---|
| | | GS 33 | GS 39 | GS 65 | GS 75 |
| Riband | Untreated | 5.35 | 8.65 | 11.39 | 15.73 |
| Riband | Azoxystrobin | 9.4 | 9.17 | 13.05 | 18.20 |
| Riband | Kresoxim-methyl + epoxiconazole | 7.74 | 9.23 | 12.29 | 19.47 |
| Riband | Epoxiconazole | 7.85 | 9.54 | 12.03 | 17.27 |
| Charger | Untreated | 7.98 | 10.70 | 12.65 | 18.10 |
| Charger | Azoxystrobin | 5.96 | 9.81 | 14.38 | 18.93 |
| Charger | Kresoxim-methyl + epoxiconazole | 7.82 | 2.10 | 13.68 | 18.67 |
| Charger | Epoxiconazole | 8.65 | 10.10 | 14.27 | 18.50 |
| Spark | Untreated | 8.38 | 8.31 | 12.21 | 16.63 |
| Spark | Azoxystrobin | 4.89 | 8.16 | 10.00 | 18.17 |
| Spark | Kresoxim-methyl + epoxiconazole | 6.61 | 8.89 | 13.56 | 17.73 |
| Spark | Epoxiconazole | 5.49 | 9.23 | 12.17 | 18.27 |
| Riband | Mean | 7.58 | 9.15 | 12.19 | 17.67 |
| Charger | Mean | 7.60 | 9.95 | 13.75 | 18.55 |
| Spark | Mean | 6.34 | 8.65 | 11.99 | 17.70 |
| Mean | Untreated | 7.24 | 9.22 | 12.08 | 16.82 |
| Mean | Azoxystrobin | 6.75 | 9.05 | 12.48 | 18.43 |
| Mean | Kresoxim-methyl + epoxiconazole | 7.39 | 9.11 | 13.18 | 18.62 |
| Mean | Epoxiconazole | 7.33 | 9.62 | 12.82 | 18.01 |
| SED (22 df) | | | | | |
| Cultivar | | 1.044 | 0.431 | 0.767 | 0.494 |
| Fungicide | | 1.206 | 0.497 | 0.886 | 0.571 |
| Interaction | | 2.088 | 0.862 | 1.535 | 0.988 |
| *P* | | | | | |
| Cultivar | | 0.401 | 0.021 | 0.063 | 0.152 |
| Fungicide | | 0.949 | 0.659 | 0.647 | 0.020 |
| Interaction | | 0.250 | 0.602 | 0.390 | 0.331 |

Table 2.5. Yield, specific weight and harvest index, Boxworth.

| Cultivar | Fungicide | Yield (t/ha) | Specific weight (kg/hl) |
|---|---|---|---|
| Riband | Untreated | 6.14 | 65.6 |
| Riband | Azoxystrobin | 9.28 | 73.9 |
| Riband | Kresoxim-methyl + epoxiconazole | 10.10 | 75.8 |
| Riband | Epoxiconazole | 9.68 | 75.1 |
| Charger | Untreated | 7.51 | 74.4 |
| Charger | Azoxystrobin | 8.97 | 75.4 |
| Charger | Kresoxim-methyl + epoxiconazole | 9.44 | 76.8 |
| Charger | Epoxiconazole | 9.04 | 78.0 |
| Spark | Untreated | 6.79 | 77.7 |
| Spark | Azoxystrobin | 6.22 | 78.8 |
| Spark | Kresoxim-methyl + epoxiconazole | 7.92 | 79.3 |
| Spark | Epoxiconazole | 7.69 | 79.5 |
| Riband | Mean | 8.80 | 73.1 |
| Charger | Mean | 8.74 | 76.2 |
| Spark | Mean | 7.15 | 78.8 |
| Mean | Untreated | 6.81 | 73.2 |
| Mean | Azoxystrobin | 8.16 | 76.0 |
| Mean | Kresoxim-methyl + epoxiconazole | 9.15 | 77.3 |
| Mean | Epoxiconazole | 8.80 | 77.5 |
| SED (22 df) | | | |
| Cultivar | | 0.377 | 0.55 |
| Fungicide | | 0.435 | 0.63 |
| Interaction | | 0.754 | 1.10 |
| *P* | | | |
| Cultivar | | <0.001 | <0.001 |
| Fungicide | | <0.001 | <0.001 |
| Interaction | | 0.055 | 0.003 |

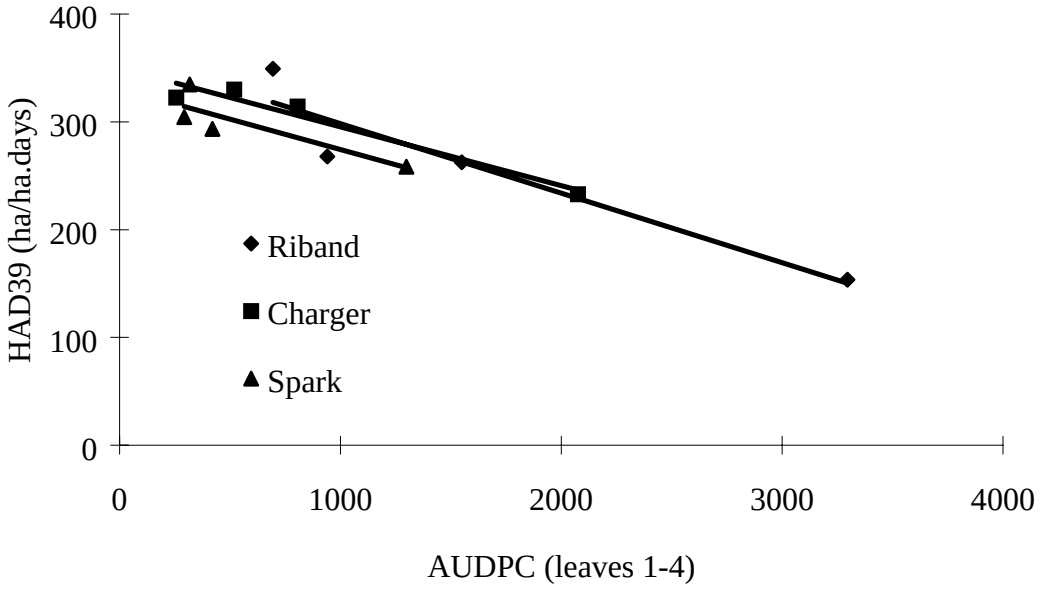


Figure 2.5. Relationship between canopy size integrated over time (HAD39) and disease (total AUDPC for leaves 1-4), Boxworth. $R^2$ values: Riband 0.889, Charger 0.946, Spark 0.744.

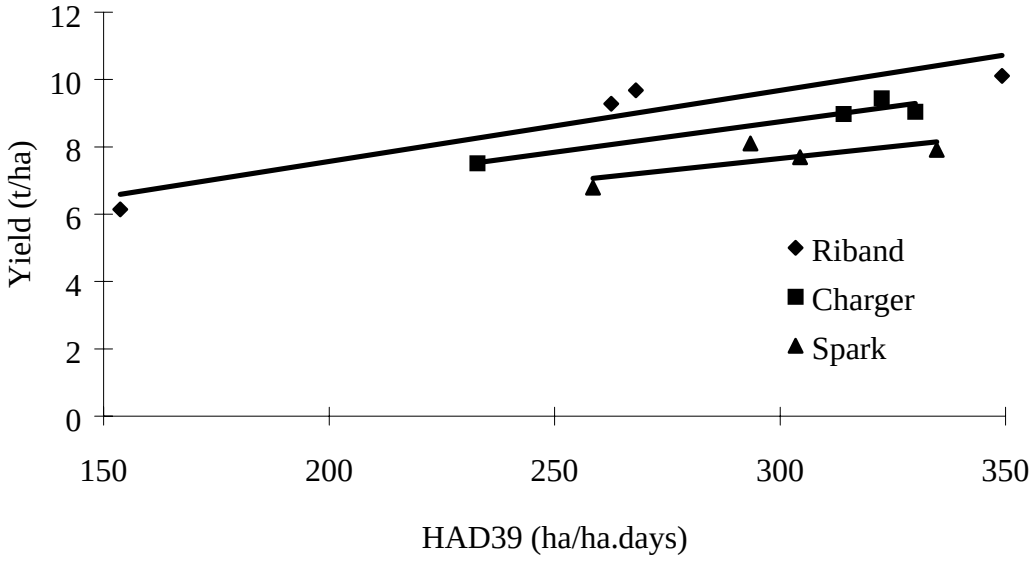


Figure 2.6. Relationship between yield and canopy size integrated over time (HAD39), Boxworth. $R^2$ values: Riband 0.878, Charger 0.931, Spark 0.582.

**Results - Rosemaund**

In contrast with Boxworth, there was a severe *S. tritici* epidemic at Rosemaund, and other diseases (mildew, brown rust) were recorded at trace levels only ( less than 1%) (Figure 2.7). There were significant differences between cultivars and fungicides in disease (AUDPC) on each leaf layer, but interactions between cultivar and disease were not statistically significant (Table 2.6). All fungicide significantly reduced disease, but azoxystrobin was less effective than either of the other fungicides on each of the top four leaves. Kresoxim-methyl plus epoxiconazole and epoxiconazole alone did not differ in disease control on the top three

leaves but, on leaf 4, there was an indication (not statistically significant) that epoxiconazole alone was the more effective.

On all three cultivars, green canopy was lowest in untreated plots from early June onwards (Figure 2.8). Azoxystrobin gave a smaller canopy than the other two fungicides on each cultivar from day 170 (June 20) onwards. There was a larger canopy ($P<0.05$) in early July from kresoxim-methyl plus epoxiconazole than from epoxiconazole alone.

There were significant differences in shoot number between cultivars, with Spark having the highest shoot numbers and Riband the lowest. For example, shoot numbers/$m^2$ at GS 87 were Spark 727, Charger 638 and Riband 465 (SED 36.5, 22 df). There were no statistically significant effects of fungicide on shoot number, nor were there any significant interactions between fungicide and cultivar.

Differences in total crop dry matter from fungicide treatment were evident from anthesis (Table 2.7, Figure 2.9). All fungicides significantly increased dry matter at GS 65, GS 75 and GS 87. The only statistically significant difference between fungicides was that kresoxim-methyl plus epoxiconazole had higher total dry matter than azoxystrobin at GS 87.

The water-soluble carbohydrates concentration at GS 65 was significantly higher in Riband than in Spark (Table 2.8). All fungicides increased water-soluble carbohydrate content in stems, but the three fungicide programmes did not differ significantly. At GS 87, levels had fallen sharply, but there were no significant differences between cultivars or fungicide regimes.

There were significant differences in yield associated with fungicides, but not between cultivars, and the interaction between cultivar and fungicide was significant (Table 2.9). All fungicides increased mean yield across cultivars, and all differed significantly from each other, with the largest increase from kresoxim-methyl plus epoxiconazole, followed by epoxiconazole alone. Azoxystrobin gave a significantly lower yield than either of the other two fungicides on each cultivar, whereas the yield advantage of kresoxim-methyl plus epoxiconazole over epoxiconazole alone was significant only on Spark. Spark gave the highest specific weights and Riband the lowest. It was increased by each fungicide, with significantly lower mean increase from azoxystrobin than from the other fungicides.

Grain number per ear differed between cultivars, with significantly higher grain numbers of Charger than Riband or Spark (Table 2.9). Effects of fungicides on grain number per ear were not significant. Harvest index was increased by all fungicides, with a larger increase from kresoxim-methyl plus epoxiconazole and epoxiconazole alone than from azoxystrobin. Spark had a lower harvest index than Riband or Charger. Nitrogen harvest index results corresponded closely with harvest index.

There were significant regressions on each cultivar of canopy duration (expressed as HAD39) on disease (expressed as total AUDPC for *S. tritici* on the top four leaves) (Figure 2.10). The percentage of variance accounted for by the regressions were 0.987 for Spark, 0.980 for Charger and 0.942 for Riband). Relationships between yield and canopy size were also highly significant ($R^2$ = 0.987 for Riband, 0.998 for Charger and 0.883 for Spark; Figure 2.11).

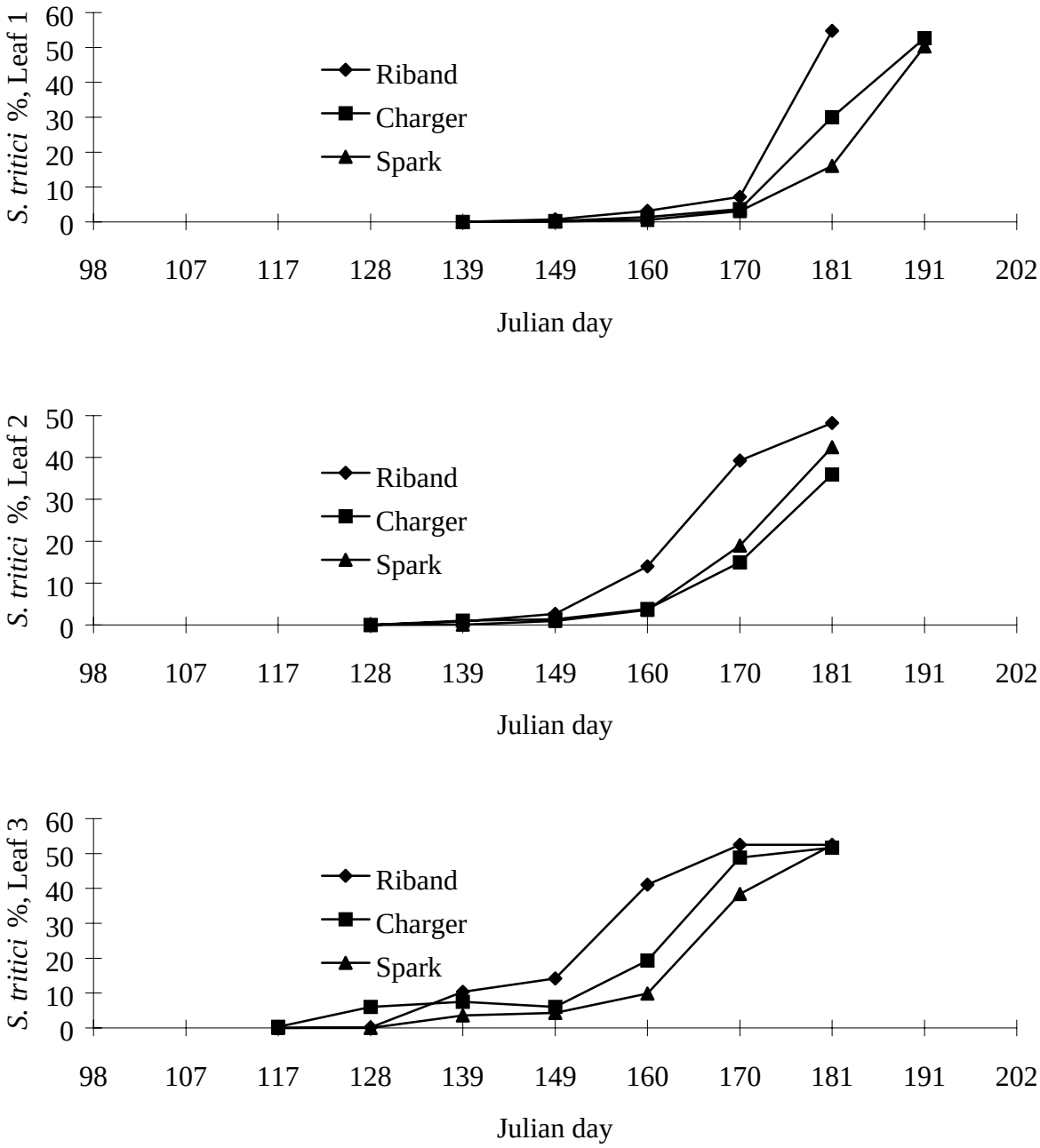


Figure 2.7. *Septoria tritici* development in untreated plots, Rosemaund.

Table 2.6. Area under *Septoria tritici* disease progress curve for each fungicide on leaves 1-4, Rosemaund.

| Cultivar | Fungicide | Area under disease progress curve (AUDPC) | | | |
|---|---|---|---|---|---|
| | | Leaf 1 | Leaf 2 | Leaf 3 | Leaf 4 |
| Riband | Untreated | 1568.1 | 1874.4 | 2055.3 | 2473.1 |
| Riband | Azoxystrobin | 1147.2 | 1184.0 | 1455.6 | 1892.2 |
| Riband | Kresoxim-methyl + epoxiconazole | 337.9 | 466.1 | 600.1 | 1412.0 |
| Riband | Epoxiconazole | 375.2 | 497.5 | 579.0 | 879.9 |
| Charger | Untreated | 1214.0 | 1174.7 | 1726.9 | 2595.0 |
| Charger | Azoxystrobin | 657.6 | 984.8 | 1260.8 | 1855.0 |
| Charger | Kresoxim-methyl + epoxiconazole | 230.9 | 266.7 | 480.2 | 1275.6 |
| Charger | Epoxiconazole | 223.6 | 309.9 | 542.6 | 1219.3 |
| Spark | Untreated | 1014.3 | 1408.7 | 1402.7 | 1679.1 |
| Spark | Azoxystrobin | 558.0 | 682.3 | 774.3 | 1193.3 |
| Spark | Kresoxim-methyl + epoxiconazole | 174.2 | 215.8 | 261.7 | 931.8 |
| Spark | Epoxiconazole | 222.4 | 295.1 | 370.6 | 791.0 |
| Riband | Mean | 857.1 | 1005.5 | 1172.5 | 1664.3 |
| Charger | Mean | 581.5 | 684.0 | 1002.6 | 1736.2 |
| Spark | Mean | 492.2 | 650.5 | 702.3 | 1148.8 |
| Mean | Untreated | 1265.5 | 1485.9 | 1728.3 | 2249.0 |
| Mean | Azoxystrobin | 787.6 | 950.3 | 1163.6 | 1646.8 |
| Mean | Kresoxim-methyl + epoxiconazole | 247.7 | 316.2 | 447.3 | 1206.5 |
| Mean | Epoxiconazole | 273.7 | 367.5 | 497.4 | 963.4 |
| SED (22 df) | | | | | |
| Cultivar | | 60.9 | 116.7 | 104.1 | 117.0 |
| Fungicide | | 70.4 | 134.7 | 120.2 | 135.1 |
| Interaction | | 121.9 | 233.4 | 208.3 | 234.0 |
| *P* | | | | | |
| Cultivar | | <0.001 | 0.011 | <0.001 | <0.001 |
| Fungicide | | <0.001 | <0.001 | <0.001 | <0.001 |
| Interaction | | 0.087 | 0.564 | 0.662 | 0.314 |

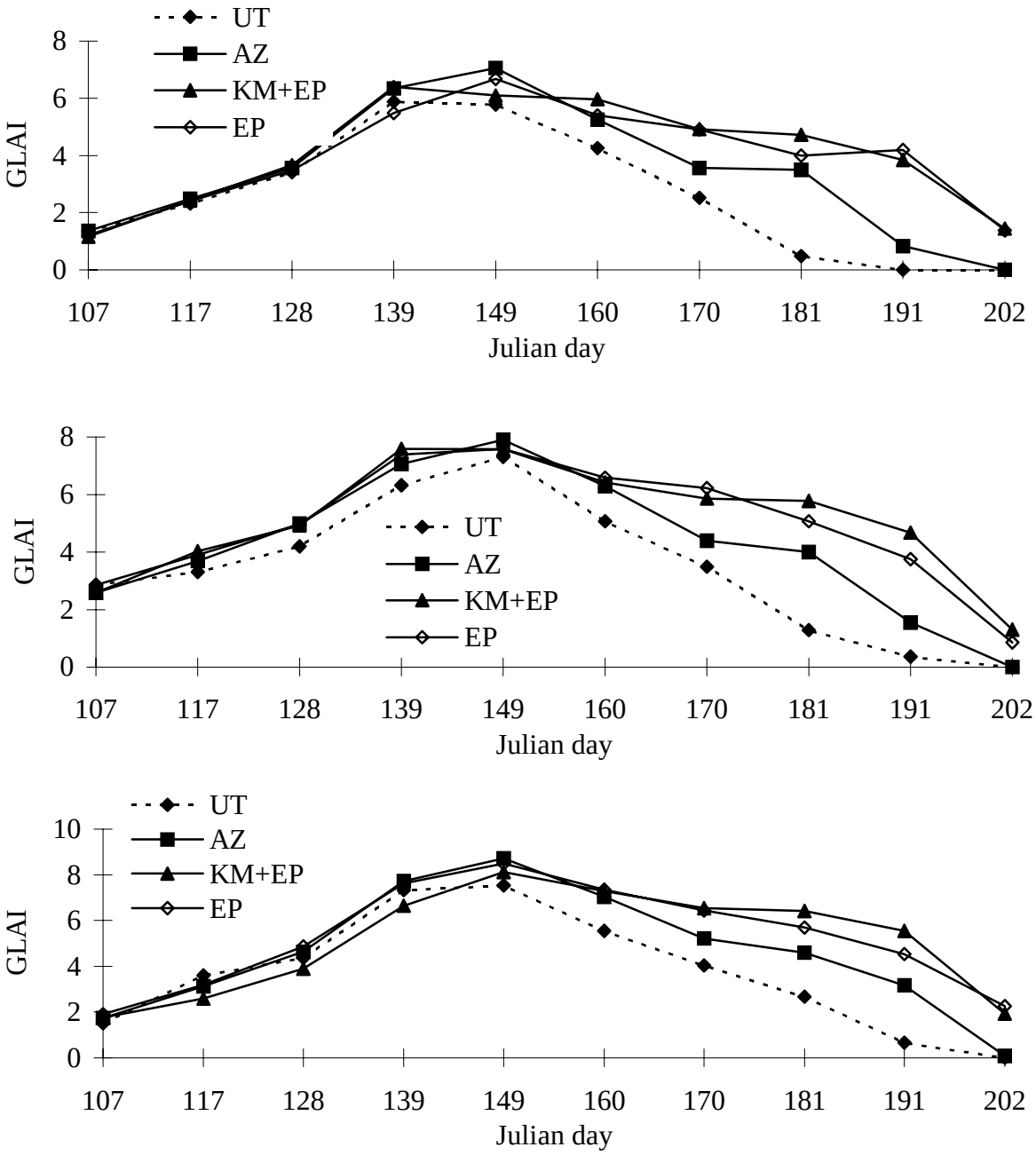








Figure 2.8. Green leaf area index (GLAI), Rosemaund; Riband (upper), Charger (middle), Spark (lower).

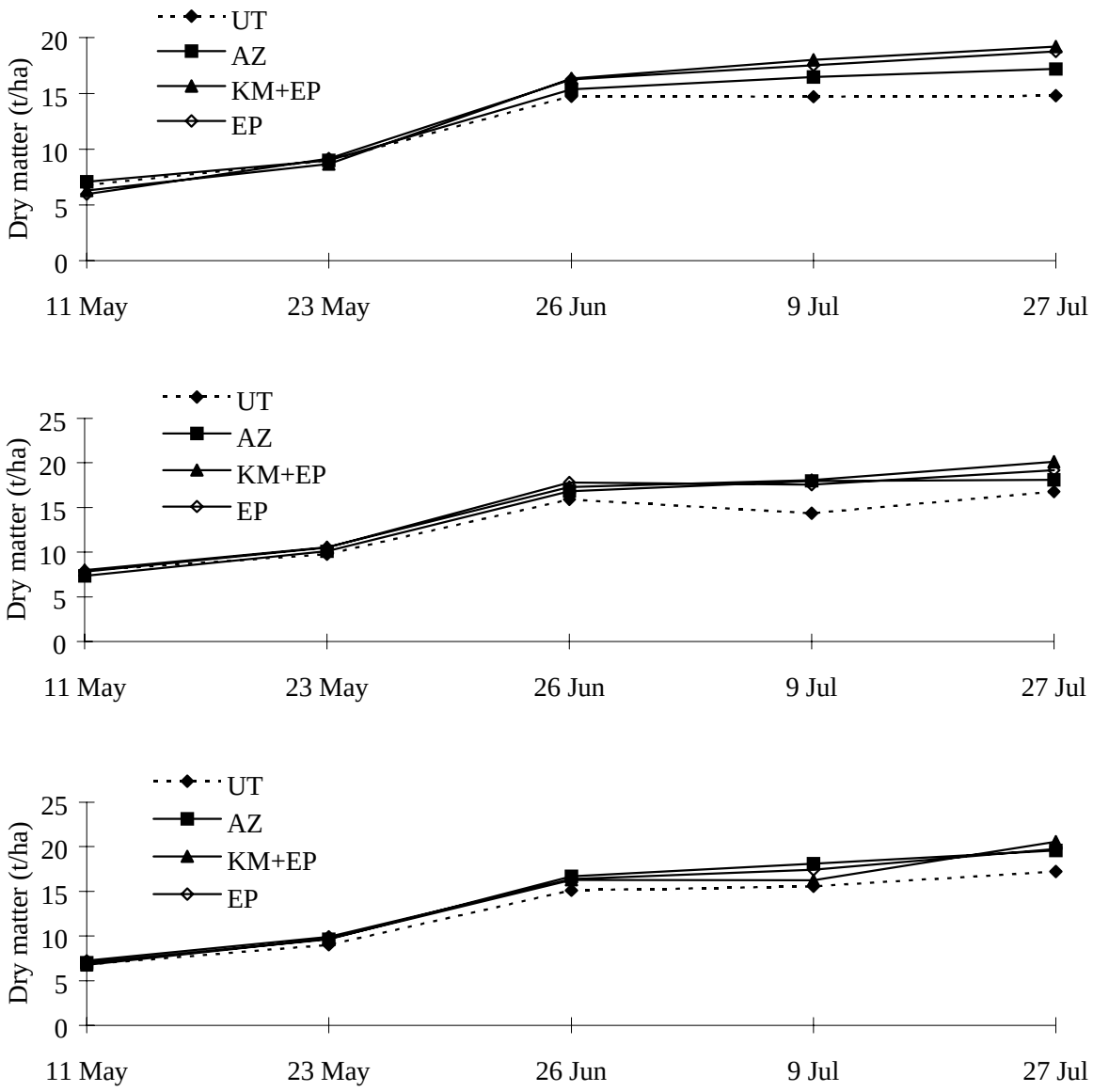


Figure 2.9. Total crop dry matter, Rosemaund; Riband (upper), Charger (middle), Spark (lower).

Table 2.7. Total crop dry matter, Rosemaund

| Cultivar | Fungicide | Total dry matter (t/ha) | | | | |
|---|---|---|---|---|---|---|
| | | 11 May (GS 33) | 23 May (GS 39) | 26 June (GS 65) | 9 July (GS 75) | 27 July (GS 87) |
| Riband | Untreated | 6.79 | 8.99 | 14.76 | 14.69 | 14.80 |
| Riband | Azoxystrobin | 7.07 | 9.00 | 15.37 | 16.46 | 17.21 |
| Riband | K-m* + epoxiconazole | 6.29 | 8.68 | 16.35 | 18.01 | 19.20 |
| Riband | Epoxiconazole | 5.97 | 9.17 | 16.24 | 17.53 | 18.77 |
| Charger | Untreated | 7.92 | 9.77 | 15.90 | 14.35 | 16.77 |
| Charger | Azoxystrobin | 7.35 | 10.10 | 16.85 | 17.98 | 18.10 |
| Charger | K-m* + epoxiconazole | 7.98 | 10.54 | 17.29 | 18.07 | 20.14 |
| Charger | Epoxiconazole | 7.82 | 10.52 | 17.81 | 17.57 | 19.19 |
| Spark | Untreated | 6.81 | 9.02 | 15.11 | 15.56 | 17.22 |
| Spark | Azoxystrobin | 7.01 | 9.64 | 16.68 | 18.10 | 19.59 |
| Spark | K-m* + epoxiconazole | 6.76 | 9.70 | 16.29 | 16.23 | 20.55 |
| Spark | Epoxiconazole | 7.23 | 9.93 | 16.38 | 17.41 | 19.75 |
| Riband | Mean | 6.53 | 8.96 | 11.26 | 16.67 | 17.50 |
| Charger | Mean | 7.77 | 10.23 | 11.50 | 16.99 | 18.55 |
| Spark | Mean | 6.95 | 9.57 | 12.74 | 16.83 | 19.28 |
| Mean | Untreated | 7.17 | 9.26 | 11.01 | 14.87 | 16.26 |
| Mean | Azoxystrobin | 7.15 | 9.58 | 11.79 | 17.52 | 18.30 |
| Mean | K-m* + epoxiconazole | 7.01 | 9.64 | 12.23 | 17.44 | 19.96 |
| Mean | Epoxiconazole | 7.01 | 9.87 | 12.30 | 17.50 | 19.23 |
| SED (22 df) | | | | | | |
| Cultivar | | 0.218 | 0.237 | 0.263 | 0.450 | 0.526 |
| Fungicide | | 0.252 | 0.274 | 0.303 | 0.520 | 0.607 |
| Interaction | | 0.436 | 0.475 | 0.525 | 0.900 | 1.052 |
| *P* | | | | | | |
| Cultivar | | <0.001 | <0.001 | <0.001 | 0.782 | 0.009 |
| Fungicide | | 0.866 | 0.192 | 0.001 | <0.001 | <0.001 |
| Interaction | | 0.141 | 0.714 | 0.752 | 0.137 | 0.893 |

*K-m = kresoxim-methyl

Table 2.8. Stem water-soluble carbohydrates, Rosemaund

| Cultivar | Fungicide | GS 65 (26 June) | GS 87 (27 July) |
|---|---|---|---|
| Riband | Untreated | 2.03 | 0.15 |
| Riband | Azoxystrobin | 2.45 | 0.65 |
| Riband | Kresoxim-methyl + epoxiconazole | 3.12 | 0.65 |
| Riband | Epoxiconazole | 3.06 | 0.38 |
| Charger | Untreated | 2.27 | 0.34 |
| Charger | Azoxystrobin | 2.99 | 0.65 |
| Charger | Kresoxim-methyl + epoxiconazole | 2.91 | 0.68 |
| Charger | Epoxiconazole | 3.24 | 0.73 |
| Spark | Untreated | 1.82 | 0.51 |
| Spark | Azoxystrobin | 2.68 | 0.89 |
| Spark | Kresoxim-methyl + epoxiconazole | 2.02 | 0.61 |
| Spark | Epoxiconazole | 2.00 | 0.70 |
| Riband | Mean | 2.85 | 0.46 |
| Charger | Mean | 2.66 | 0.60 |
| Spark | Mean | 2.13 | 0.68 |
| Mean | Untreated | 2.04 | 0.33 |
| Mean | Azoxystrobin | 2.71 | 0.73 |
| Mean | Kresoxim-methyl + epoxiconazole | 2.68 | 0.64 |
| Mean | Epoxiconazole | 2.77 | 0.60 |
| SED (22 df) | | 0.268 | 0.125 |
| Cultivar | | 0.310 | 0.144 |
| Fungicide | | 0.536 | 0.249 |
| Interaction | | | |
| *P* | | | |
| Cultivar | | 0.003 | 0.233 |
| Fungicide | | 0.012 | 0.061 |
| Interaction | | 0.270 | 0.845 |

Table 2.9. Yield, specific weight and harvest index, Rosemaund

| Cultivar | Fungicide | Yield (t/ha) | Specific weight (kg/hl) | Grains per ear | Harvest index | Nitrogen harvest index |
|---|---|---|---|---|---|---|
| Riband | Untreated | 4.44 | 65.7 | 31.4 | 37.9 | 43.6 |
| Riband | Azoxystrobin | 6.90 | 71.2 | 27.5 | 41.6 | 44.9 |
| Riband | K-m* + epoxiconazole | 9.47 | 74.9 | 38.6 | 52.4 | 57.0 |
| Riband | Epoxiconazole | 9.01 | 74.4 | 39.0 | 50.2 | 55.5 |
| Charger | Untreated | 5.18 | 72.1 | 20.1 | 36.3 | 41.9 |
| Charger | Azoxystrobin | 7.35 | 74.4 | 25.1 | 43.7 | 49.7 |
| Charger | K-m* + epoxiconazole | 8.46 | 76.0 | 24.3 | 48.3 | 56.8 |
| Charger | Epoxiconazole | 8.10 | 76.1 | 28.8 | 49.0 | 55.6 |
| Spark | Untreated | 5.25 | 75.2 | 21.3 | 29.9 | 36.4 |
| Spark | Azoxystrobin | 6.73 | 78.2 | 21.9 | 36.2 | 44.5 |
| Spark | K-m* + epoxiconazole | 8.70 | 80.0 | 21.6 | 36.8 | 42.5 |
| Spark | Epoxiconazole | 7.86 | 79.5 | 20.1 | 39.4 | 47.0 |
| Riband | Mean | 7.45 | 71.6 | 24.6 | 45.5 | 50.3 |
| Charger | Mean | 7.27 | 74.6 | 34.1 | 44.3 | 51.0 |
| Spark | Mean | 7.14 | 78.2 | 21.2 | 35.6 | 42.6 |
| Mean | Untreated | 4.96 | 71.0 | 24.3 | 34.7 | 40.7 |
| Mean | Azoxystrobin | 6.99 | 74.6 | 24.8 | 40.5 | 46.4 |
| Mean | K-m* + epoxiconazole | 8.87 | 76.9 | 28.2 | 45.8 | 52.1 |
| Mean | Epoxiconazole | 8.33 | 76.7 | 29.3 | 46.2 | 52.7 |
| SED (22 df) | | | | | | |
| Cultivar | | 0.182 | 0.43 | 2.71 | 1.53 | 1.80 |
| Fungicide | | 0.210 | 0.49 | 3.13 | 1.77 | 2.08 |
| Interaction | | 0.363 | 0.85 | 5.42 | 3.06 | 3.60 |
| *P* | | | | | | |
| Cultivar | | 0.237 | <0.001 | <0.001 | <0.001 | <0.001 |
| Fungicide | | <0.001 | <0.001 | 0.315 | <0.001 | <0.001 |
| Interaction | | 0.005 | 0.003 | 0.505 | 0.399 | 0.217 |

*K-m = kresoxim-methyl

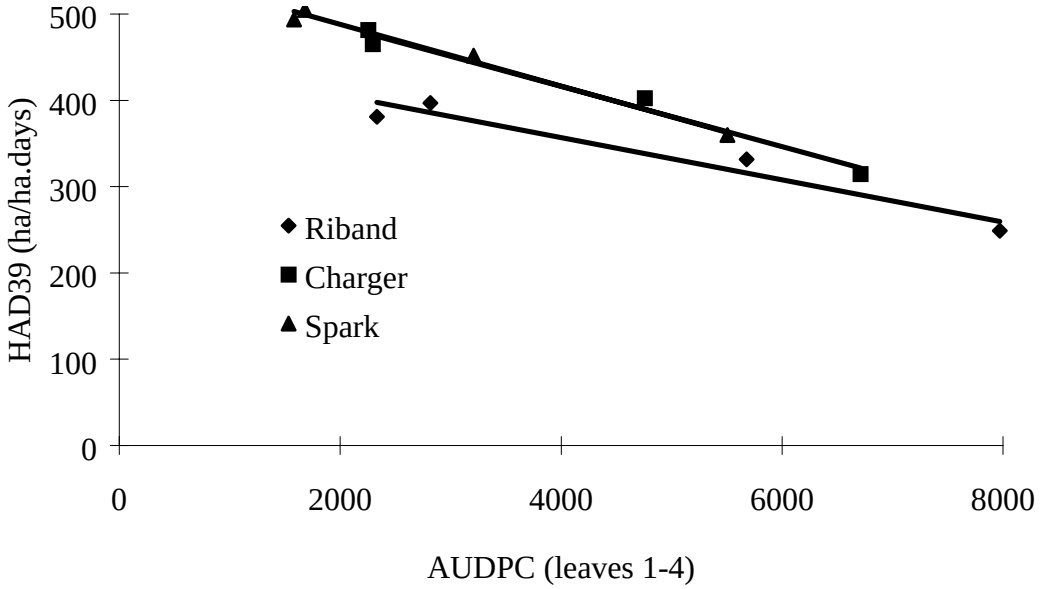


Figure 2.10. Relationship between canopy size integrated over time (HAD39) and disease (total AUDPC for leaves 1-4), Rosemaund. $R^2$ values: Riband 0.942, Charger 0.980, Spark 0.987.

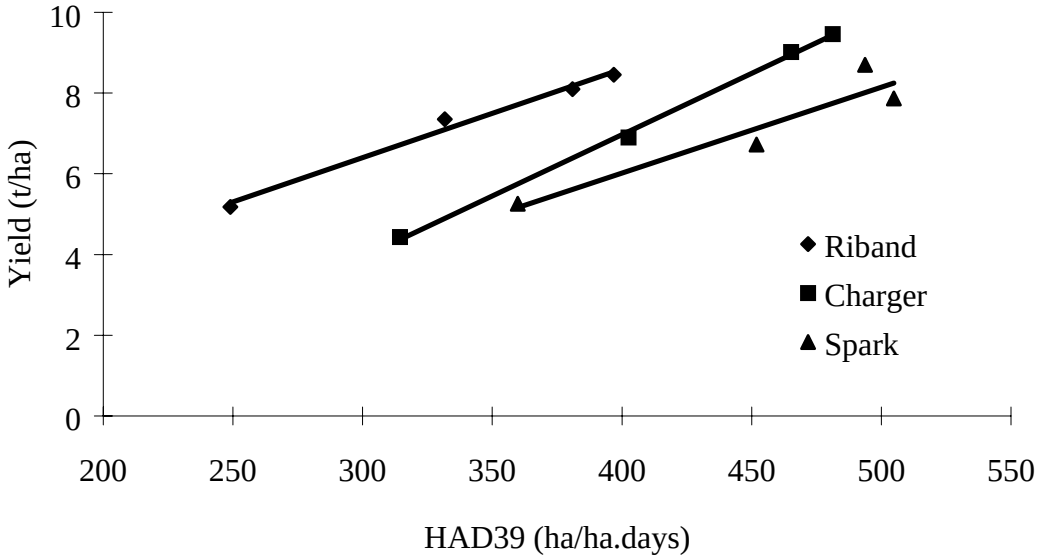


Figure 2.11. Relationship between yield and canopy size integrated over time (HAD39), Rosemaund. $R^2$ values: Riband 0.987, Charger 0.998, Spark 0.883.

**Discussion**

The two experiments provided a marked contrast in terms of disease severity, and in effects of fungicides on disease control, crop growth and yield. There was a severe *Septoria tritici* epidemic at Rosemaund, with marked differences in disease between the three cultivars and large yield increases from fungicide (up to 5.03 t/ha on Riband, 3.28 t/ha on Charger and 3.55 t/ha on Spark). In contrast, at Boxworth, there were moderately severe epidemics of *S. tritici* and brown rust on Riband, but low disease levels on Charger and Spark. Yield increases were large on Riband (up to 3.96 t/ha), but smaller on the other cultivars (1.93 and 1.13 t/ha on Charger and Spark respectively).

The benefits from using a strobilurin fungicide were shown clearly through a comparison of kresoxim-methyl plus epoxiconazole with epoxiconazole alone. At the high disease site, Rosemaund, the mean yield advantage of the kresoxim-methyl mixture over epoxiconazole alone was 0.54 t/ha, and the difference was statistically significant on each of the three cultivars, despite the contrasting disease severities. There were also indications, though not statistically significant, that the treatment with kresoxim-methyl had less disease on each of the top three leaves. Canopy size was greater where the mixture was used, although there was no measurable effect on total biomass, grain number per ear, or harvest index.

At Boxworth, there was yield advantage from the kresoxim-methyl mixture over epoxiconazole on each cultivar (mean 0.35 t/ha), though this was not statistically significant. There was no clear evidence of improved control of the low levels of disease from the mixture, but the mixture gave larger green canopies from mid-June until the end of green canopy life.

The performance of azoxystrobin was markedly inferior to that from kresoxim-methyl plus epoxiconazole or epoxiconazole alone, and shows the need for a triazole fungicide in mixture with a strobilurin under conditions of severe disease. At Rosemaund, azoxystrobin had a smaller canopy from mid-June onwards, and an indication (not statistically significant) of smaller total biomass at GS 87. This resulted in a lower yield than from kresoxim-methyl plus epoxiconazole or from epoxiconazole alone, with a lower grain number per ear and harvest index. At the low disease site at Boxworth, azoxystrobin gave a lower yield than kresoxim-methyl plus epoxiconazole, although no differences were detected in canopy or crop biomass.

Although there were clear differences between cultivars and fungicides, there was only one significant cultivar x fungicide interaction in the experiment. This was in the yields at Rosemaund, where Riband gave a lower untreated yield but higher treated yields than other cultivars. Although yield increases from the strobilurins were smaller at Boxworth, particularly on the less disease-prone cultivars, there were still clear indications of a yield benefit from strobilurins on all cultivars under conditions of low disease, indicating benefits from use of strobilurins occur in both high and low disease situations.

The relationship between yield and green canopy from GS 39 (HAD39) was good on each cultivar at Rosemaund, and on the more susceptible cultivars (Riband and Charger) at Boxworth, but was markedly poorer on the cultivar with low disease, Spark. This suggests that, where disease caused substantial loss of green canopy, yield was strongly related to the increases in green canopy resulting from fungicide use, and that there was no evidence that canopy efficiency (radiation use efficiency) was altered. The relationship between disease and HAD39 was also good at Rosemaund and, on Riband and Charger, at Boxworth. This suggests that loss of canopy was largely associated with disease, and that the effect of fungicides on canopy survival was primarily associated with disease control. Under low disease conditions, on Spark at Boxworth, relationships were poorer, particularly between yield and canopy. However, no clear evidence was found in the experiment to account for any other factors, such as physiological effects, which may have affected the response of the cultivar to the fungicides.

## APPENDIX 3

### Experiment 3: Interaction between strobilurin fungicides and seed rate

### Introduction

Early experimental work with strobilurin fungicides showed that, in some instances, strobilurin application to winter wheat during stem elongation resulted in greater duration of green canopy than resulted from application of triazole fungicides on the same dates, although there was little difference between fungicides in disease control (Jones, 2000). It was suggested that this may be one contributory factor to the greater yields given by strobilurin fungicides.

It has been shown that the optimum canopy size for winter wheat was to reach a maximum GAI between 5 and 6, whereas canopies with GAI up to 9, or even higher are common in current farming practice (Sylvester-Bradley *et al*., 1998). In a canopy with GAI 5 or 6, there will be greater light penetration to the lower stem leaves, so it might be expected that the effect of strobilurin fungicides in keeping lower leaves green might have a greater effect on yield in such a canopy than in a denser canopy. Differences in canopy size are related primarily to shoot number, so manipulation of shoot number provides an ideal test system in which to determine whether there is an interaction between canopy size and the effect of strobilurin fungicides on disease, growth and yield.

### Methods

An experiment was established at ADAS Rosemaund, Herefordshire, in 1999/2000, on a stoneless silty clay loam of the Bromyard series. The previous crop was lupins. Two seed rates were used, 100 seeds/$m^2$, which is below the optimum for the sowing date of 16 October, and 350 seeds/$m^2$, which represents a conventional commercial seed rate (Spink *et al*., 2000). Fungicides are detailed in Table 3.1. Fungicides were applied in 225 litre/ha water, using an MDM Oxford Precision Sprayer fitted with 03-F110 nozzles. The experiment was on cv. Consort, which is susceptible to the target pathogen, *Septoria tritici* (Anonymous, 1999). The experimental design was a two factor randomised block, with three replicates. The area of each plot was 4 m x 24 m, of which one 2 m x 24 m strip was used for disease assessment and yield estimation, and the other for destructive sampling for growth analysis.

Table 3.1. Fungicides

| Treat-ment | Active ingredient | Product | Application rate of product/ha |
|---|---|---|---|
| 1 | --- | Untreated | --- |
| 2 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.5 + 0.5 litre |
| 3 | Trifloxystrobin + epoxiconazole | Twist | 1.0 + 0.5 litre |
| 4 | Kresoxim-methyl + epoxiconazole | Landmark | 0.5 litre |
| 5 | Epoxiconazole | Opus | 0.5 litre |

Each treatment was applied on two occasions, at GS 31 (28 April) and GS 39 (24 May) Tottman & Broad, 1987). Other agronomic inputs followed good commercial practice.

Foliar diseases were assessed as visual estimates of the percentage leaf area infected by each disease on each leaf layer, on 10 tillers taken at random from each sub plot at 7 day (+/-1)

intervals from the date of the first fungicide application until all leaves were senescent. Percentage green leaf area was also estimated. To provide a cumulative measure of the effect of disease during the life of the stem leaves, the area under disease progress curve (AUDPC) was calculated for each treatment. This can be visualised on a graph showing disease progress over time, as the area under the line showing disease development for that treatment.

Growth analysis was done at flag leaf emergence (GS 39), when 50% of all shoots reached mid-anthesis (GS 65), at the milky-ripe stage (GS 75) and at mid-senescence (GS 87), calculated at 750 ºCd after GS 39 (Foulkes & Scott, 1998). On each occasion, an area of 1.0 m x 6 rows (0.81 $m^2$) was sampled, leaving 3 rows on each side and at least 0.5 m between samples to avoid edge effects (Austin & Blackwell, 1980). The plants were cut at ground level and taken to the laboratory for analysis, except at GS 31, when whole plants were dug up, taken to the laboratory and plant number counted before the roots were cut off. The number and dry weight of shoots were assessed separately for potentially fertile, dead and dying shoots. A shoot was classified as dying when no further leaf was emerging and the most recently emerged leaf was yellowing (Thorne & Wood, 1987).

Green area index (GAI) was calculated by determining actual leaf size on each occasion that diseases were assessed, using two of the ten leaves taken from each plot. Leaf length and width were measured , to the nearest 0.1 mm, and leaf area determined using a form factor of 0.83 (Bryson *et al*., 1997). GAI was calculated using the mean number of shoots per plot from assessments between mid-anthesis and mid-senescence. The leaf areas were then integrated over time from GS 39 until the end of all green canopy to give healthy area duration from GS 39 (HAD39; Bryson *et al.*, 1997). This provides a measure of green canopy size during the period in which photosynthesis is contributing primarily to grain filling rather than to canopy structure.

The water soluble carbohydrate (WSC) content of the stems was determined at mid-anthesis, the development stage at it is near to its maximum (Foulkes *et al*., 1998), and at mid-senescence. This was determined on 8 shoots randomly selected from each plot and flash dried at 105°C for 2 hours. The WSC content was then assessed using the spectrophotometry method described by Thomas (1977).

At harvest, samples of 0.81 $m^2$ were removed from each sub plot. Final crop dry weight and ear number were recorded. The ears were then threshed, grain dry weight measured and harvest index calculated. Grain yield was measured from the 2 m x 24 m strip allocated within each sub plot using a plot combine harvester. Grain was analysed for moisture content and specific weight using GAC 2000 grain analysis computer (Dickey-John Corporation). The thousand grain weight was determined on grain samples taken from the combine. The number of grains per ear was then calculated using the combine grain yield, the thousand grain weight and the number of ears/$m^2$.

## Results

The dominant disease at this site was *Septoria tritici* leaf spot, and the only other disease recorded was brown rust, which affected up to 1.5% of the top two leaves on fungicide-treated plots by 21 July; untreated plots had no green canopy by this time. There was no effect of seed rate on disease severity, so disease data are shown as the mean for each fungicide across seed rates. *S. tritici* development on the top four leaves is shown in Figure 3.1. There was a clear difference between all fungicide treatments and the untreated control on each leaf layer. One clear trend was that *S. tritici* was more severe on leaf 4 in the kresoxim-methyl + epoxiconazole treatment than in other treatments, a difference that was statistically significant in all except one of the weekly assessments from day 147 (26 May) until day 175 (23 June).

This difference was not observed for this fungicide treatment on the top three leaves. The other difference which was evident on each of leaves 1-3 was that there were significant differences between fungicide programmes only towards the end of the life of each leaf, with significantly higher disease severity in the epoxiconazole treatment than in the three strobilurin plus epoxiconazole treatments on the final one or two assessment dates on each of these leaf layers. The three strobilurin mixtures showed only small differences in efficacy on leaves 2-4, but trifloxystrobin plus epoxiconazole had least disease on the flag leaves, followed by azoxystrobin plus epoxiconazole.

The area under disease progress curve (AUDPC) data show that, on all leaf layers, trifloxystrobin plus epoxiconazole had the lowest AUDPC (Figure 3.2), but this was not significantly different from azoxystrobin plus epoxiconazole on any leaf layer (Table 3.2). The AUDPC for kresoxim-methyl plus epoxiconazole was significantly higher than for trifloxystrobin plus epoxiconazole on leaves 3 and 4, as was the AUDPC for epoxiconazole on leaf 3. On each leaf layer, *S. tritici* was more severe on the low seed rate than the higher seed rate, although the difference was statistically significant on leaf 2 only.

Crop canopy, determined from measurement of sub-samples of leaves each week, showed a clear effect of seed rate throughout, and of fungicide from day 147 (26 May) (Figure 3.3). Mean differences between seed rates were statistically significant up to late June (day 175).

Differences between fungicide treatments were not statistically significant prior to day 161 (9 June). On that date, azoxystrobin plus epoxiconazole and trifloxystrobin plus epoxiconazole had significantly greater indices than the untreated control. The effect of fungicide increased progressively, until green canopy was lost completely in untreated plots by 15 July, on which date fungicide-treated plots had green lamina area indices of up to 4.5. Differences between fungicides emerged in July. Epoxiconazole alone had a smaller canopy than epoxiconazole in mixture with azoxystrobin or trifloxystrobin in all assessments from 7 July (day 189), and smaller than kresoxim-methyl plus epoxiconazole from 14 July onwards. Statistically significant interactions between seed rate and fungicide were not found on any date.

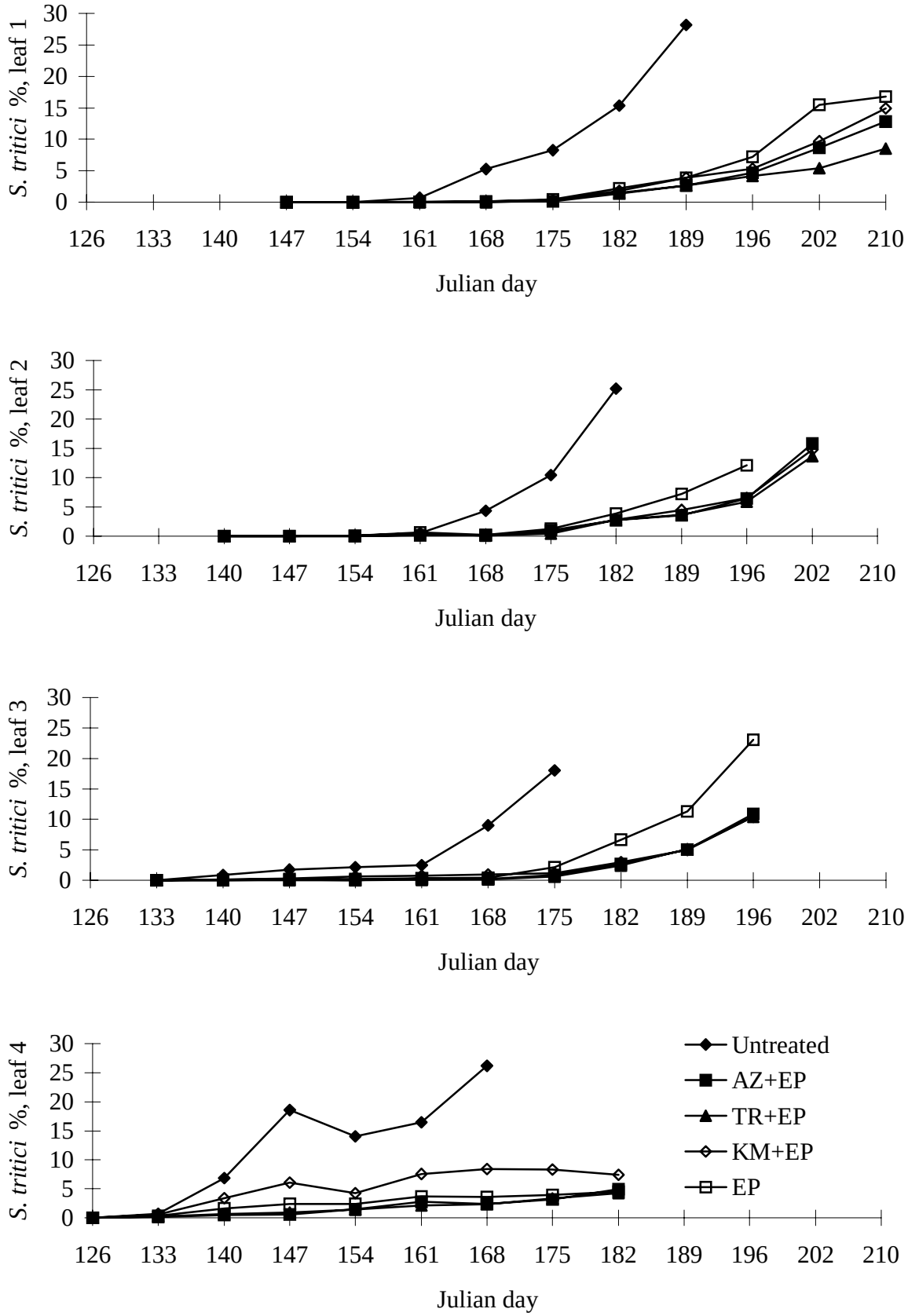


Figure 3.1. *Septoria tritici* development on the upper four leaves in each fungicide treatment (mean across seed rates). AZ = azoxystrobin, TR = trifloxystrobin, KM = kresoxim-methyl, EP = epoxiconazole.

Table 3.2. Area under disease progress curve for each fungicide (mean across seed rates) on leaves 1-4.

| Seed rate/$m^2$ | Fungicide | Area under disease progress curve (AUDPC) | | | |
|---|---|---|---|---|---|
| | | leaf 1 | leaf 2 | leaf 3 | leaf 4 |
| 100 | | 382.7 | 449.7 | 339.7 | 334.4 |
| 350 | | 322.8 | 360.4 | 318.6 | 265.4 |
| | Untreated | 969.5 | 930.6 | 672.3 | 854.8 |
| | AZ+EP | 172.0 | 247.0 | 207.0 | 95.1 |
| | TR+EP | 134.3 | 203.9 | 154.9 | 93.9 |
| | KM+EP | 206.0 | 263.9 | 272.9 | 298.4 |
| | EP | 281.8 | 379.7 | 338.8 | 157.2 |
| 100 | Untreated | 1077.7 | 1107.3 | 669.1 | 911.1 |
| 100 | AZ+EP | 188.3 | 273.4 | 250.8 | 119.1 |
| 100 | TR+EP | 149.2 | 215.6 | 166.4 | 105.5 |
| 100 | KM+EP | 217.3 | 277.7 | 251.1 | 372.4 |
| 100 | EP | 280.8 | 374.4 | 361.4 | 163.8 |
| 350 | Untreated | 861.4 | 753.9 | 675.6 | 798.5 |
| 350 | AZ+EP | 155.6 | 220.7 | 163.3 | 71.2 |
| 350 | TR+EP | 119.4 | 192.1 | 143.3 | 82.3 |
| 350 | KM+EP | 194.6 | 250.1 | 294.7 | 224.5 |
| 350 | EP | 282.8 | 385.1 | 316.3 | 150.6 |
| SED (18 df) | | | | | |
| seed rate | | 61.7 | 41.9 | 32.5 | 38.2 |
| fungicide | | 97.5 | 66.2 | 51.4 | 60.5 |
| interaction | | 137.9 | 93.6 | 72.8 | 85.5 |
| *P* | | | | | |
| seed rate | | 0.344 | 0.047 | 0.525 | 0.088 |
| fungicide | | <0.001 | <0.001 | <0.001 | <0.001 |
| interaction | | 0.798 | 0.075 | 0.757 | 0.756 |

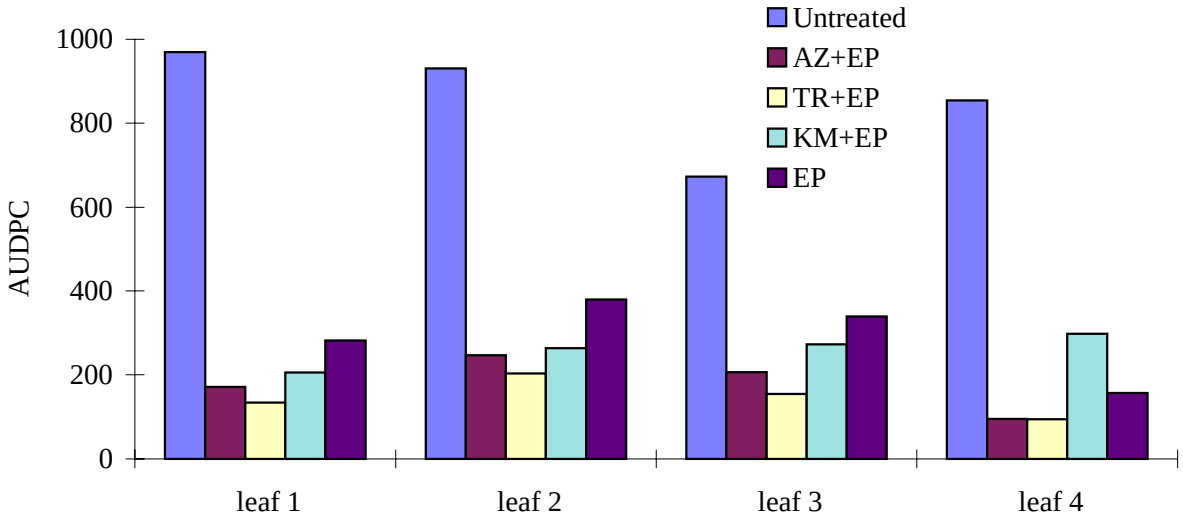


Figure 3.2. Area under disease progress curve for each fungicide (mean across seed rates) on leaves 1-4. AZ = azoxystrobin, TR = trifloxystrobin, KM = kresoxim-methyl, EP = epoxiconazole.

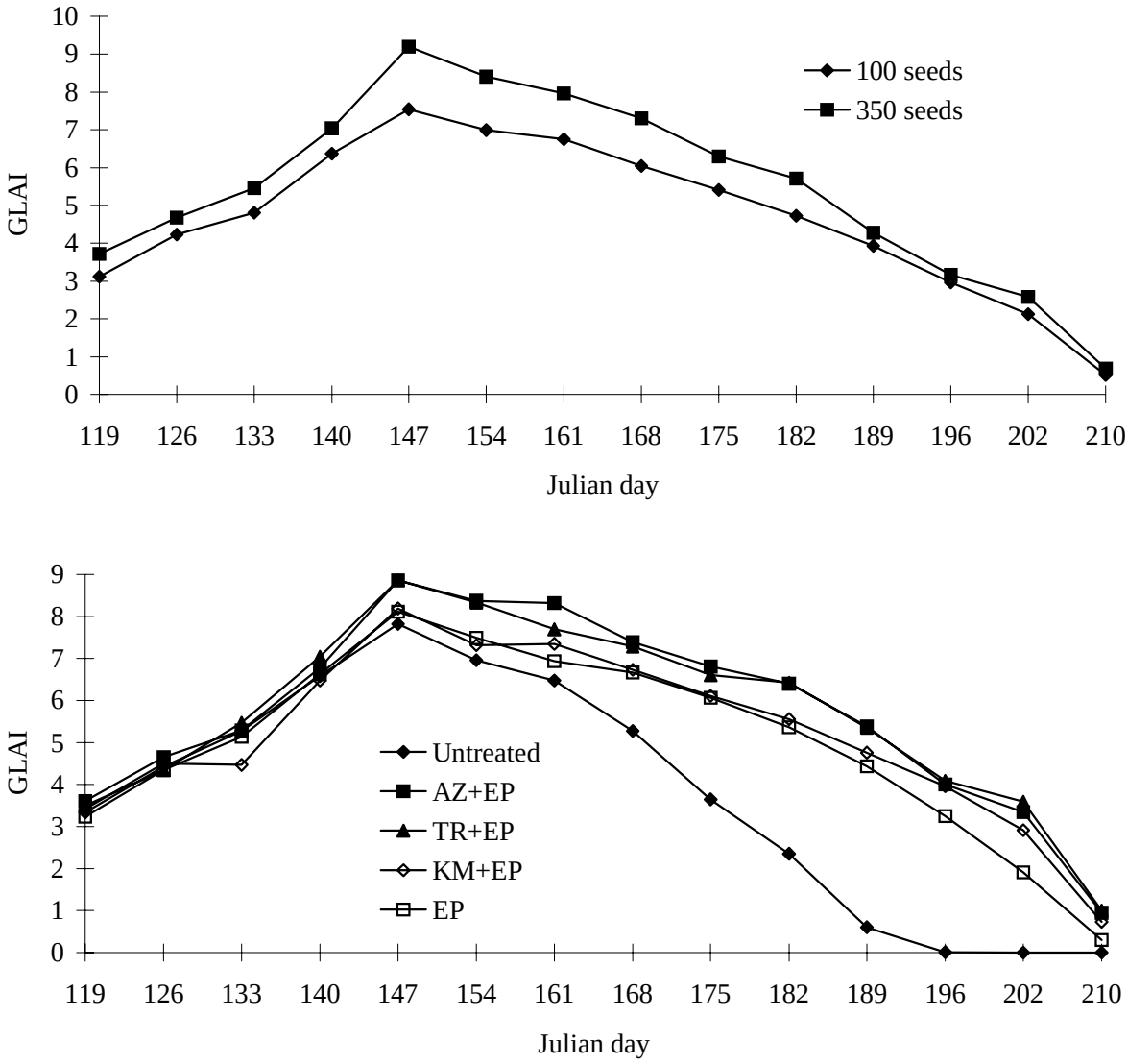


Figure 3.3. Green leaf area index (GLAI), mean for each seed rate (upper) and fungicide (lower). AZ = azoxystrobin, TR = trifloxystrobin, KM = kresoxim-methyl, EP = epoxiconazole.

Seed rate ($P = 0.002$) and fungicide ($P < 0.001$) both had significant effects on canopy integrated over time from GS 39 (HAD39), but the interaction between seed rate and fungicide was not significant ($P = 0.951$). The HAD39 for the lower and higher seed rates were 348.2 and 410.0 respectively (SED = 16.82). All fungicides increased HAD39 (SED = 26.6). Epoxiconazole had a significantly lower value than either azoxystrobin or trifloxystrobin plus epoxiconazole, but kresoxim-methyl plus epoxiconazole did not differ significantly from the other fungicides (Figure 3.4).

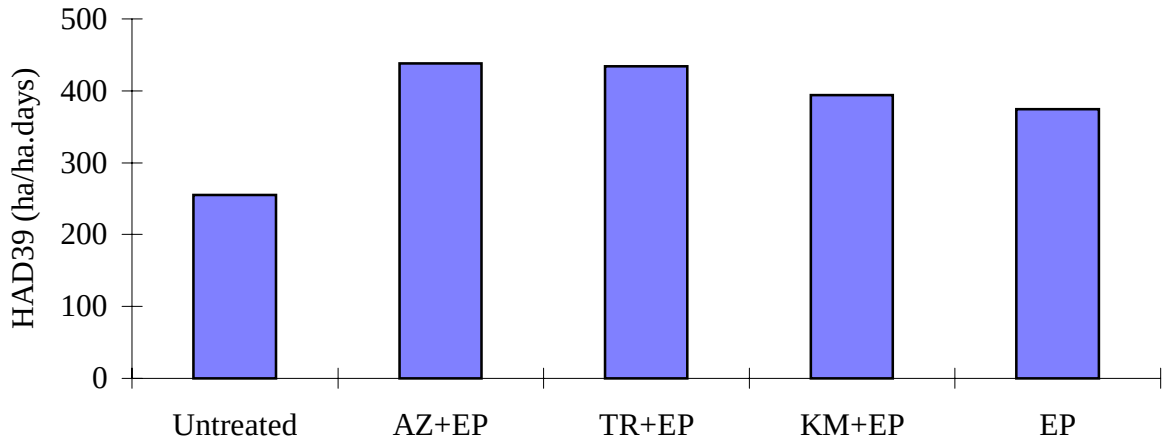


Figure 3.4. Healthy area duration from GS 39. AZ = azoxystrobin, TR = trifloxystrobin, KM = kresoxim-methyl, EP = epoxiconazole.

Shoot numbers were higher on each date in the plots sown at 350 seeds/$m^2$ (Figure 3.5), although the difference was statistically significant only at GS 65, GS 75 and GS 87. There were significant effects of fungicide on two instances, where one fungicide treatment had a lower population than others. These were kresoxim-methyl plus epoxiconazole treatment at GS 65 and epoxiconazole at GS 87. Interactions between seed rate and fungicide were not significant.

Seed rate had a significant effect on total biomass in the earlier three growth analyses, at GS 39, GS 65 and GS 75, and the apparent differences at the later assessments were not statistically significant (Figure 3.6, Table 3.3). In contrast, fungicide had a significant effect on biomass only from GS 87 onwards. At this stage, all fungicide treatments gave higher biomass than the untreated, and epoxiconazole alone was lower than the three strobilurin plus epoxiconazole mixtures. In the pre-harvest assessment (GS 93), the only differences that were statistically significant were that the azoxystrobin plus epoxiconazole mixture had higher total biomass than the untreated control. As with shoot number, there were no statistically significant interactions between seed rate and fungicide.

Water-soluble carbohydrate levels at GS 65 were higher in fungicide-treated than untreated plots ($P$=0.063; Table 3.4). By GS 87, the untreated plots had significantly less than any fungicide treatment, and epoxiconazole had less than trifloxystrobin plus epoxiconazole.

There was a mean benefit in yield of 1.13 t/ha from the higher seed rate compared with the lower rate Table 3.5). All fungicides increased yield compared with the untreated control, and the yield from epoxiconazole was significantly lower than that from each of the strobilurin plus epoxiconazole mixtures. Specific weight and thousand grain weight were increased by each fungicide treatment, but not by seed rate (Table 3.5). Azoxystrobin plus epoxiconazole and trifloxystrobin plus epoxiconazole had higher thousand grain weights than the other two fungicide treatments. There were no significant differences in numbers of grains per ear. Harvest index was significantly higher in the lower seed rate plots, and was increased by each fungicide treatment, with significantly higher indices from azoxystrobin plus epoxiconazole and trifloxystrobin than from epoxiconazole alone (Table 3.5). Interactions between seed rate and fungicide were not statistically significant in yield, grain quality, grain number per ear and harvest index.

The relationship between disease (as AUDPC) and HAD39 showed that 84.7% of the variation in HAD39 was accounted for by disease (Figure 3.7). However, this regression was

heavily weighted by the two results from untreated plots and, where these were removed from the analysis, the regression accounted for only 63.8% of the variance.

The relationship between yield and canopy duration, expressed as HAD39, showed a highly significant regression, which accounted for 93% of the total variance (Figure 3.8).

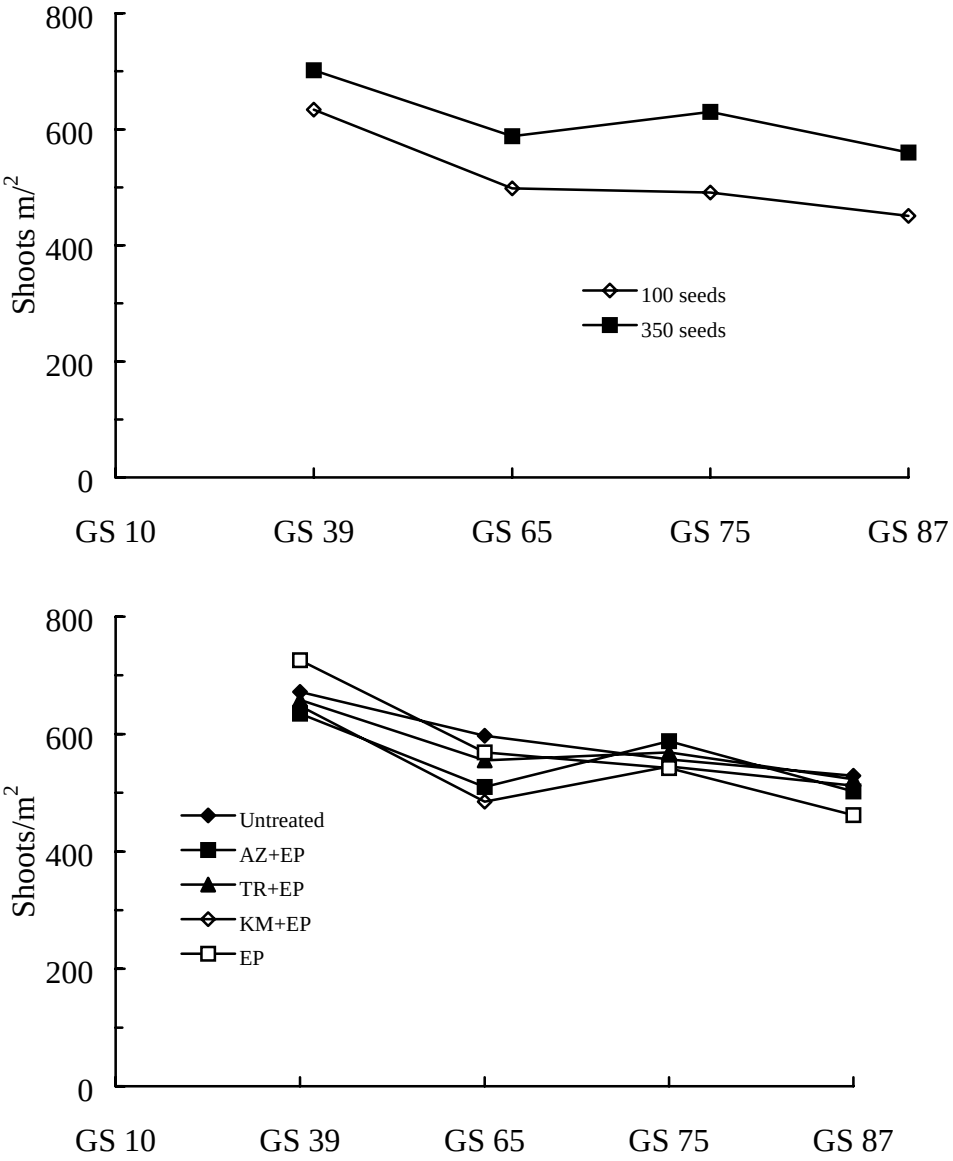


Figure 3.5. Shoot numbers, mean for each seed rate (upper) and fungicide (lower). AZ = azoxystrobin, TR = trifloxystrobin, KM = kresoxim-methyl, EP = epoxiconazole.

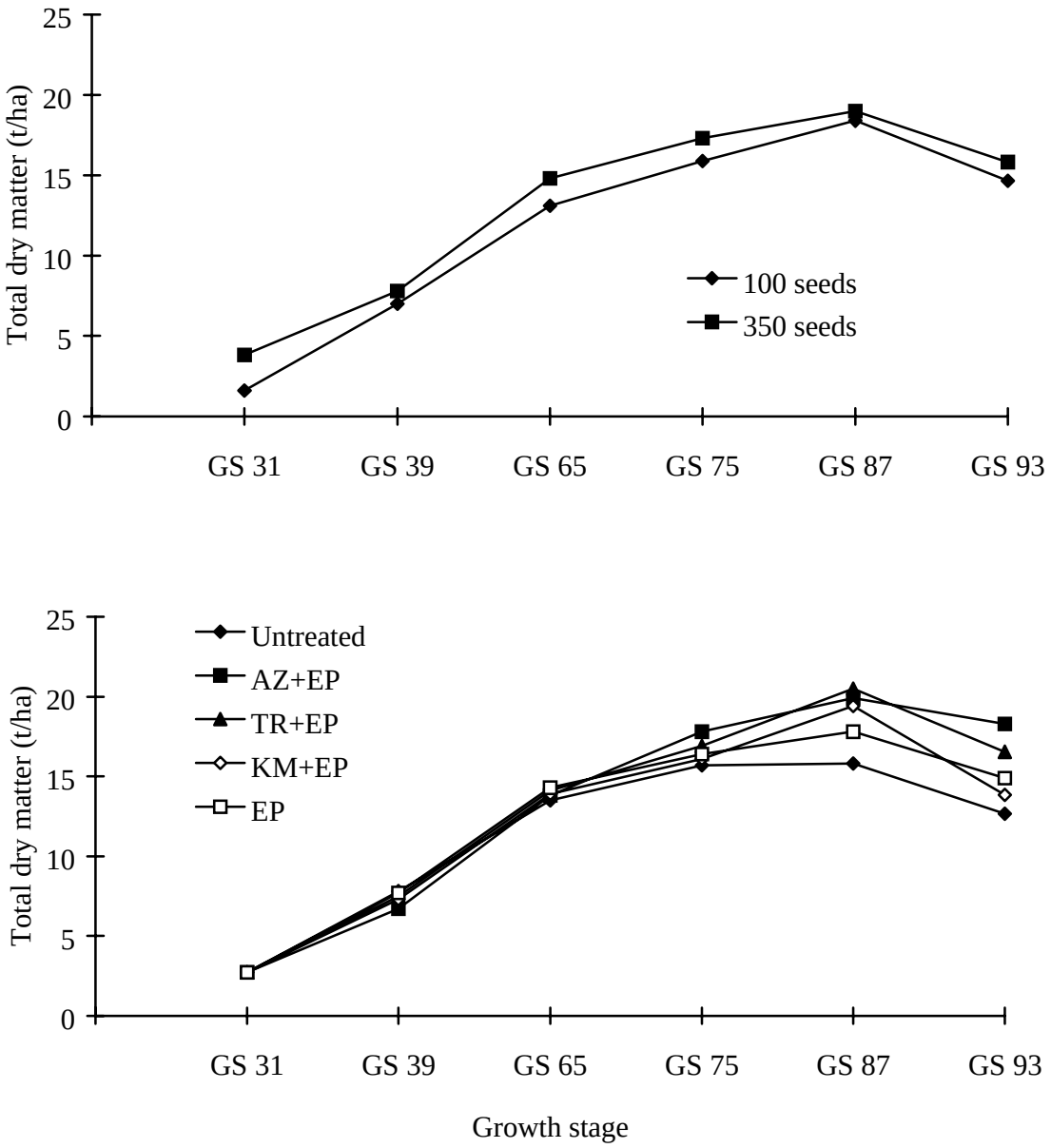


Figure 3.6. Total crop dry matter, mean for each seed rate (upper) and fungicide (lower). AZ = azoxystrobin, TR = trifloxystrobin, KM = kresoxim-methyl, EP = epoxiconazole.

Table 3.3. Total crop dry matter.

| Seed rate/$m^2$ | Fungicide | Total dry matter (t/ha) | | | | |
|---|---|---|---|---|---|---|
| | | GS 39 (22 May) | GS 65 (19 June) | GS 75 (3 July) | GS 87 (22 July) | GS 93 (30 August) |
| 100 | | 7.0 | 13.1 | 15.9 | 18.4 | 14.7 |
| 350 | | 7.8 | 14.8 | 17.3 | 19.0 | 15.8 |
| | Untreated | 7.8 | 13.5 | 15.7 | 15.8 | 12.7 |
| | AZ+EP | 6.7 | 13.8 | 17.8 | 19.9 | 18.3 |
| | TR+EP | 7.5 | 14.1 | 16.9 | 20.5 | 16.5 |
| | KM+EP | 7.3 | 13.9 | 16.1 | 19.4 | 13.8 |
| | EP | 7.7 | 14.3 | 16.4 | 17.8 | 14.9 |
| 100 | Untreated | 7.9 | 13.1 | 14.9 | 15.1 | 12.0 |
| 100 | AZ+EP | 6.6 | 12.9 | 16.9 | 19.6 | 18.0 |
| 100 | TR+EP | 7.0 | 13.4 | 16.3 | 20.0 | 15.4 |
| 100 | KM+EP | 6.6 | 12.7 | 15.3 | 18.7 | 14.3 |
| 100 | EP | 7.0 | 13.2 | 15.8 | 18.5 | 13.7 |
| 350 | Untreated | 7.8 | 13.9 | 16.3 | 16.4 | 13.3 |
| 350 | AZ+EP | 6.9 | 14.7 | 18.7 | 20.3 | 18.6 |
| 350 | TR+EP | 7.9 | 14.9 | 17.6 | 20.9 | 17.7 |
| 350 | KM+EP | 8.0 | 15.1 | 16.9 | 20.1 | 13.4 |
| 350 | EP | 8.4 | 15.3 | 17.1 | 17.1 | 16.1 |
| SED (18 df) | | | | | | |
| seed rate | | 0.26 | 0.41 | 0.46 | 0.53 | 1.37 |
| fungicide | | 0.41 | 0.64 | 0.73 | 0.83 | 2.15 |
| interaction | | 0.58 | 0.92 | 1.03 | 1.17 | 3.05 |
| *P* | | | | | | |
| seed rate | | 0.007 | <0.001 | 0.005 | 0.261 | 0.406 |
| fungicide | | 0.111 | 0.758 | 0.069 | <0.001 | 0.120 |
| interaction | | 0.281 | 0.782 | 0.997 | 0.431 | 0.931 |

Table 3.4. Stem water-soluble carbohydrates

| Seed rate/$m^2$ | Fungicide | Water-soluble carbohydrates (t/ha) | |
|---|---|---|---|
| | | GS 65 | GS 87 |
| 100 | | 2.62 | 0.34 |
| 350 | | 2.81 | 0.27 |
| | Untreated | 2.23 | 0.07 |
| | AZ+EP | 2.68 | 0.40 |
| | TR+EP | 2.88 | 0.45 |
| | KM+EP | 3.12 | 0.32 |
| | EP | 2.65 | 0.28 |
| 100 | Untreated | 2.00 | 0.06 |
| 100 | AZ+EP | 2.40 | 0.42 |
| 100 | TR+EP | 2.87 | 0.52 |
| 100 | KM+EP | 3.05 | 0.35 |
| 100 | EP | 2.78 | 0.34 |
| 350 | Untreated | 2.46 | 0.08 |
| 350 | AZ+EP | 2.96 | 0.39 |
| 350 | TR+EP | 2.90 | 0.37 |
| 350 | KM+EP | 3.19 | 0.30 |
| 350 | EP | 2.52 | 0.21 |
| SED (18 df) | | | |
| seed rate | | 0.178 | 0.039 |
| fungicide | | 0.282 | 0.062 |
| interaction | | 0.399 | 0.088 |
| *P* | | | |
| seed rate | | 0.306 | 0.099 |
| fungicide | | 0.063 | <0.001 |
| interaction | | 0.612 | 0.625 |

Table 3.5. Yield and grain quality.

| Seed rate/$m^2$ | Fungicide | Yield (t/ha) | Specific weight (kg/hl) | Thousand grain weight (g) | Grains per ear | Harvest index |
|---|---|---|---|---|---|---|
| 100 | | 7.80 | 75.7 | 45.3 | 33 | 0.50 |
| 350 | | 8.67 | 76.2 | 45.9 | 28 | 0.48 |
| | Untreated | 5.52 | 72.4 | 33.8 | 26 | 0.41 |
| | AZ+EP | 9.08 | 76.9 | 51.2 | 35 | 0.53 |
| | TR+EP | 9.12 | 77.1 | 50.5 | 31 | 0.52 |
| | KM+EP | 9.01 | 76.8 | 47.3 | 28 | 0.51 |
| | EP | 8.43 | 76.5 | 45.2 | 32 | 0.49 |
| 100 | Untreated | 4.98 | 71.9 | 32.6 | 30 | 0.42 |
| 100 | AZ+EP | 8.47 | 76.8 | 51.7 | 39 | 0.54 |
| 100 | TR+EP | 8.64 | 76.9 | 49.8 | 33 | 0.54 |
| 100 | KM+EP | 8.73 | 76.5 | 47.5 | 32 | 0.52 |
| 100 | EP | 8.15 | 76.4 | 44.9 | 31 | 0.51 |
| 350 | Untreated | 6.07 | 72.9 | 35.0 | 23 | 0.40 |
| 350 | AZ+EP | 9.69 | 77.1 | 50.7 | 32 | 0.51 |
| 350 | TR+EP | 9.61 | 77.3 | 51.1 | 28 | 0.51 |
| 350 | KM+EP | 9.29 | 77.0 | 47.0 | 23 | 0.50 |
| 350 | EP | 8.71 | 76.6 | 45.6 | 32 | 0.48 |
| SED (18 df) | | | | | | |
| seed rate | | 0.151 | 0.31 | 0.77 | 3.1 | 0.007 |
| fungicide | | 0.239 | 0.50 | 1.21 | 4.9 | 0.011 |
| interaction | | 0.337 | 0.70 | 1.71 | 6.9 | 0.015 |
| *P* | | | | | | |
| seed rate | | <.001 | 0.166 | 0.444 | 0.111 | 0.002 |
| fungicide | | <.001 | <.001 | <.001 | 0.372 | <0.001 |
| interaction | | 0.532 | 0.924 | 0.634 | 0.862 | 0.970 |

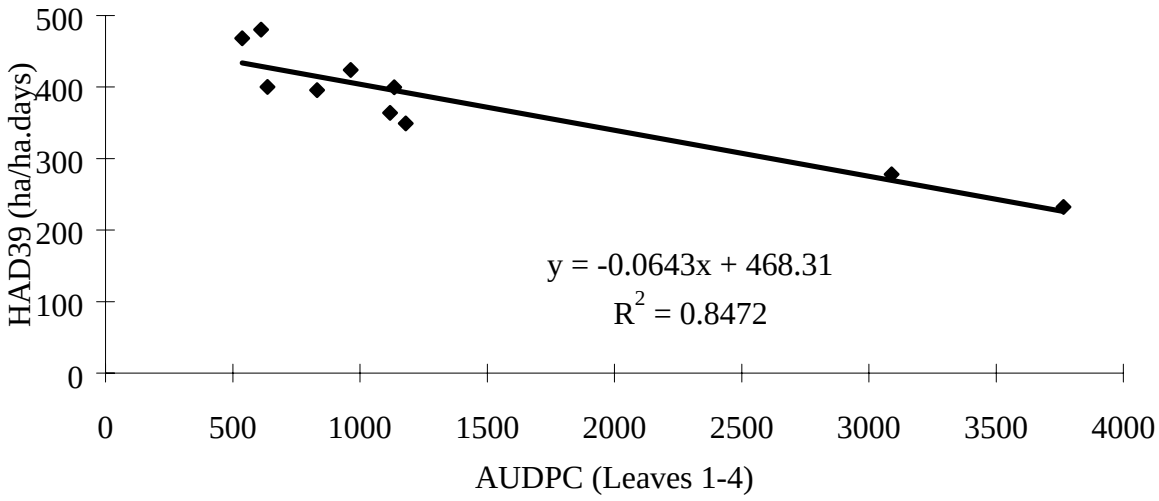


Figure 3.7. Relationship between canopy size integrated over time (HAD39) and disease.

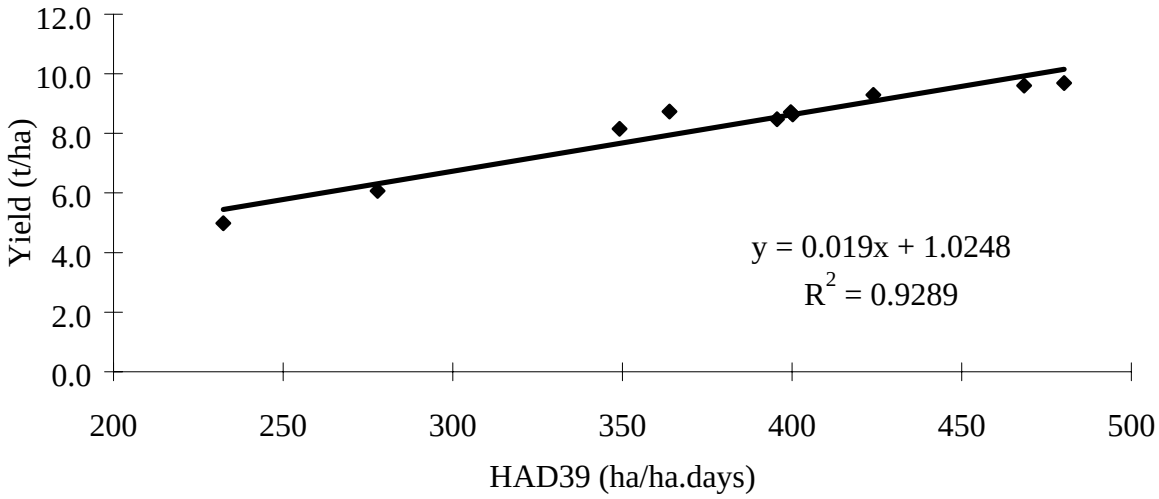


Figure 3.8. Relationship between yield and canopy size integrated over time (HAD39).

**Discussion**

A consistent feature throughout the results of this experiment was the lack of statistical interactions between seed rate and fungicide. There were effects of both seed rate and fungicide, generally in line with what would be expected, but no evidence that the crop established from a low seed rate showed a different response to fungicides to the crop from the 'conventional' seed rate. The crop from the lower seed rate had lower shoot numbers, smaller canopy size until late June, lower total biomass until early July (GS 75), lower yield and higher harvest index. There was no effect of seed rate on disease or on water-soluble carbohydrates. Fungicides gave the expected reduction in disease, increased green canopy and crop biomass and higher yields.

Comparing fungicides, few differences were evident in terms of control of *Septoria tritici*, but there were some small effects. On leaf 4, where eradicant activity would have been tested most severely, kresoxim-methyl plus epoxiconazole was less effective than epoxiconazole alone or in mixture with either of the other strobilurins. There has been much discussion among agronomists about the efficacy of the epoxiconazole in the kresoxim-methyl co-formulation (Landmark), compared with the straight epoxiconazole product Opus. It has been suggested that, because of the different type of formulation required for the mixture, epoxiconazole in Landmark was less effective than the same rate of active ingredient applied as Opus. Most of the results in this experiment show no difference between Landmark and Opus in *S. tritici* control, which is consistent with earlier HGCA-funded work (Jones, 2000). However, the eradicant activity of Landmark was slightly poorer, which only became evident where maximum eradicant activity was required. It is likely that this difference would also be evident if the dose applied had been reduced to the point at which it was only just sufficient to control the disease.

The other difference in disease control was that, on each of the top three leaves, the epoxiconazole mixtures with trifloxystrobin or azoxystrobin gave greater persistence of disease control than epoxiconazole alone or in mixture with kresoxim-methyl. This resulted in higher green leaf area indices in the last three weeks of green canopy life. There was a reasonably close relationship between green canopy integrated over time (HAD39) and disease, but a very close relationship between yield and HAD39. This suggests that the increased yield which resulted from the fungicide treatments was directly related to the increase in green canopy size and, therefore, it is unlikely that more efficient function of the canopy (perhaps through increased radiation use efficiency) was a significant factor. The slightly poorer relationship between disease and HAD39 may indicate that some of the increase in canopy may have resulted from activity other than direct fungicidal activity on the principal pathogen *S. tritici*. This could, perhaps, be associated with delaying colonisation of the leaf by saprophytes, which has been shown with azoxystrobin (Bertelsen *et al*., 2001) On the other hand, this may be a reflection of the difficulty in making measurements of the actual amount of disease, as opposed to total necrotic area, particularly towards the end of the life of a leaf.

Overall, this experiment did not provide any evidence to support the hypothesis that there may be a greater benefit from strobilurins in thin crops, where the lower stem leaves make a greater contribution to yield, than in thick crops. The actual crop canopy which resulted from sowing 100 seeds/$m^2$ was not quite as small as some commercial crop canopies which result from late sowing or adverse conditions (e.g. following severe slug damage), but sufficiently small to indicate that the benefits from strobilurins will not be fundamentally different on a thin crop. The experiment also showed that, under conditions of severe foliar disease, the strobilurins gave greater persistence of disease control, resulting in greater green canopy duration, and that yield was closely correlated with canopy size. Under these conditions, there was no evidence in this experiment of any physiological effects of strobilurins.

## APPENDIX 4

### Experiment 4: Interaction between strobilurin fungicides and nitrogen utilisation

### Introduction

Early field experiments with strobilurin fungicides showed increases in green canopy and yield which could not be related directly to improvements in disease control. This was observed particularly with kresoxim-methyl plus epoxiconazole, compared with epoxiconazole alone, but also with azoxystrobin plus epoxiconazole (Jones and Bryson, 1998; Jones, 2000). This raised interest in whether the strobilurins have direct physiological effects on plants which contribute to green canopy duration and thereby to yield.

There is evidence from glasshouse, growth room or detached leaf studies that kresoxim-methyl can affect various physiological processes in plants. Dark inactivation of nitrate reductase was reduced (Köhle *et al.*, 1997), and degradation of nitrate reductase inhibited (Glaab and Kaiser, 1999) which may increase nitrogen uptake and lead to a larger canopy size and, possibly, increased radiation use efficiency. Grossman and Retzlaff (1997) and Grossman *et al.* (1999) showed that kresoxim-methyl inhibited ethylene biosynthesis and increased abscisic acid levels, which may delay senescence. They also showed that stomatal aperture was reduced, which could reduce transpiration and, consequently, water stress on the plants.

The two main components of yield are the number of grains/$m^2$ and grain weight. The number of grains/$m^2$ is a function of the number of ears/$m^2$ and the number of fertile florets remaining following floret death pre-anthesis. Jones and Bryson (1998) showed that application of kresoxim-methyl plus epoxiconazole at GS 31 rather than GS 32 gave a higher yield, with a greater effect on grains/$m^2$, than on grain weight. This could have been associated with larger green canopy and increased assimilate production around the time of anthesis. This could be advantageous, since the maximum potential grain weight of wheat is influenced by the number of endosperm cells in the grain, which is determined at around anthesis (Brocklehurst, 1977). Endosperm cell numbers are affected by the level of assimilates available during the period of division, so the potential yield is dependent upon assimilate availability at around anthesis. Therefore, fungicides which give greater protection of canopy size up to anthesis could affect yield through increases in grain number as well as grain weight, whereas delaying canopy senescence after this stage can only increase yield through increased grain weight, which may be limited by the number of grains which have been produced.

In addition to the possibility of direct physiological effects on the crop, it has also been suggested by Bertlesen *et al*. (2001) that the high frequency of defence reactions against attempted fungal infection could result in the associated energy expenditure adversely affecting yield. They hypothesised that the yield advantage observed in field experiments for azoxystrobin treated crops could be due to the initiation of fewer defence reactions by the plants, particularly when compared with treatment with epoxiconazole.

This experiment was designed to investigate whether, under field conditions, direct effects of strobilurin fungicides on nitrogen uptake and utilisation could be detected, and whether there were effects on the crop nitrogen requirement.

**Materials and methods**

Experiments were established at ADAS Boxworth, Cambridge, in 1998/99 and 1999/2000, on a clay soil of the Hanslope series. In both years, the previous crop was winter beans. The crops were drilled at a seed rate of 140 seeds/$m^2$ on 15 October in 1999 and 6 October in 2000. The experiments were on cv. Spark, which has a rating of 7 for resistance to *Septoria tritici* (Anomymous, 1998).

The experimental design was the same in both years, and was fully randomised with three replicates. There were five nitrogen treatments (0, 80, 160, 240 and 320 kg/ha), four fungicide treatments (kresoxim-methyl, azoxystrobin, epoxiconazole and a control with no fungicide, referred to as 'no strobilurin') and three replicates. There were an additional 18 plots (6 per replicate) for destructive sampling. The total number of plots was 78, and the area of each plot was 4 m x 24 m. Treatments are detailed in Tables 4.1 - 4.3. In addition, there were overall applications of fungicides thought unlikely to have any physiological effects, to minimise the risk of disease and increase the probability of detecting any non-pathological effects of the treatment fungicides. These are detailed in Table 4.4.

Table 4.1. Treatments

| Treatment no. | Fungicide | N treatment |
|---|---|---|
| 1 | No strobilurin | N1 |
| 2 | No strobilurin | N2 |
| 3 | No strobilurin | N3 |
| 4 | No strobilurin | N4 |
| 5 | No strobilurin | N5 |
| 6 | Azoxystrobin | N1 |
| 7 | Azoxystrobin | N2 |
| 8 | Azoxystrobin | N3 |
| 9 | Azoxystrobin | N4 |
| 10 | Azoxystrobin | N5 |
| 11 | Kresoxim-methyl | N1 |
| 12 | Kresoxim-methyl | N2 |
| 13 | Kresoxim-methyl | N3 |
| 14 | Kresoxim-methyl | N4 |
| 15 | Kresoxim-methyl | N5 |
| 16 | Epoxiconazole | N1 |
| 17 | Epoxiconazole | N2 |
| 18 | Epoxiconazole | N3 |
| 19 | Epoxiconazole | N4 |
| 20 | Epoxiconazole | N5 |

Table 4.2. Fungicides

| | Fungicide treatment | Product | Rate/ha |
|---|---|---|---|
| 1 | No strobilurin | - | --- |
| 2 | Azoxystrobin | Amistar | 1.00 litre |
| 3 | Kresoxim-methyl | Stroby | 0.25 kg |
| 4 | Epoxiconazole | Opus | 1.00 litre |

Each fungicide treatment was applied on two occasions, at GS 31-32 (30 April 1999 and 8 May 2000) and GS 39 (29 May 1999 and 24 May 2000) (Tottman & Broad, 1987). Other agronomic inputs followed good commercial practice.

Table 4.3. Nitrogen treatments

| N treatment | kg/ha N at each application | | |
|---|---|---|---|
| | 1st | 2nd | Total |
| N1 | 0 | 0 | 0 |
| N2 | 40 | 40 | 80 |
| N3 | 80 | 80 | 160 |
| N4 | 120 | 120 | 240 |
| N5 | 160 | 160 | 320 |

Nitrogen was applied on 22 April and 8 May in 1999, and 18 April and 25 April in 2000.

Table 4.4. Overall fungicide applications

| Active ingredient | Fungicide product | Application rate of product/ha | Date of application |
|---|---|---|---|
| Chlorothalonil | Bravo | 1.00 | 16 April 1999 |
| Quinoxyfen | Fortress | 0.15 | 16 April 1999 |
| Chlorothalonil | Bravo | 1.00 | 19 May 1999 |
| Chlorothalonil | Bravo | 1.00 | 19 April 2000 |
| Quinoxyfen | Fortress | 0.15 | 19 April 2000 |
| Chlorothalonil | Bravo | 1.00 | 30 May 2000 |

Foliar diseases were assessed as visual estimates of the percentage leaf area infected by each disease on each leaf layer, on 10 tillers taken at random from each sub plot at 7 day (+/-1) intervals from the date of the first fungicide application until all leaves were senescent. Percentage green leaf area was also estimated. The crop was not assessed for take-all, but as in both years the experiment was on a first wheat, take-all would not be expected to be important.

Growth analysis was done weekly on fungicide Treatments 1-3 from the onset of stem extension (GS 31), until harvest. On each occasion, the area within a quadrat was sampled (two 0.5 $m^2$ quadrats in 1999, and one 0.72 $m^2$ quadrat in 2000), leaving 3 rows on each side and at least 0.5 m between samples to avoid edge effects (Austin & Blackwell, 1980). The plants were cut at ground level and taken to the laboratory for analysis, except at GS 31 where plants were removed from the field intact and plant number counted before cutting off the roots at ground level. The number and dry weight of shoots were assessed separately for potentially fertile, dead and dying shoots. A shoot was classified as dying when no further leaf was emerging and the most recently emerged leaf was yellowing (Thorne & Wood, 1987).

Green area index (GAI) was calculated by determining actual leaf size on each occasion that diseases were assessed, using two of the ten leaves taken from each plot. Leaf length and width were measured , to the nearest 0.1 mm, and leaf area determined using a form factor of 0.83 (Bryson *et al*., 1997). GAI was calculated using the mean number of shoots per plot from assessments between mid-anthesis and mid-senescence. The leaf areas were then integrated over time from GS 39 until the end of all green canopy to give healthy area duration from GS 39 (HAD39; Bryson *et al.*, 1997). This provides a measure of green canopy size during the period in which photosynthesis is contributing primarily to grain filling rather than to canopy structure.

GAI was also calculated from the quadrat sampled crop at each sampling occasion in 1999, and at GS 61 in 2000. The projected green areas of separated leaves, stems and ears were recorded from a 10% sub-sample, using a LiCor 3100 leaf area meter, and the green areas for the fractions summed to calculate GAI.

Fitted curves were applied to green area data from FLAME measurements on both years, and from GAI measurements in 1999. Logistic curves of the form $A + C/(1 + EXP(-B*(x - M)))$ were fitted to values for green area per leaf, per shoot, and per $m^2$, and the estimates of the M parameters of these curves was taken to correspond to the date of 50% green area loss, and the C parameter to correspond to maximum green area. The A parameter was constrained to 0. The parameters were compared across treatments by using a t-test. An example curve is shown in Figure 4.10.

A SPAD meter (Minolta SPAD-502 Tokyo, Japan) was used to measure leaf 'greenness' both seasons. Ten measurements were made per leaf, on ten leaves per plot, by clamping the SPAD sensor over the leaf lamina. Measurements were taken at weekly intervals on all leaves from 25 May until senescence 1999, and in 2000, at GS 65 on leaves 1-3.

Samples were taken in 2000 to predict the potential mature grain weight from grain water content, as described by Macbeth (1996). Samples were taken 28 days after the beginning of anthesis (GS 61) from nitrogen treatments 0 and 160, and fungicide treatments azoxystrobin, kresoxim-methyl and No Strobilurin. Ten ears were randomly selected and cut below the collar. The ears for each sample were immediately wrapped in polyethylene film, sealed in a labelled polyethythene bag, and placed in a cool box for transport back to the laboratory.

In the laboratory, grains were immediately excised from florets 1 and 2 in three spikelets: the central spikelet and the two below it. The total number of excised grains per ten ear sample was recorded. Fresh weights were recorded, and the grains were then dried at 80ºC for 48 hours, within open tins, and the dry weight recorded. These results were used to calculate the potential maximum grain weights.

Potential mature grain weight was then calculated as follows:
$PGW = 44.02 + (0.51 \times GWC) - (0.24 \times GNNO) - (0.01 \times S/m^2)$
Where PGW = potential grain weight
GWC = grain water content
GNNO = grain number per ear
$S/m^2$ = shoots per $m^2$

In both seasons, at harvest, a 0.5 $m^2$ sample was removed from each plot. Final crop dry weight and ear number were recorded. The ears were then threshed, grain dry weight measured and harvest index calculated. Grain yield was measured from an accurately measured length of each plot using a plot combine harvester. Grain was analysed for moisture content and specific weight using GAC 2000 grain analysis computer (Dickey-John Corporation). The thousand grain weight was determined on grain samples taken from the

combine. The number of grains per ear was then calculated using the combine grain yield, the thousand grain weight and the number of ears/$m^2$.

## Results

*Disease*

Despite attempts to eliminate disease from the experiments, *Septoria tritici* infection reached significant levels in both years. This may have been in part due to rainfall, which occurred shortly after the fungicide was sprayed. In 1999, the application of the experimental fungicides was on the 29 May, and this was followed by heavy rainfall (27mm) on 30 May. In 2000, the final application of the experimental fungicides on 24 May, and the final overall fungicide on 30 May, were both made on days with some rainfall. The disease was particularly prevalent in the 'No Strobilurin' treatment (Figure 4.1).

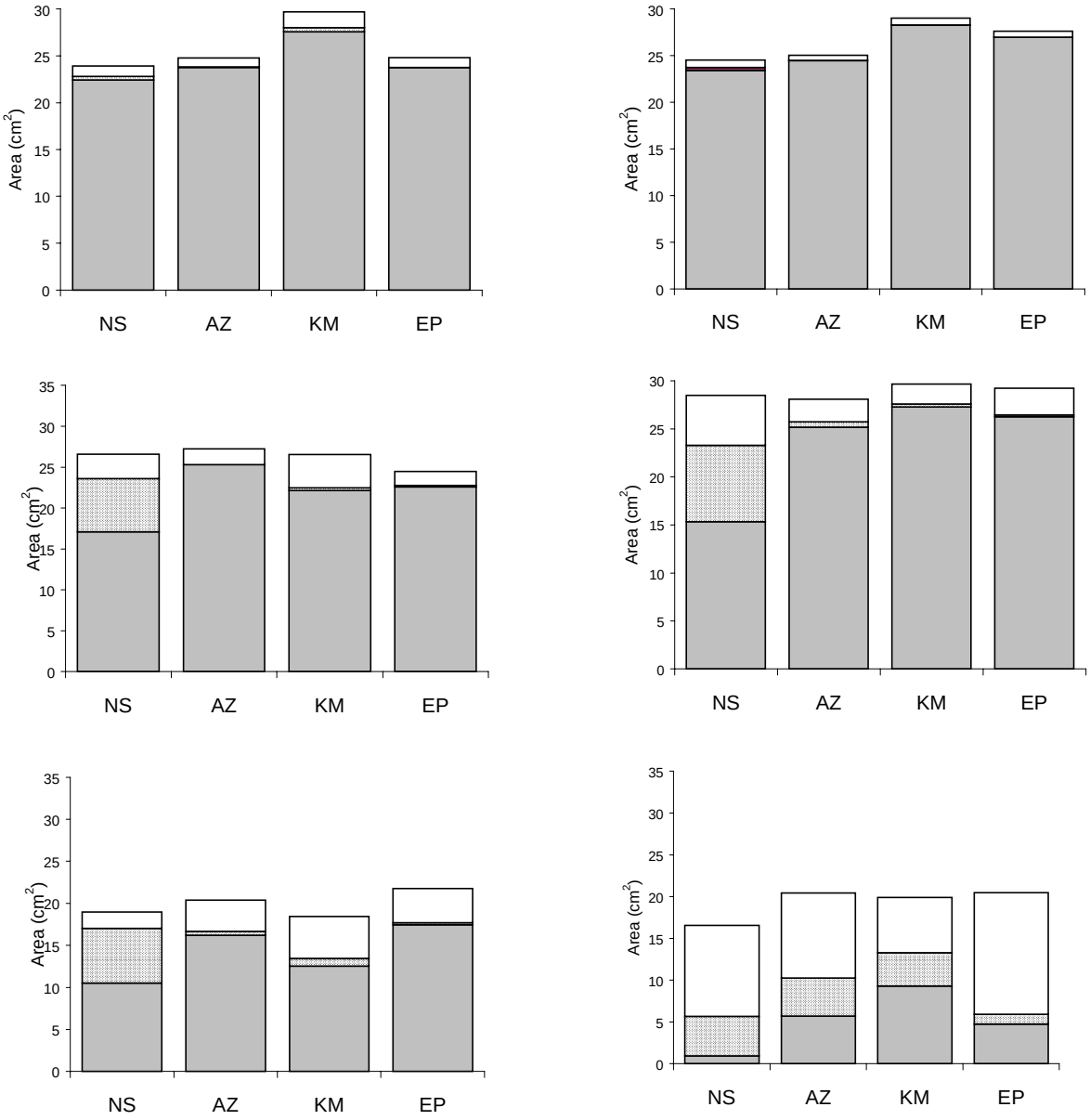


Figure 4.1. Area of leaves 1-3 at GS 75, shown as (from bottom to top in each bar) green area, non-green area with no disease symptoms, and symptom area. NS = no strobilurin, AZ = azoxystrobin, KM = kresoxim-methyl, EP = epoxiconazole

These figures demonstrate that on the whole, strobilurins did not affect the amount of non-diseased, non-green area in leaves. This could indicate that the strobilurins have no real effect on reducing natural senescence, and that in the absence of disease, no difference in the maintenance of green area would be expected.

In 1999, disease progress became rapid on about 9 June (Figure 4.2), at which point the crop could no longer be considered disease-free. Since an examination of the physiological effects of strobilurin fungicides would be compromised once disease had occurred, any observed differences between fungicide treatments pre-anthesis provide the best evidence for any physiological effects.

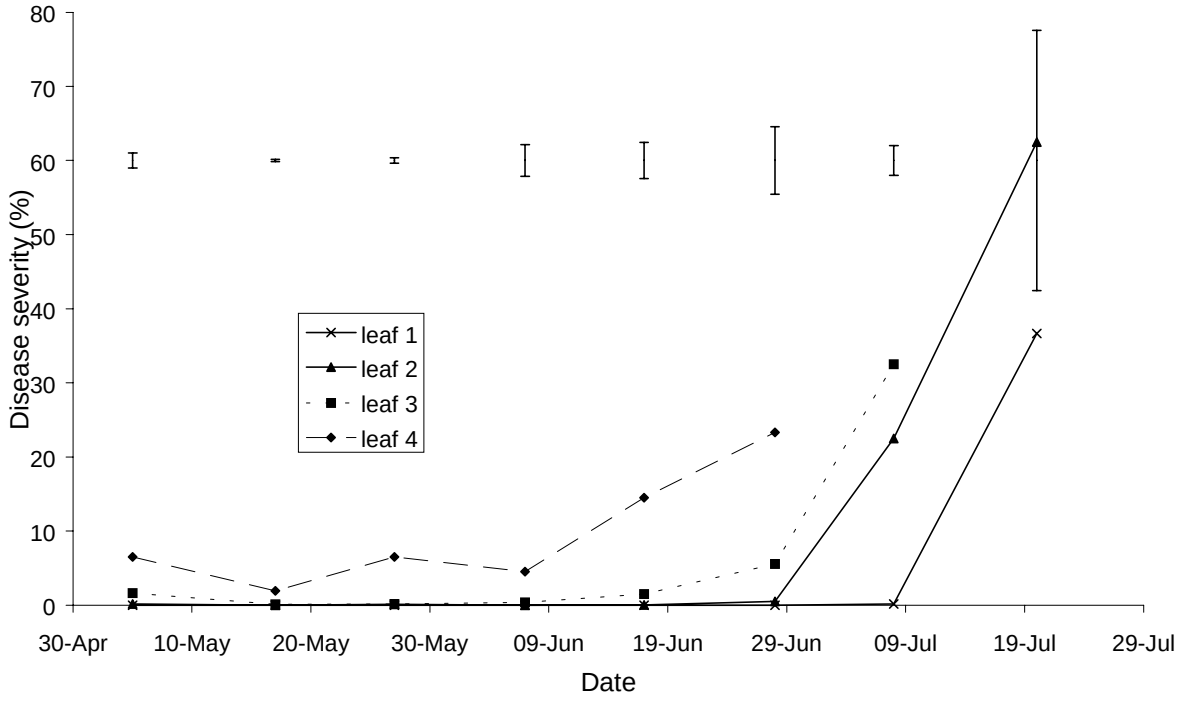


Figure 4.2. *Septoria tritici* progress curves 1999, 160N treatment. Error bars are s.e.d. with 6 df (last point 2 df, penultimate point 4 df).

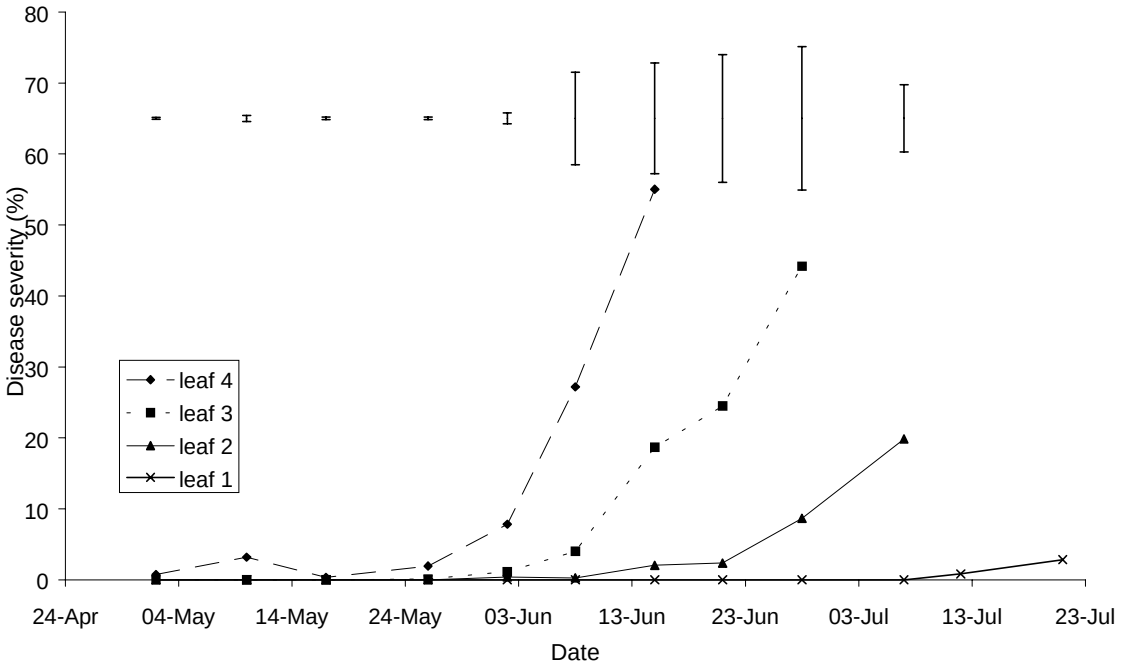


Figure 4.3. *Septoria tritici* progress curves 2000, 160N treatment. Error bars are s.e.d. with 6 df (last point 2 df, penultimate two points 4 df).

As in the previous year, the crop could only be considered disease-free until about 3 June in 2000 (Figure 4.3).

The nitrogen treatment also had an effect on disease severity, particularly in the No Strobilurin treated plots (Figures 4.4 and 4.5).

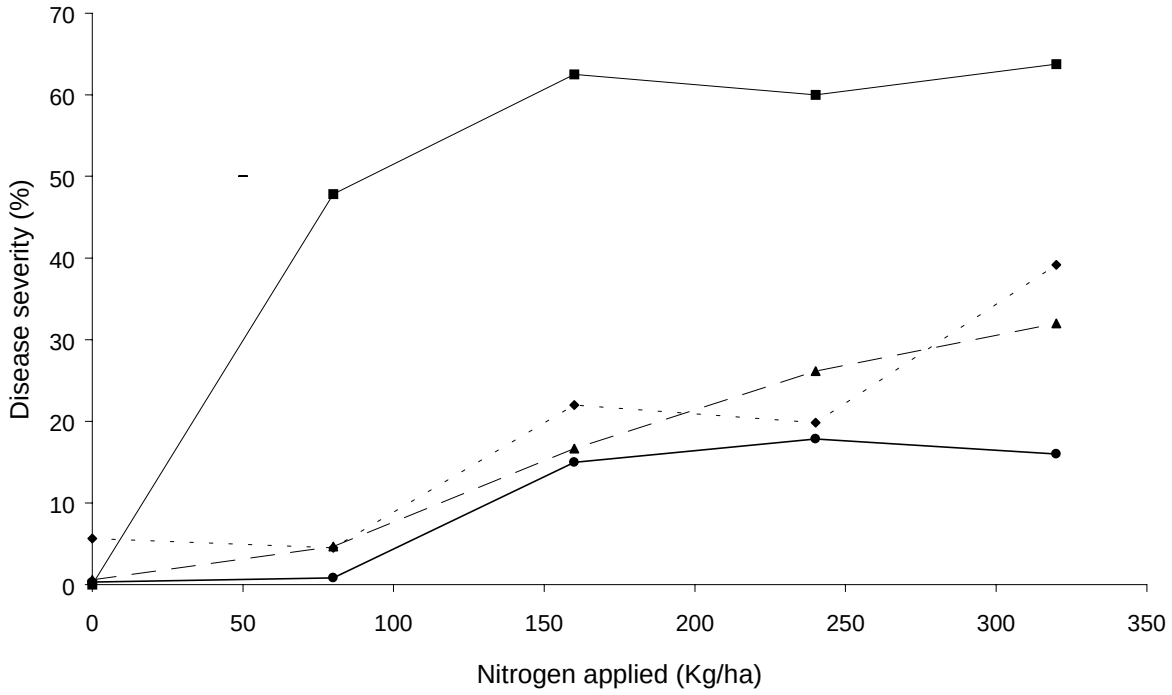


Figure 4.4. Disease severity with increasing levels of nitrogen application in 1999. No Strobilurin (—▲—), Azoxystrobin (⁻ ⁻■⁻ ⁻), Kresoxim-methyl (----◆----), Epoxiconazole (—●—).

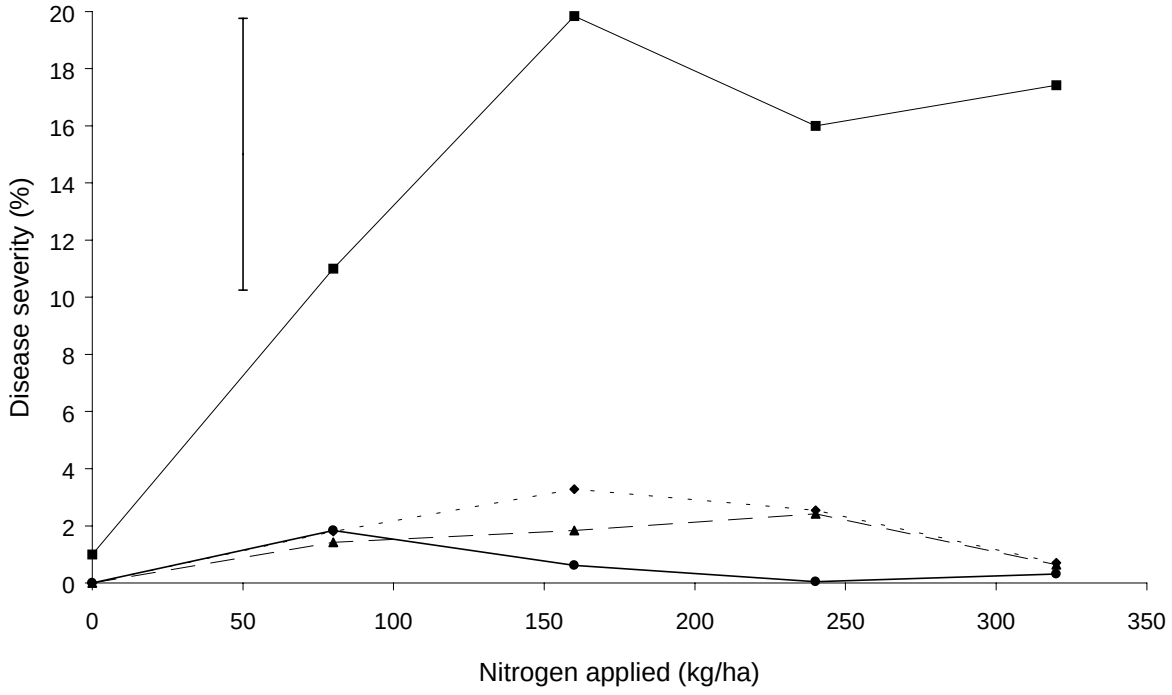


Figure 4.5. Disease severity with increasing levels of nitrogen application in 2000. . No Strobilurin (—▲—), Azoxystrobin (⁻ ⁻■⁻ ⁻), Kresoxim-methyl (----◆----), Epoxiconazole (—●—).

*Shoot numbers*

Shoot numbers were recorded at weekly intervals in two nitrogen treatments (160N and 0N), and three fungicide treatments (No Strobilurin, Azoxystrobin, and Kresoxim-methyl). Final ear numbers (taken from pre-harvest analysis on all treatment combinations) showed a difference between N treatments in both years ($P = 0.001$ in 1999, and $P < 0.001$ in 2000), with higher N levels producing more ears than the 0N and 80N treatments (Figures 4.6 and 4.7). There was also a statistically significant effect of fungicide ($P = 0.025$) in 2000, with epoxiconazole treated crops producing more ears than the other treatments at 80 and 160N levels. This could be as a result of eradicant activity of epoxiconazole allowing for an increase in shoot survival when it is not limited by nitrogen. This effect was not observed in 1999.

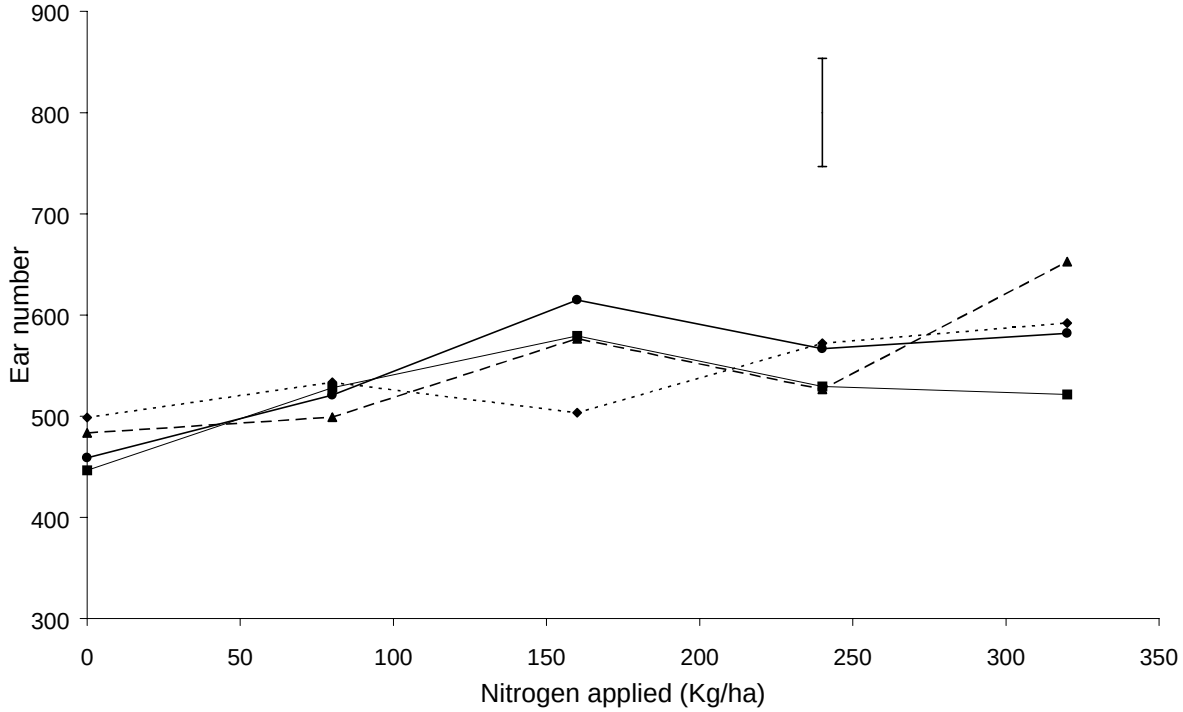


Figure 4.6. Final ear number in 1999. No Strobilurin (—▲—), Azoxystrobin (– –■– –), Kresoxim-methyl (----◆----), Epoxiconazole (—●—). Error bar is S.E.D. with 37 df.

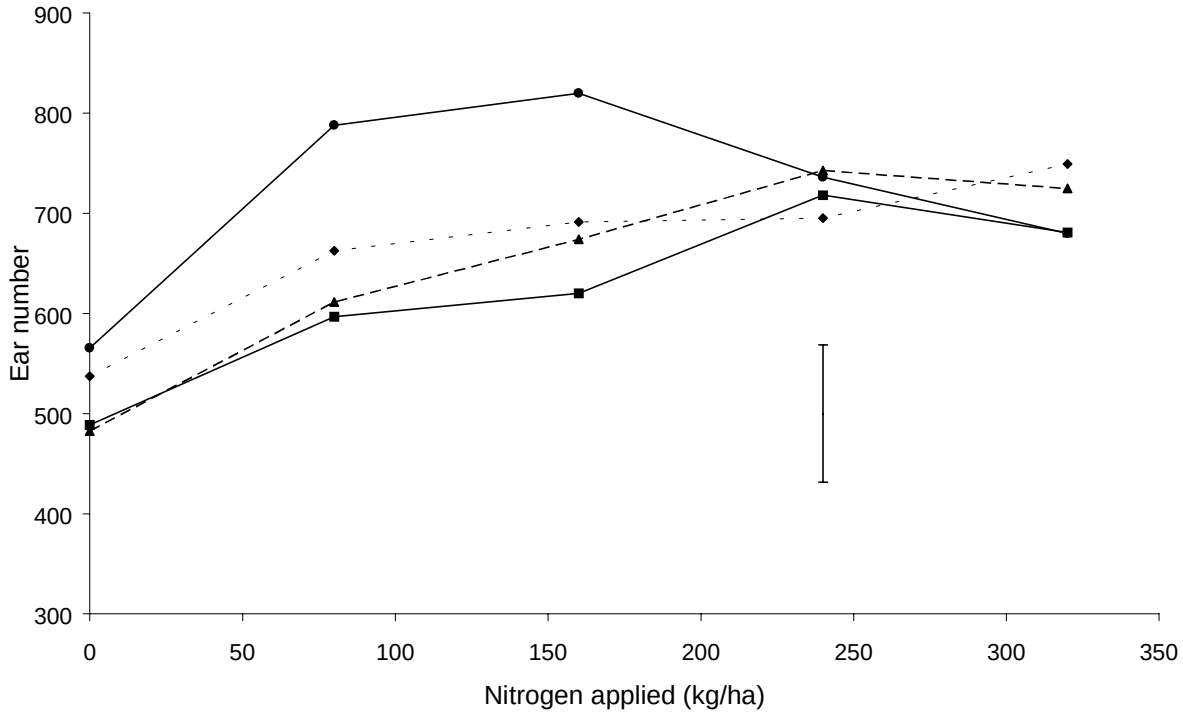


Figure 4.7. Final ear number in 2000. No Strobilurin (—▲—), Azoxystrobin (- -■- -), Kresoxim-methyl (----◆----), Epoxiconazole (—●—). Error bar is S.E.D. with 38 df.

*N uptake*

There were early differences in N uptake between N treatments, the 160N treatment showing a more rapid uptake than the 0N treatment in both seasons.

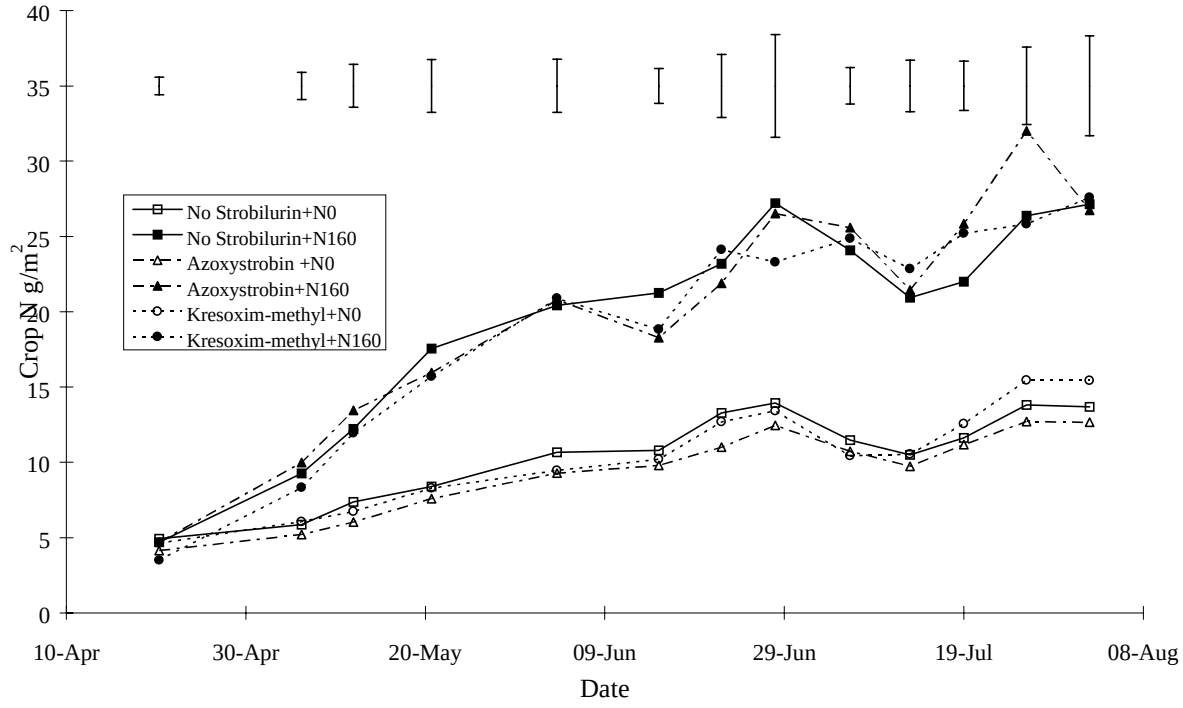


Figure 4.8 Total N uptake 1999. Error bars are S.E.D. with 10 df.

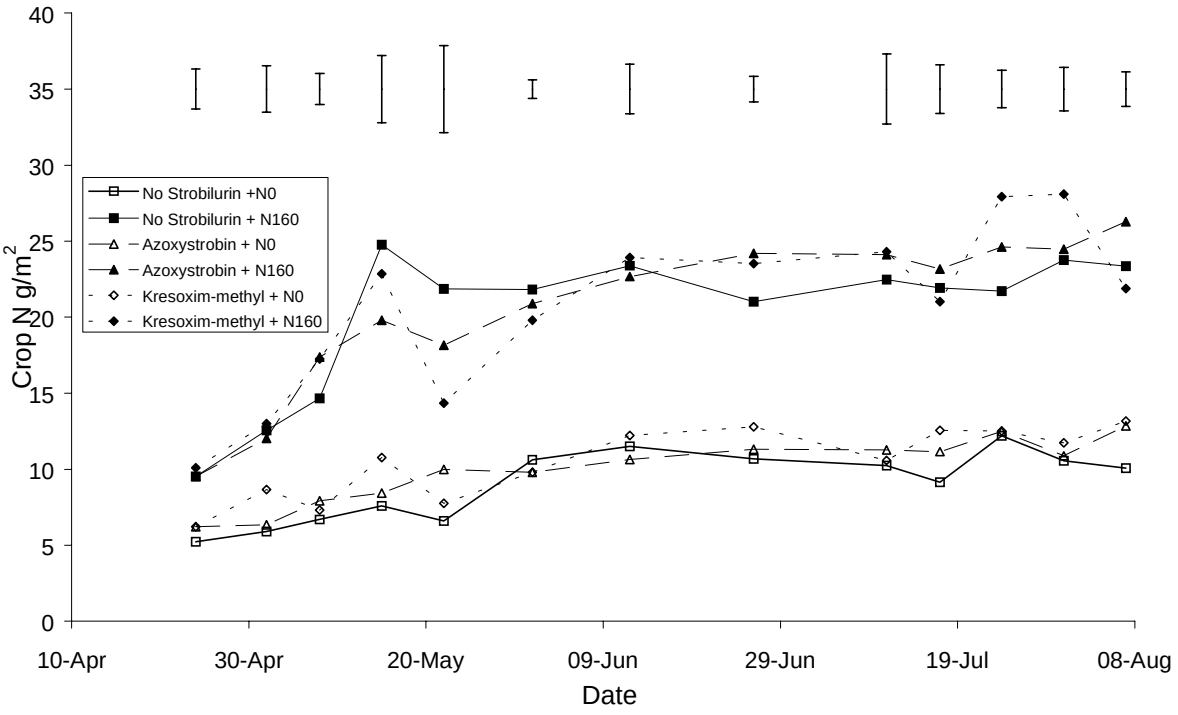


Figure 4.9. Total N uptake 2000. Error bars are S.E.D. with 10 df.

The 160N treatment showed more rapid early uptake in 2000 than in 1999, possibly due to a combination of higher rainfall in May, as well as earlier N application (the second N application was made on 6 May in 1999, and on 25 April in 2000) (Figures 4.8 and 4.9).

As disease was in the crop from about the 9 June in 1999, and 3 June in 2000, statistical comparison of N uptake between fungicide treatments was made using samples taken before these dates. Regression analysis of crop N over time was used to compare rates and starting values for fungicide treatments within each N level.

In 1999, at 160N, there was no statistically significant difference between fungicide treatment in N uptake ($P = 0.451$, 2 df), although at 0N there was a difference between fungicide treatments ($P = 0.045$, 2 df).

In 2000, the results were similar, with no difference between fungicide treatment in at either level of N ($P = 0.075$, 2 df at 0N, and $P = 0.264$, 2 df at 160N).

*Nitrogen in grain at harvest*

There was no evidence of strobilurins affecting the grain N% in either year, at any level of applied nitrogen. Grain N% increased with increasing N application, as expected.

The only significant difference was in 1999 for the 320N treatment, in which the 'No Strobilurin' had a significantly lower N% than both Azoxystrobin and Epoxiconazole. Kresoxim-methyl was also significantly lower than Azoxystrobin.

*Green area*

GAI was calculated both from the projected areas of a sub-sample from weekly quadrat analysis (performed on all samples in 1999, and at GS 61 in 2000), and leaf area index (LAI)

from data obtained from FLAME measurements. Maximum LAI was determined by fitting a logistic curve to the data. An example curve is shown in Figure 4.10.

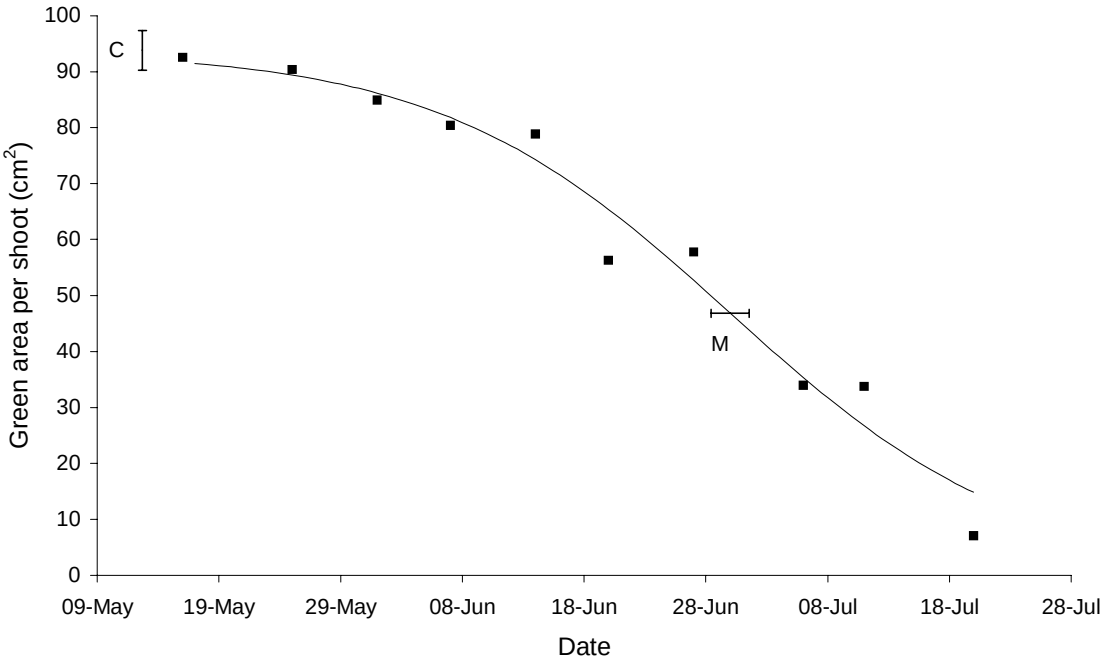


Figure 4.10. Fitted senescence curve for Azoxystrobin 0N, from FLAME measurements in 2000. Parameters C (maximum green area), and M (mid-point of senescence), are shown with their associated s.e.; 27 df.

The maximum green area per shoot for each treatment from FLAME measurements are shown in Tables 4.7 and 4.8. Results showed that increased levels of nitrogen produced larger canopies. Across fungicides, however very few differences were shown. No differences were shown in 1999, and in 2000, only Azoxystrobin had a greater green area per shoot (113 $cm^2$) than No Strobilurin (100 $cm^2$) at 80N.

As both shoot number and leaf expansion were not affected by the strobilurin treatment, leaf senescence was analysed to test any effects on canopy persistence.

Results from projected GAI data in 1999 showed that increased levels of nitrogen delayed mid-senescence. Results generally showed that strobilurins delayed senescence when compared to the No Strobilurin treatment – particularly at higher N levels. At 160N, both strobilurin treatments experienced a delayed senescence compared to the No Strobilurin, whereas at 0N, no differences were apparent. These results were generally consistent whether data were analysed as GAI, or in leaf layers, although on a per shoot basis, senescence was only statistically significantly delayed for azoxystrobin compared to No Strobilurin.

However, GAI was not measured in all fungicide treatments. FLAME data were available for all treatments, and when compared to Epoxiconazole, there were no significant differences for the strobilurins in LAI senescence. The parameters for each treatment from FLAME measurements per shoot are shown in Tables 4.5 and 4.6. Statistically significant differences were compared between treatments by using a t-test.

In both years there was a strong relationship shown between GAIs measured by projected area and LAIs measured by FLAME at GS 61 ($R^2$ = 0.97 in 1999, and 0.93 in 2000). However, in

both years, the FLAME values were larger than the projected GAI areas (an average of 10.4% larger in 1999, and as much as 40.0% larger in 2000). This is likely to have been due different criteria for shoot selection and perhaps to the disease incidence on the leaves, as diseased areas were removed from samples before measuring their projected areas, whereas the % incidence of disease was estimated when taking FLAME measurements, and these values applied to the data. FLAME measurements may therefore provide a more accurate measurement of green area when disease is present.

The equation of the curve was re-arranged to allow calculation of the date of the onset of senescence (2% loss in green area from the maximum) and the end of senescence (when 2% of green area remained). The duration of senescence was then calculated as the difference between these two dates.

The equation
$y = C/(1+EXP(-B*(x-M)))$ was rearranged to
$x = M+(Log((C/y)-1)/(-B))$
where y had been calculated as 2% below C and 2% of C to calculate the onset and end of senescence respectively.

Table 4.5. Senescence parameters from FLAME data 1999 (followed by s.e.), and the onset and duration of senescence calculated from these parameters.

| Fungicide treatment | Nitrogen treatment | Mid-point of senescence (Julian date) | Onset of senescence (Julian date) | Duration of senescence (days) | Maximum green area ($cm^2$/shoot) |
|---|---|---|---|---|---|
| Azoxystrobin | 0 | 193 (*) | 188 | 11.6 | 66.5 (*) |
| | 80 | 191 (1.71) | 185 | 12.5 | 83.0 (4.58) |
| | 160 | 193 (1.59) | 186 | 14.1 | 85.3 (4.08) |
| | 240 | 193 (*) | 188 | 11.7 | 85.0 (*) |
| | 320 | 194 (*) | 188 | 12.3 | 88.8 (*) |
| Kresoxim-methyl | 0 | 196 (*) | 189 | 13.4 | 66.2 (*) |
| | 80 | 191 (1.76) | 185 | 13.3 | 81.3 (4.66) |
| | 160 | 193 (1.13) | 187 | 12.5 | 83.3 (2.83) |
| | 240 | 196 (*) | 189 | 13.9 | 82.5 (*) |
| | 320 | 192 (0.68) | 187 | 10.4 | 87.3 (1.67) |
| Epoxiconazole | 0 | 190 (1.36) | 183 | 14.8 | 67.6 (3.29) |
| | 80 | 190 (1.77) | 181 | 18.1 | 84.6 (5.01) |
| | 160 | 192 (85.5) | 186 | 11.0 | 85.5 (2.72) |
| | 240 | 194 (*) | 188 | 12.6 | 86.4 (*) |
| | 320 | 193 (*) | 188 | 11.7 | 89.5 (*) |
| No Strobilurin | 0 | 189 (2.37) | 181 | 17.1 | 68.5 (5.65) |
| | 80 | 191 (2.02) | 186 | 10.2 | 76.1 (4.48) |
| | 160 | 188 (1.12) | 178 | 18.8 | 90.3 (3.61) |
| | 240 | 189 (1.24) | 182 | 13.7 | 85.1 (4.17) |
| | 320 | 190 (1.10) | 184 | 12.3 | 81.7 (3.07) |

* denotes that no s.e. could be obtained.

Table 4.6. Senescence parameters from FLAME data 2000 (followed by s.e.), and the onset and duration of senescence calculated from these parameters.

| Fungicide treatment | Nitrogen treatment | Mid-point of senescence (Julian date) | Onset of senescence (Julian date) | Duration of senescence (days) | Maximum green area ($cm^{2/}$shoot) |
|---|---|---|---|---|---|
| Azoxystrobin | 0 | 182 (1.57) | 162 | 40.5 | 94 (3.56) |
| | 80 | 186 (2.00) | 163 | 46.8 | 113 (5.23) |
| | 160 | 190 (1.76) | 169 | 41.9 | 111 (4.45) |
| | 240 | 192 (1.50) | 170 | 45.9 | 114 (3.81) |
| | 320 | 197 (2.07) | 172 | 49.0 | 112 (4.89) |
| Kresoxim-methyl | 0 | 183 (1.49) | 165 | 36.8 | 95 (3.45) |
| | 80 | 184 (1.86) | 164 | 40.3 | 111 (4.91) |
| | 160 | 189 (1.27) | 173 | 32.4 | 110 (3.27) |
| | 240 | 191 (1.04) | 180 | 21.7 | 107 (2.78) |
| | 320 | 190 (1.53) | 176 | 27.8 | 108 (3.90) |
| Epoxiconazole | 0 | 189 (1.90) | 170 | 31.0 | 91 (3.64) |
| | 80 | 189 (1.90) | 170 | 38.8 | 105 (4.60) |
| | 160 | 192 (1.38) | 175 | 34.5 | 109 (3.34) |
| | 240 | 196 (1.68) | 168 | 36.9 | 112 (3.85) |
| | 320 | 197 (1.65) | 177 | 39.8 | 113 (3.67) |
| No Strobilurin | 0 | 179 (1.56) | 160 | 38.7 | 93 (3.64) |
| | 80 | 182 (1.29) | 166 | 30.8 | 100 (3.26) |
| | 160 | 182 (0.98) | 168 | 28.8 | 104 (2.52) |
| | 240 | 182 (1.43) | 177 | 29.6 | 114 (4.13) |
| | 320 | 182 (1.39) | 167 | 30.4 | 113 (3.94) |

Senescence occurred later, and was more rapid in 1999 than in 2000 In general, the end of senescence occurred on a similar calendar date in both years. In 1999, senescence tended to start earlier in the No Strobilurin treatment than in the other fungicide treatment plots, resulting in a longer duration of senescence at the 0 and 160N treatments (Table 4.5). Fungicide treatment appeared to have little effect on the date of the end of senescence. In 2000, epoxiconazole experienced a later onset of senescence at the lower N treatments, which resulted in a shorter duration of senescence compared to the other fungicide treatments (Table 4.6). In general, the No Strobilurin treatment finished senescence earlier than the other three fungicide treatments.

The fit of the curves was improved in 2000, as senescence was less rapid, and so the number of samples taken during this phase was greater. The date of the mid-point of senescence was plotted against the nitrogen treatment (Figure 4.11), and showed a delayed senescence at the higher N levels for all fungicide treatments except the No Strobilurin.

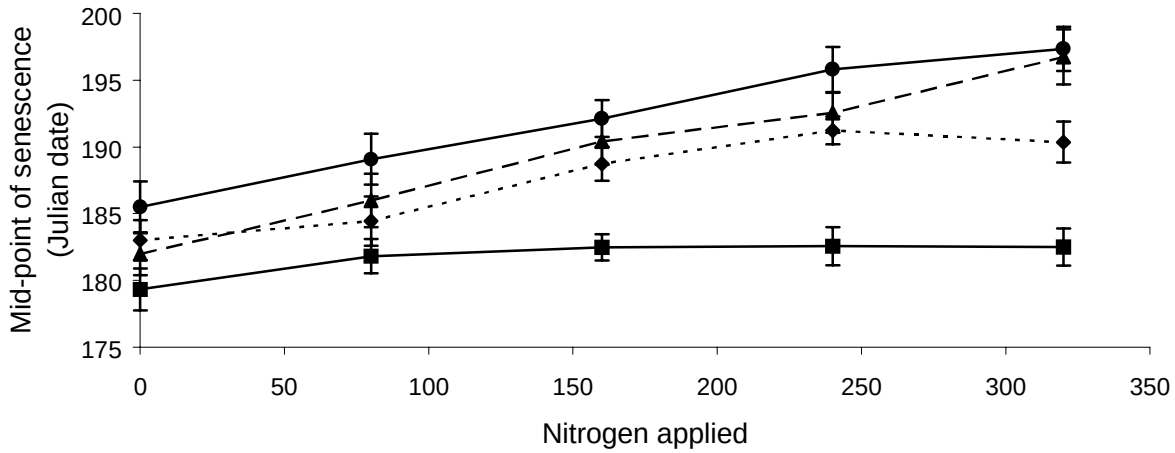


Figure 4.11. The mid point of senescence (Julian date), derived from fitted curves, in 2000. No Strobilurin (—▲—), Azoxystrobin (⁻ ⁻■⁻ ⁻), Kresoxim-methyl (----◆----), Epoxiconazole (—●—). Error bars are s.e. for each fitted point; 27df.

These effects on senescence, however, do not appear to be unrelated to disease incidence, as disease was present in both years (particularly *Septoria tritici*), and was most severe in the No Strobilurin treatment, and least severe in the epoxiconazole.

A calculation of green area lost per unit area of disease was made, in order to standardise the disease levels to enable comparisons across treatments. This was calculated from the FLAME measurements on leaf 2 taken on all plots at GS 75 as follows:

Maximum total leaf area – Total leaf area / Diseased area

It would be predicted that if the strobilurins have a physiological effect of delaying senescence, that the plots treated with strobilurins would show less depletion in green area with the same amount of disease.

Results showed statistically significant differences between fungicide treatment in the amount of green area loss per $cm^2$ of disease ($P = 0.006$). However, the results were not as expected, with the No Strobilurin treatment showing the least reduction in green area per unit area disease.

*Biomass*

There was an early effect of N treatment, but very little evidence for a fungicide effect, except at the end of the season, when disease is likely to have affected growth (Figures 4.12 and 4.13). Pre-harvest samples showed no differences in total above-ground biomass between fungicide treatments at any nitrogen level.

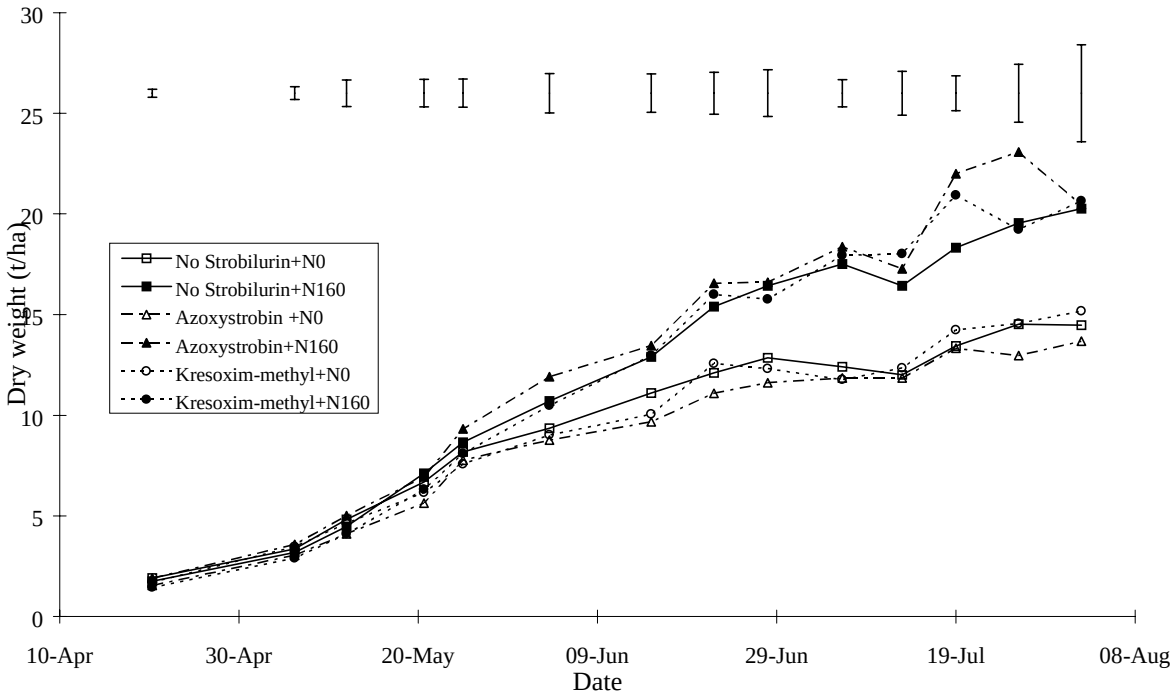


Figure 4.12. Dry matter production in 1999. Error bars are S.E.D. with 10 df

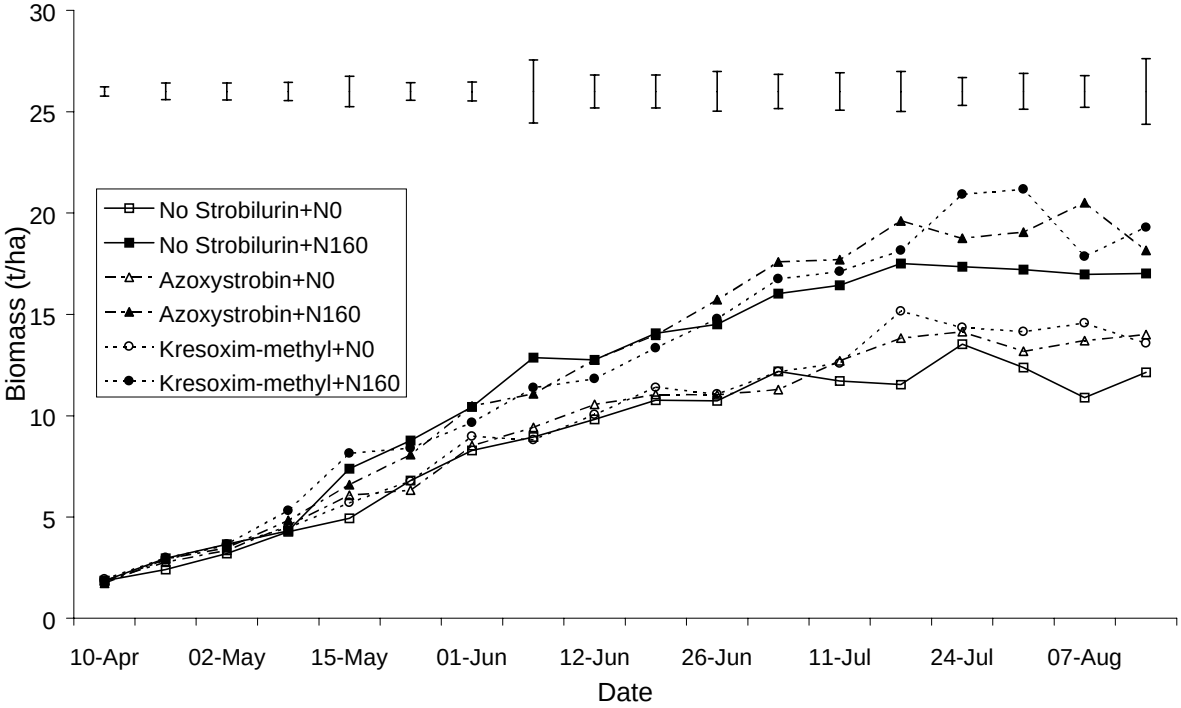


Figure 4.13. Dry matter production in 2000 Error bars are S.E.D. with 10 df

*Radiation Use Efficiency (RUE)*

RUE was calculated by fitting a linear relationship between cumulative biomass accumulation (from weekly growth analysis measurements from the beginning of May until disease was present in early June), and cumulative radiation interception over the same period (LAI was calculated from FLAME measurements and shoot counts for the 0N and 160N, No Strobilurin, Azoxystrobin and Kresoxim-methyl, treatment combinations). PAR interception

was calculated from records of incident radiation by applying a standard extinction co-efficient for the variety Spark of 0.43 $k_{PAR}$ (J. Whaley, personal communication). RUE was then calculated as the slope of the linear relationship, restricted to pass through zero. This approach is widely used (Sinclair and Muchow, 1999). It should be noted however, that in these calculations, LAI was used instead of GAI, as no measurements of stem plus sheath green area were available. This would lead to an under-estimation of light interception, and hence and over-estimation of RUE.

In both experimental years, the nitrogen treatment caused differences in RUE, the 160N treatment generally having a higher RUE than the 0N treatment ($P = 0.013$) (Table 4.7). However, no differences were shown between fungicide treatments, demonstrating that the strobilurins did not increase RUE ($P = 0.718$).

Table 4.7 Radiation use efficiency before disease incidence

| Nitrogen | Fungicide | RUE (g/MJ) | |
|---|---|---|---|
| | | 1999 | 2000 |
| 0 | No Strobilurin | 3.44 | 2.21 |
| 0 | Azoxystrobin | 3.69 | 2.31 |
| 0 | Kresoxim-methyl | 3.24 | 2.28 |
| 160 | No Strobilurin | 3.68 | 3.02 |
| 160 | Azoxystrobin | 2.96 | 2.94 |
| 160 | Kresoxim-methyl | 3.61 | 2.88 |

| SED | | |
|---|---|---|
| Year | (4 df) | 0.069 |
| Nitrogen | (20 df) | 0.117 |
| Fungicide | (20 df) | 0.143 |
| Interaction | (20 df) | 0.202 |

RUE was calculated post anthesis in a similar manner, from GS 61 until complete senescence using an extinction coefficient for post ear-emergence Spark (0.47 $k_{PAR}$, J. Whaley personal communication). Calculated RUE was lower than the pre-anthesis values for all treatment combinations. In both experimental years, the nitrogen treatment caused differences in RUE, the 160N treatment generally having a higher RUE than the 0N treatment ($P = 0.004$) (Table 4.8). However, no differences were shown between fungicide treatments, demonstrating that the strobilurins did not increase RUE ($P = 0.785$), although as the data were associated with high standard errors, small differences would be difficult to detect. In this way, despite the strobilurins appearing to have a higher post-anthesis RUE at 160N in 2000, variability in the data resulted in these differences not being statistically significant.

Table 4.8 Radiation use efficiency post-anthesis

| Nitrogen | Fungicide | RUE (g/MJ) | |
|---|---|---|---|
| | | 1999 | 2000 |
| 0 | No Strobilurin | 0.95 | 1.55 |
| 0 | Azoxystrobin | 1.85 | 1.59 |
| 0 | Kresoxim-methyl | 1.30 | 1.45 |
| 160 | No Strobilurin | 2.75 | 1.99 |
| 160 | Azoxystrobin | 1.22 | 2.78 |
| 160 | Kresoxim-methyl | 2.56 | 2.82 |

| SED | | |
|---|---|---|
| Year | (4 df) | 0.491 |
| Nitrogen | (17 df) | 0.270 |
| Fungicide | (17 df) | 0.330 |
| Interaction | (17 df) | 0.467 |

*Yield*

No significant yield advantage was shown for either kresoxim-methyl or azoxystrobin over epoxiconazole. At nitrogen treatment levels of over 160N, the No Strobilurin treatment yielded significantly less than the other fungicide treatments in both seasons (with the exception of the 320N treatment in 2000, for which there were no statistically significant differences).

Curves were fitted to the combine yield data, to determine whether there was a significant interaction between nitrogen level and fungicide. In both years there was a significant effect of both fungicide and nitrogen level and, in 1999, there was also a significant interaction between fungicide and nitrogen (Figures 4.14 and 4.15).

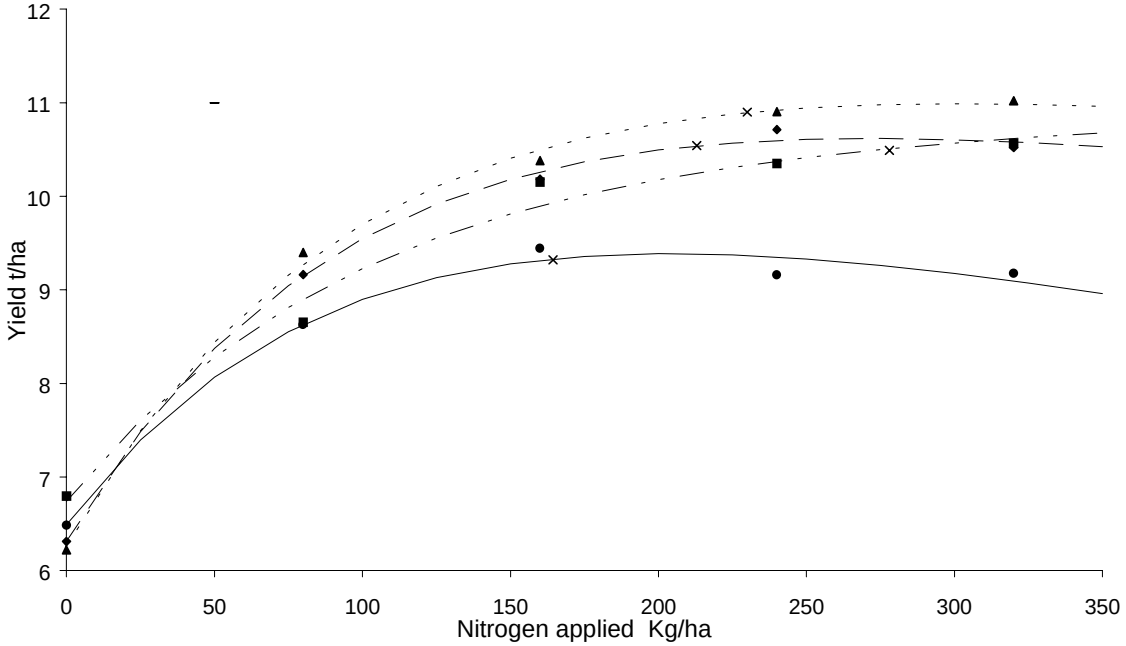


Figure 4.14. Fitted curves for combine yield with increasing N in 1999. X denotes Nitrogen optima. No Strobilurin (—▲—), Azoxystrobin (- -■- -), Kresoxim-methyl (----◆----), Epoxiconazole (—●—). Error bar is s.e.d. with 38 df.

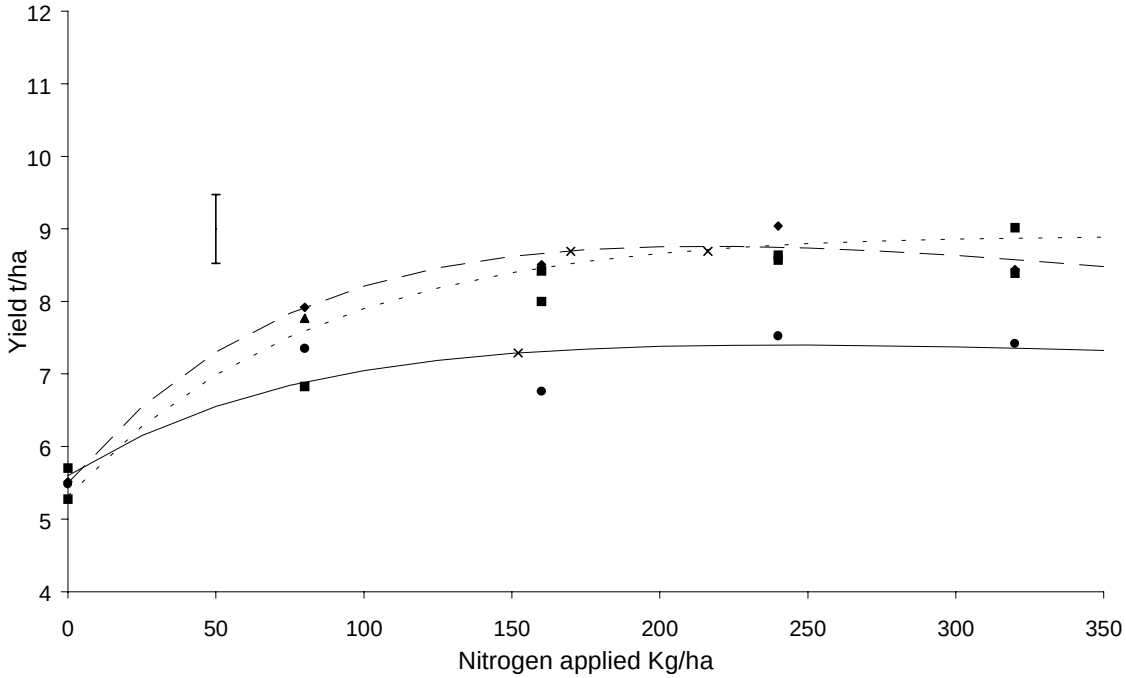


Figure 4.15. Fitted curves for combine yield with increasing N in 2000. X denotes Nitrogen optima. No Strobilurin (—▲—), Azoxystrobin (- -■- -), Kresoxim-methyl (----◆----), Epoxiconazole (—●—). Error bar is s.e.d. with 38 df.

From this analysis it was possible to calculate nitrogen optima. The N optimum (calculated on the basis of a 3:1 ratio) was not significantly increased by the strobilurins, although N optima with strobilurins were consistently higher than those for both the epoxiconazole and the control treatment (Table 4.9). N optima could not be calculated from the kresoxim-methyl

data in 2000, as the slope of the curve remained positive above the highest level of N applied (320 kg/ha).

Table 4.9. N optimum (followed by standard errors in parentheses).

| Fungicide | N optimum (kg/ha) | | |
|---|---|---|---|
| | 1999 | 2000 | Mean |
| No Strobilurin | 164 (19) | 152 (37) | 158 |
| Azoxystrobin | 230 (34) | 216 (56) | 223 |
| Kresoxim-methyl | 278 (107) | * | * |
| Epoxiconazole | 213 (27) | 170 (29) | 191 |
| Mean | 221 | 179 | 200 |

*Yield components*

No statistically significant difference was shown between fungicide treatments at any nitrogen level for ear number per $m^2$, grain number per $m^2$, or grain number per ear.

A statistically significant difference was shown in thousand grain weight at the higher nitrogen levels in both years, but not at 0N, nor 80N (Table 4.10). The level of significance increased with the amount of nitrogen applied. Where significant differences occurred, the No Strobilurin treatment consistently had the lowest mean grain weight and, at the highest two N levels, kresoxim-methyl produced a lower grain weight than either epoxiconazole or azoxystrobin (although this was only significant in 2000).

Table 4.10. Thousand grain weights.

| Nitrogen | Fungicide | Thousand grain weight (g) 1999 | 2000 |
|---|---|---|---|
| 0 | Azoxystrobin | 39.2 | 40.5 |
| | Kresoxim-methyl | 37.1 | 39.0 |
| | Epoxiconazole | 36.7 | 40.4 |
| | No Strobilurin | 37.6 | 39.8 |
| | Mean | 37.6 | 39.9 |
| 80 | Azoxystrobin | 37.5 | 40.2 |
| | Kresoxim-methyl | 38.4 | 39.6 |
| | Epoxiconazole | 37.4 | 40.7 |
| | No Strobilurin | 37.0 | 37.1 |
| | Mean | 37.6 | 39.4 |
| 160 | Azoxystrobin | 36.9 | 40.5 |
| | Kresoxim-methyl | 36.6 | 38.7 |
| | Epoxiconazole | 37.7 | 41.4 |
| | No Strobilurin | 33.8 | 36.0 |
| | Mean | 36.3 | 39.1 |
| 240 | Azoxystrobin | 40.4 | 41.5 |
| | Kresoxim-methyl | 37.2 | 36.3 |
| | Epoxiconazole | 39.6 | 40.6 |
| | No Strobilurin | 34.5 | 33.1 |
| | Mean | 37.9 | 37.9 |
| 320 | Azoxystrobin | 38.7 | 40.2 |
| | Kresoxim-methyl | 37.2 | 34.9 |
| | Epoxiconazole | 38.4 | 39.2 |
| | No Strobilurin | 34.5 | 32.1 |
| | Mean | 37.2 | 36.6 |
| Mean | Azoxystrobin | 38.6 | 40.6 |
| | Kresoxim-methyl | 37.3 | 37.7 |
| | Epoxiconazole | 38.0 | 40.5 |
| | No Strobilurin | 35.5 | 35.6 |
| | Mean | 37.3 | 38.6 |
| SED (38 df) | | | |
| Fungicide | | 0.55 | 0.47 |
| Nitrogen | | 0.62 | 0.53 |
| Interaction | | 1.24 | 1.06 |

Potential mature grain weight was measured in the 0N and 160N treatments in 2000, for No Strobilurin, kresoxim-methyl, and azoxystrobin (Table 4.11). No statistically significant difference was shown between fungicides at either N level. A difference was shown between the nitrogen treatments, with the 0N treated plots having a higher potential grain weight than the 160N.

Table 4.11. Potential mature grain weights (mg).

| Nitrogen | Fungicide | Predicted mature grain weight (mg) |
|---|---|---|
| 0 | Azoxystrobin | 48.3 |
| | Kresoxim-methyl | 48.0 |
| | No Strobilurin | 48.5 |
| | Mean | 48.3 |
| 160 | Azoxystrobin | 44.0 |
| | Kresoxim-methyl | 44.6 |
| | No Strobilurin | 44.0 |
| | Mean | 44.2 |
| Mean | Azoxystrobin | 46.2 |
| | Kresoxim-methyl | 46.4 |
| | No Strobilurin | 46.3 |
| | Overall Mean | 46.3 |
| SED (10 df) | | |
| Fungicide | | 0.82 |
| Nitrogen | | 0.67 |
| Interaction | | 1.15 |

In 1999, results for harvest index (taken to be the ratio of grain dry weight to total above-ground dry weight at harvest) were associated with high standard errors, as such, no statistically significant differences were evident between fungicide treatments ($P = 0.295$) (Table 4.12). In 2000, however, statistically significant differences were shown between fungicide treatments ($P < 0.001$), with the No Strobilurin treatment showing a lower harvest index than the other treatments. An effect of N treatment was observed in 1999 ($P = 0.002$), as harvest index was shown to increase with higher levels of N. No difference between N level was shown in 2000 ($P = 0.323$).

Table 4.13 Harvest Index

| Nitrogen | Fungicide | Harvest index 1999 | Harvest index 2000 |
|---|---|---|---|
| 0 | Azoxystrobin | 0.45 | 0.38 |
| | Kresoxim-methyl | 0.45 | 0.39 |
| | Epoxiconazole | 0.40 | 0.40 |
| | No Strobilurin | 0.43 | 0.38 |
| | Mean | 0.43 | 0.39 |
| 80 | Azoxystrobin | 0.48 | 0.38 |
| | Kresoxim-methyl | 0.48 | 0.39 |
| | Epoxiconazole | 0.48 | 0.39 |
| | No Strobilurin | 0.45 | 0.37 |
| | Mean | 0.47 | 0.38 |
| 160 | Azoxystrobin | 0.50 | 0.40 |
| | Kresoxim-methyl | 0.51 | 0.39 |
| | Epoxiconazole | 0.45 | 0.44 |
| | No Strobilurin | 0.47 | 0.38 |
| | Mean | 0.48 | 0.40 |
| 240 | Azoxystrobin | 0.49 | 0.40 |
| | Kresoxim-methyl | 0.50 | 0.41 |
| | Epoxiconazole | 0.58 | 0.40 |
| | No Strobilurin | 0.48 | 0.35 |
| | Mean | 0.51 | 0.39 |
| 320 | Azoxystrobin | 0.57 | 0.41 |
| | Kresoxim-methyl | 0.50 | 0.40 |
| | Epoxiconazole | 0.51 | 0.41 |
| | No Strobilurin | 0.48 | 0.36 |
| | Mean | 0.52 | 0.39 |
| Mean | Azoxystrobin | 0.50 | 0.39 |
| | Kresoxim-methyl | 0.49 | 0.40 |
| | Epoxiconazole | 0.49 | 0.41 |
| | No Strobilurin | 0.46 | 0.37 |
| | Mean | 0.48 | 0.39 |
| SED (38 df) | | | |
| Fungicide | | 0.019 | 0.007 |
| Nitrogen | | 0.021 | 0.008 |
| Interaction | | 0.043 | 0.017 |

**Discussion**

Strobilurins have been hypothesised here to affect the green area of treated crops through either a) increased N uptake, and hence leaf expansion, resulting in a larger canopy, b) increased photosynthesis, or c) delayed senescence.

Despite efforts to keep the crop disease-free (use of a resistant cultivar and base-line fungicides at a low disease site), the crop was affected to some extent by *Septoria tritici* in both experimental years and, therefore, physiological effects of the strobilurin fungicides were difficult to test once the disease had reached significant levels.

Analysis of the crop growth before disease (up to GS 55-59) showed little effect of fungicide treatment. There was no increase in nitrogen uptake observed at either N application level monitored. This would indicate that, if disease had been successfully prevented, any effects of delayed senescence for the strobilurin treated plots would not have been due to an increase in early nitrogen uptake.

In addition, there was no difference between strobilurins and epoxiconazole in maximum GAI. It would appear, therefore, that the strobilurins had no effect on N uptake and canopy expansion. There was also no evidence of any effect on radiation use efficiency either pre-anthesis or post-anthesis. Differences between treatments in the rate of senescence appeared to be related to the level of disease in each treatment. Since no increase in nitrogen uptake was observed associated with the strobilurins, it is not possible to determine whether there were any physiological effects on senescence.

No increases were observed in any yield component due to strobilurins over epoxiconazole and, as a result, there was no yield advantage of either strobilurin over epoxiconazole in either experimental year. However, even if disease had been more effectively controlled, it would be difficult to verify that the 'untreated' control plots in a field experiment are absolutely disease-free. For example, it has recently been shown that azoxystrobin may also control the root pathogen causing take-all (Jenkyn *et al*., 2000). Consequently, even if there are no observed effects on foliar pathogens, this does not necessarily indicate that any observed yield effects are due to physiological activity. In this way, physiological effects on both yield components and on senescence are difficult to separate from disease-effects in field experiments.

## APPENDIX 5

### Experiment 5: Dose-response for azole fungicides in mixture with strobilurins

### Introduction

Previous work funded by the HGCA had shown that the strobilurin fungicide azoxystrobin, when used alone, did not give satisfactory control of *Septoria tritici* at sites with severe disease (Jones, 2000; Jones & Bryson, 1998). This related to the lack of eradicant activity of azoxystrobin, in contrast with triazole fungicides which showed eradicant activity against this pathogen. It was clear that, in commercial practice, strobilurins would need to be used in mixture with triazole fungicides to give acceptable control of *S. tritici*. This experiment was designed to evaluate the relative efficacies of triazole fungicides in mixture with azoxystrobin.

### Methods

Experiments were established at ADAS Rosemaund, Herefordshire, in each of 1997/98, 1998/99 and 1999/2000, on a stoneless silty clay loam of the Bromyard series. The previous crops were oilseed rape, beans and oilseed rape respectively. In each experiment, azoxystrobin was evaluated alone, and in mixture with each of a series of triazole fungicides at various doses. Trifloxystrobin was also evaluated in mixture with epoxiconazole in 1999/2000. Owing to constraints on the size of the experiments, it was not possible to evaluate more than one dose of each strobilurin fungicide, and the dose selected for each strobilurin was half of the commercially recommended rate, with triazoles at one quarter, half and full rate. One eighth rate was also included in 1999/2000 to increase the precision of dose-response estimation. Treatments are detailed in Tables 5.1 - 5.3, and fungicide active ingredients are given in Table 5.4. Fungicides were applied in 225 litre/ha water, using an MDM Oxford Precision Sprayer fitted with 03-F110 nozzles. The experiments were on cv. Riband in the first two years, and on cv. Consort in 1999/2000; both cultivars are susceptible to the target pathogen, *Septoria tritici* (Anonymous, 1999). The experimental design was a randomised block with three replicates of each treatment except for Nos 1 and 2, and also No. 3 in 1999/2000, which were replicated six times. Plot sizes were in the range 24-48 $m^2$.

Each treatment was applied on two occasions, at GS 31-32 and GS 39 (Tottman & Broad, 1987). Application dates and growth stages are listed in Table 5.5. Other agronomic inputs followed good commercial practice.

Foliar diseases were assessed, as visual estimates of the percentage leaf area infected by each disease on each leaf layer, on 10 tillers taken at random from each plot approximately 21 and 35 days after the date of the second fungicide application. Percentage green leaf area was also estimated.

Grain yield was measured from each whole plot using a plot combine harvester. Grain was analysed for moisture content and specific weight using GAC 2000 grain analysis computer (Dickey-John Corporation).

For each experiment, fitted curves were calculated for the dose-response for disease on each leaf and for yield, using the exponential function $y = a + be^k$ (Paveley, 2000). An example is shown in Figure 5.1. Within this model, parameter a is the lower asymptote which represents the lowest level of disease achievable, and is a measure of the efficacy of the fungicide. Parameter b is the difference between the untreated AUDPC and the lower asymptote, which

represents the range of disease control which could potentially be achieved by the fungicide. Parameter k is a measure of the curvature of the line.

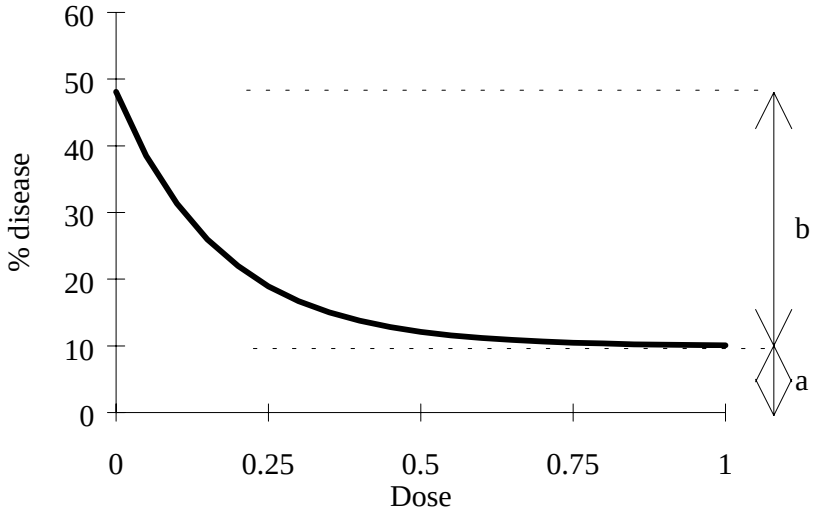


Figure 5.1. Example dose - response curve, showing parameters a and b.

In the figures below, actual data points are shown, and the solid line is the fitted curve.

Table 5.1. Treatments in 1997/98

| Treat-ment | Active ingredient | Product | Application rate of product/ha |
|---|---|---|---|
| 1 | --- | Untreated | --- |
| 2 | Azoxystrobin | Amistar | 0.50 litre |
| 3 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 0.25 litre |
| 4 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 0.50 litre |
| 5 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 1.00 litre |
| 6 | Azoxystrobin + tebuconazole | Amistar + Folicur | 0.50 + 0.25 litre |
| 7 | Azoxystrobin + tebuconazole | Amistar + Folicur | 0.50 + 0.50 litre |
| 8 | Azoxystrobin + tebuconazole | Amistar + Folicur | 0.50 + 1.00 litre |
| 9 | Azoxystrobin + cyproconazole | Amistar + Alto | 0.50 + 0.20 litre |
| 10 | Azoxystrobin + cyproconazole | Amistar + Alto | 0.50 + 0.40 litre |
| 11 | Azoxystrobin + cyproconazole | Amistar + Alto | 0.50 + 0.80 litre |
| 12 | Azoxystrobin + flusilazole | Amistar + Sanction | 0.50 + 0.10 litre |
| 13 | Azoxystrobin + flusilazole | Amistar + Sanction | 0.50 + 0.20 litre |
| 14 | Azoxystrobin + flusilazole | Amistar + Sanction | 0.50 + 0.40 litre |
| 15 | Azoxystrobin + propiconazole | Amistar + Tilt | 0.50 + 0.125 litre |
| 16 | Azoxystrobin + propiconazole | Amistar + Tilt | 0.50 + 0.250 litre |
| 17 | Azoxystrobin + propiconazole | Amistar + Tilt | 0.50 + 0.50 litre |
| 18 | Azoxystrobin + flutriafol | Amistar + Pointer | 0.50 + 0.25 litre |
| 19 | Azoxystrobin + flutriafol | Amistar + Pointer | 0.50 + 0.50 litre |
| 20 | Azoxystrobin + flutriafol | Amistar + Pointer | 0.50 + 1.00 litre |

Table 5.2. Treatments in 1998/99

| Treat-ment | Active ingredient | Product | Application rate of product/ha |
|---|---|---|---|
| 1 | --- | Untreated | --- |
| 2 | Azoxystrobin | Amistar | 0.50 litre |
| 3 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 0.25 litre |
| 4 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 0.50 litre |
| 5 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 1.00 litre |
| 6 | Azoxystrobin + flusilazole | Amistar + Sanction | 0.50 + 0.10 litre |
| 7 | Azoxystrobin + flusilazole | Amistar + Sanction | 0.50 + 0.20 litre |
| 8 | Azoxystrobin + flusilazole | Amistar + Sanction | 0.50 + 0.40 litre |
| 9 | Azoxystrobin + fluquinconazole | Amistar + Flamenco | 0.50 + 0.313 litre |
| 10 | Azoxystrobin + fluquinconazole | Amistar + Flamenco | 0.50 + 0.625 litre |
| 11 | Azoxystrobin + fluquinconazole | Amistar + Flamenco | 0.50 + 1.25 litre |
| 12 | Azoxystrobin + tetraconazole | Amistar + Eminent | 0.50 + 0.25 litre |
| 13 | Azoxystrobin + tetraconazole | Amistar + Eminent | 0.50 + 0.50 litre |
| 14 | Azoxystrobin + tetraconazole | Amistar + Eminent | 0.50 + 1.00 litre |
| 15 | Azoxystrobin + metconazole | Amistar + Caramba | 0.50 + 0.375 litre |
| 16 | Azoxystrobin + metconazole | Amistar + Caramba | 0.50 + 0.75 litre |
| 17 | Azoxystrobin + metconazole | Amistar + Caramba | 0.50 + 1.50 litre |

Table 5.3. Treatments in 1999/2000

| Treat-ment | Active ingredient | Product | Application rate of product/ha |
|---|---|---|---|
| 1 | --- | Untreated | --- |
| 2 | Azoxystrobin | Amistar | 0.50 litre |
| 3 | Trifloxystrobin | Twist | 1.00 litre |
| 4 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 0.125 litre |
| 5 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 0.25 litre |
| 6 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 0.50 litre |
| 7 | Azoxystrobin + epoxiconazole | Amistar + Opus | 0.50 + 1.00 litre |
| 8 | Azoxystrobin + fluquinconazole | Amistar + Flamenco | 0.50 + 0.156 litre |
| 9 | Azoxystrobin + fluquinconazole | Amistar + Flamenco | 0.50 + 0.313 litre |
| 10 | Azoxystrobin + fluquinconazole | Amistar + Flamenco | 0.50 + 0.625 litre |
| 11 | Azoxystrobin + fluquinconazole | Amistar + Flamenco | 0.50 + 1.25 litre |
| 12 | Azoxystrobin + tetraconazole | Amistar + Eminent | 0.50 + 0.125 litre |
| 13 | Azoxystrobin + tetraconazole | Amistar + Eminent | 0.50 + 0.25 litre |
| 14 | Azoxystrobin + tetraconazole | Amistar + Eminent | 0.50 + 0.50 litre |
| 15 | Azoxystrobin + tetraconazole | Amistar + Eminent | 0.50 + 1.00 litre |
| 16 | Azoxystrobin + metconazole | Amistar + Caramba | 0.50 + 0.188 litre |
| 17 | Azoxystrobin + metconazole | Amistar + Caramba | 0.50 + 0.375 litre |
| 18 | Azoxystrobin + metconazole | Amistar + Caramba | 0.50 + 0.75 litre |
| 19 | Azoxystrobin + metconazole | Amistar + Caramba | 0.50 + 1.50 litre |
| 20 | Trifloxystrobin + epoxiconazole | Amistar + Opus | 1.00 + 0.125 litre |
| 21 | Trifloxystrobin + epoxiconazole | Amistar + Opus | 1.00 + 0.25 litre |
| 22 | Trifloxystrobin + epoxiconazole | Amistar + Opus | 1.00 + 0.50 litre |
| 23 | Trifloxystrobin + epoxiconazole | Amistar + Opus | 1.00 + 1.00 litre |

Table 5.4. Fungicides

| Active ingredient | Product | Rate of a.i. in product | Full recommended rate of product |
|---|---|---|---|
| Azoxystrobin | Amistar | 250 g/l | 1.0 l/ha |
| Epoxiconazole | Opus | 125 g/l | 1.0 l/ha |
| Fluquinconazole | Flamenco | 100 g/l | 1.25 l/ha |
| Flusilazole | Sanction | 400 g/l | 0.4 l/ha |
| Flutriafol | Pointer | 125 g/l | 1.0 l/ha |
| Metconazole | Caramba | 60 g/l | 1.5 l/ha |
| Propiconazole | Tilt | 250 g/l | 0.5 l/ha |
| Tebuconazole | Folicur | 250 g/l | 1.0 l/ha |
| Tetraconazole | Eminent | 125 g/l | 1.0 l/ha |
| Trifloxystrobin | Twist | 125 g/l | 2.0 l/ha |

**Results**

Dose-response curves, together with actual data points, are shown in Figures 5.2 - 5.8, and dose-response parameters are listed in Tables 5.5 and 5.6. The disease data shown are for those dates and leaf layers which provided curves with the best fit. In two instances, a curve could not be fitted to the data points for yield.

*Septoria tritici* was severe in the 1998 and 1999 experiments, and moderately severe in 2000. The only other diseases recorded were traces of mildew and brown rust in 1998, both at less than 1% leaf area affected.

In 1998, there was little difference in disease control between azole fungicides at full rate. All gave marked reductions compared with azoxystrobin alone, even though azoxystrobin reduced disease severity by 38% and 30% on leaves 1 and 2 respectively (Figures 5.2 and 5.3). Differences between azoles became clearer at lower doses. On each of the top two leaves, epoxiconazole at half or quarter rate had less than 5% additional disease compared with the full rate treatment, whereas other fungicides showed a marked reduction in efficacy at quarter rate and, in the cases of flusilazole and propiconazole, at half rate. There was a greater effect of reducing fungicide rate on yield than on disease (Figure 5.4). Azoxystrobin alone increased yield by 1.86 t/ha, and there were substantial additional increases from mixture with each of the azole fungicides. The fitted curves show that, for epoxiconazole, tebuconazole and cyproconazole, the curves were approaching the upper asymptote at full rate, whereas other curves, particularly propiconazole, were still rising at full rate. There was a greater difference between epoxiconazole and other azoles in yield than in disease control, with a higher yield from quarter rate epoxiconazole than from full rate of any other fungicide.

In 1999, *S. tritici* was very severe in completely untreated plots, but azoxystrobin alone gave a greater reduction than in 1998 (Figure 5.5). Epoxiconazole was the most effective fungicide for control of *S. tritici*, but fluquinconazole was almost as effective. The yield increase from azoxystrobin alone was 2.81 t/ha, larger than in 1998. Yield increases from addition of an azole fungicide were lower than in 1998 but, as in 1998, the difference in yield between epoxiconazole and other azoles was more marked than difference in disease control (Figure 5.6). The fitted curve shows that the yield from full rate epoxiconazole was well below the upper asymptote. Fluquinconazole gave the next highest yields, but there was little benefit in yield from the other three azoles compared with azoxystrobin alone.

All fungicide gave good *S. tritici* control in 2000, but the fitted curve showed that epoxiconazole was slightly more effective than the other azoles (Figure 5.7). Trifloxystrobin alone was more effective than azoxystrobin alone but, in mixture with epoxiconazole, there was no difference between these two strobilurins. As in the previous two years, epoxiconazole plus azoxystrobin gave higher yields than any other treatment. Trifloxystrobin alone gave a higher yield increase (2.89 t/ha) than azoxystrobin alone (2.65 t/ha), but this advantage was not apparent in mixture with epoxiconazole (Figure 5.8). It should, however, be noted that the curve for trifloxystrobin plus epoxiconazole was a poor fit (45.3% of variance accounted for), so results from this mixture should be interpreted with caution.

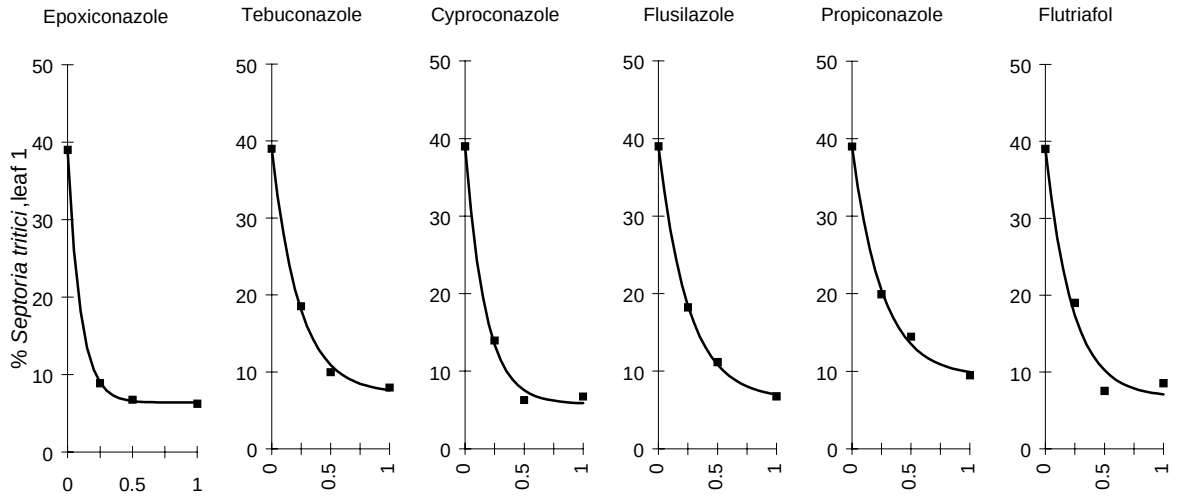


Figure 5.2. Dose-response curves for disease control on leaf 1 from triazole fungicides in mixture with 0.5 l/ha Amistar, two-spray programme (GS 31/32 + GS 39), cv. Riband, 1998. Untreated disease severity 77.7%.

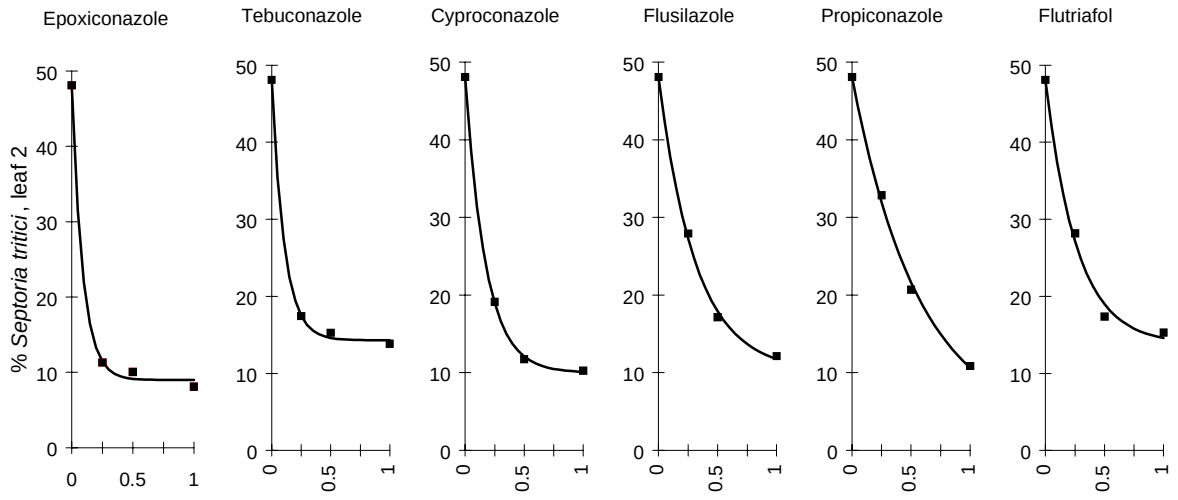


Figure 5.3. Dose-response curves for disease control on leaf 2 from triazole fungicides in mixture with azoxystrobin, two-spray programme (GS 31/32 + GS 39), cv. Riband, 1998. Untreated disease severity 79.0%.

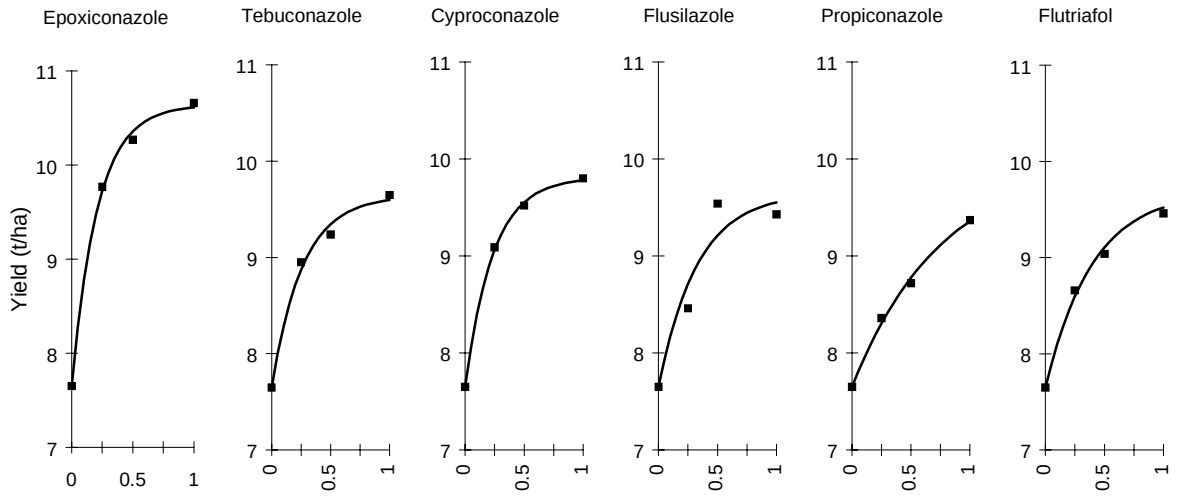


Figure 5.4. Dose-response curves for effect on yield of triazole fungicides in mixture with azoxystrobin, two-spray programme (GS 31/32 + GS 39), cv. Riband, 1998. Untreated yield 5.79 t/ha.

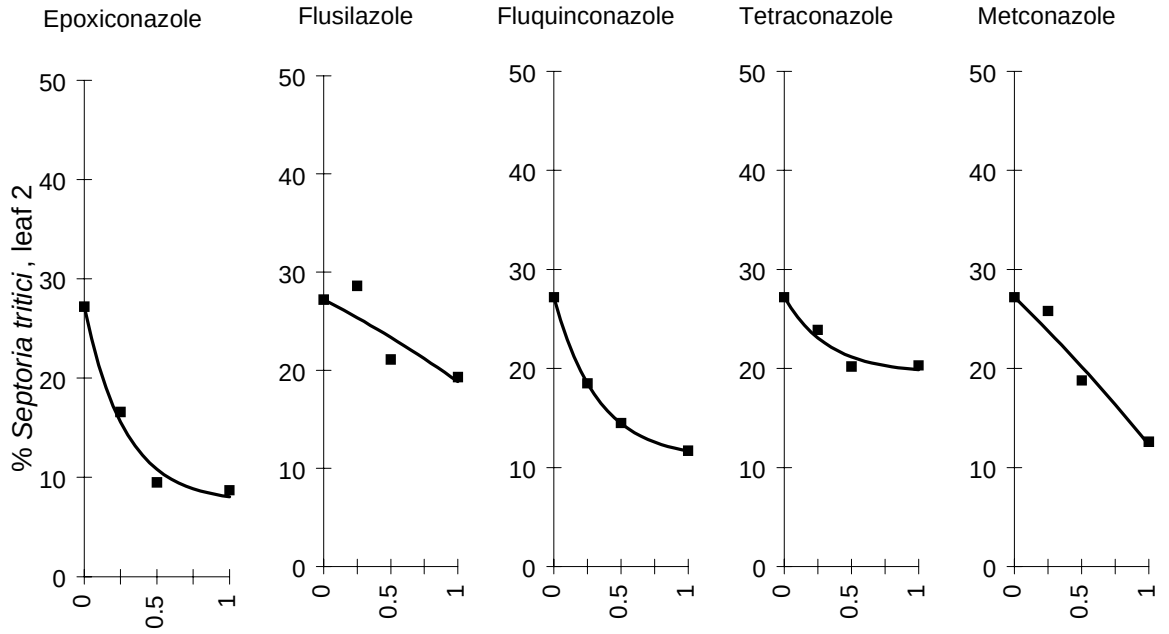


Figure 5.5. Dose-response curves for disease control on leaf 2 from triazole fungicides in mixture with azoxystrobin, two-spray programme (GS 31/32 + GS 39), cv. Riband, 1999. Untreated disease severity 100.0%.

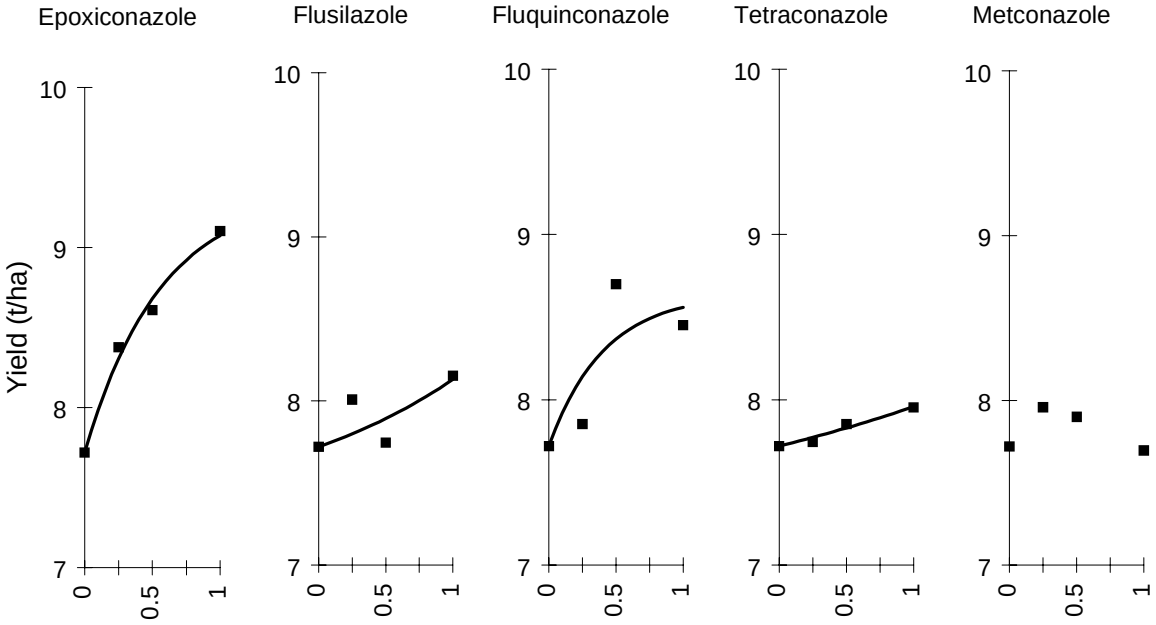


Figure 5.6. Dose-response curves for effect on yield of triazole fungicides in mixture with azoxystrobin, two-spray programme (GS 31/32 + GS 39), cv. Riband, 1999. Untreated yield 4.93 t/ha.

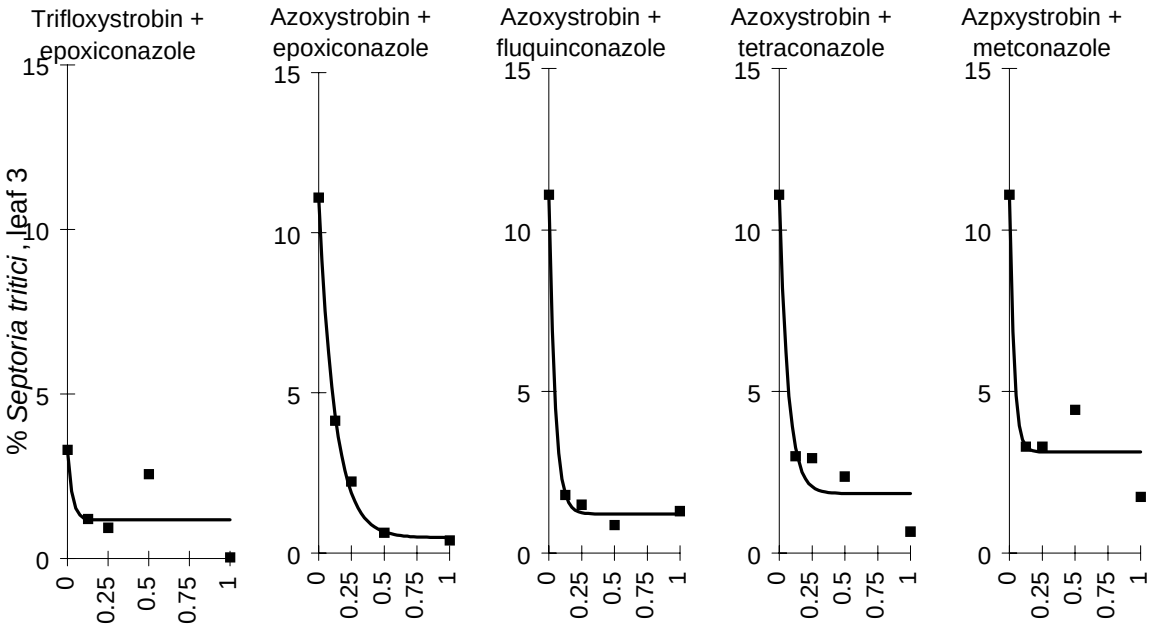


Figure 5.7. Dose-response curves for disease control on leaf 3 from triazole fungicides in mixture with trifloxystrobin or azoxystrobin, two-spray programme (GS 31/32 + GS 39), cv. Consort, 2000. Untreated disease severity 25.8%.

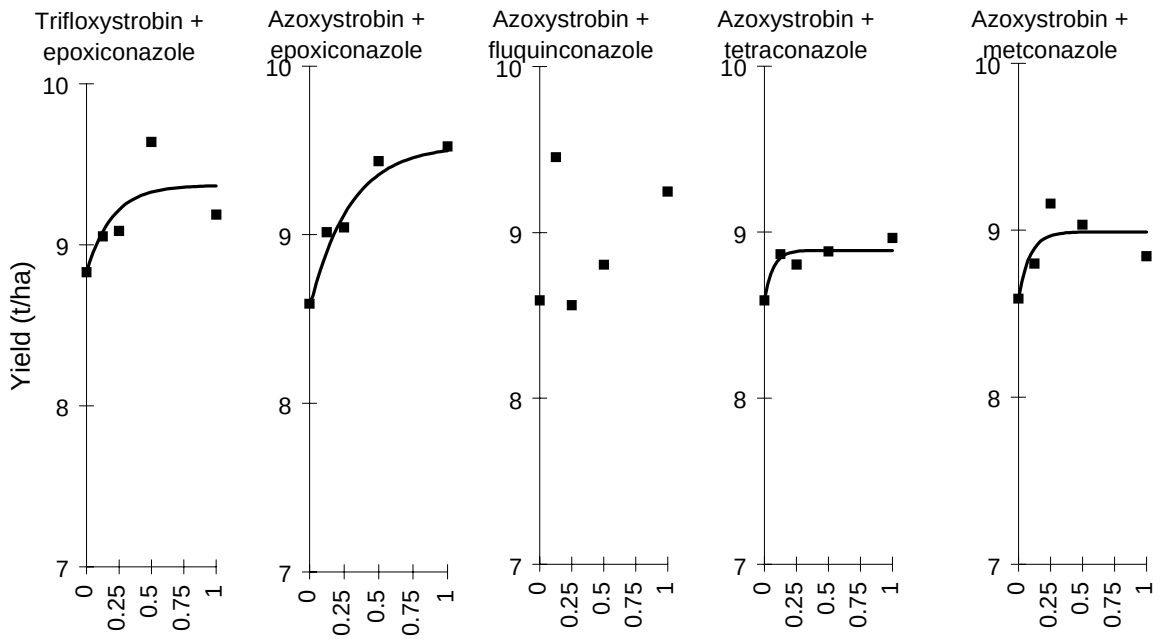


Figure 5.8. Dose-response curves for yield from triazole fungicides in mixture with trifloxystrobin or azoxystrobin, two-spray programme (GS 31/32 + GS 39), cv. Consort, 2000. Untreated yield 5.94 t/ha.

Table 5.5. Parameter estimates - disease control

| Year | Active ingredients* | Leaf | a | b | a+b | k | $R^2$ |
|---|---|---|---|---|---|---|---|
| 1998 | az + epoxiconazole | 1 | 6.4 | 32.6 | 39.0 | -10.20 | 100.0 |
| 1998 | az + tebuconazole | 1 | 7.2 | 31.8 | 39.0 | -4.30 | 99.7 |
| 1998 | az + cyproconazole | 1 | 5.8 | 33.2 | 39.0 | -5.88 | 99.5 |
| 1998 | az + flusilazole | 1 | 6.4 | 32.6 | 39.0 | -3.98 | 99.9 |
| 1998 | az + propiconazole | 1 | 9.3 | 29.7 | 39.0 | -3.90 | 99.6 |
| 1998 | az + flutriafol | 1 | 6.7 | 32.3 | 39.0 | -4.43 | 97.2 |
| 1998 | az + epoxiconazole | 2 | 9.0 | 39.1 | 48.1 | -11.07 | 99.8 |
| 1998 | az + tebuconazole | 2 | 14.3 | 33.8 | 48.1 | -9.45 | 99.9 |
| 1998 | az + cyproconazole | 2 | 10.0 | 38.1 | 48.1 | -5.79 | 100.0 |
| 1998 | az + flusilazole | 2 | 10.2 | 37.9 | 48.1 | -3.18 | 99.8 |
| 1998 | az + propiconazole | 2 | 2.9 | 45.2 | 48.1 | -1.76 | 99.7 |
| 1998 | az + flutriafol | 2 | 13.8 | 34.3 | 48.1 | -3.78 | 99.1 |
| 1999 | az + epoxiconazole | 2 | 7.5 | 19.7 | 27.2 | -3.56 | 97.8 |
| 1999 | az + flusilazole | 2 | 50.2 | -23.0 | 27.2 | 0.31 | 61.7 |
| 1999 | az + fluquinconazole | 2 | 10.9 | 16.3 | 27.2 | -3.05 | 100.0 |
| 1999 | az + tetraconazole | 2 | 19.5 | 7.7 | 27.2 | -3.05 | 92.0 |
| 1999 | az + metconazole | 2 | 82.2 | -55.0 | 27.2 | 0.24 | 93.4 |
| 2000 | tr + epoxiconazole | 3 | 1.2 | 2.1 | 3.3 | -35.66 | 34.5 |
| 2000 | az + epoxiconazole | 3 | 0.5 | 10.6 | 11.1 | -8.12 | 99.2 |
| 2000 | az + fluquinconazole | 3 | 1.2 | 9.9 | 11.1 | -22.14 | 99.7 |
| 2000 | az + tetraconazole | 3 | 1.8 | 9.3 | 11.1 | -14.99 | 95.2 |
| 2000 | az + metconazole | 3 | 3.1 | 8.0 | 11.1 | -30.40 | 90.9 |

* az = azoxystrobin
tr = trifloxystrobin

Table 5.6. Parameter estimates - yield

| Year | Active ingredients* | a | b | a+b | k | $R^2$ |
|---|---|---|---|---|---|---|
| 1998 | az + epoxiconazole | 10.6 | -3.0 | 7.6 | -4.72 | 99.7 |
| 1998 | az + tebuconazole | 9.6 | -2.0 | 7.6 | -3.82 | 98.7 |
| 1998 | az + cyproconazole | 9.8 | -2.2 | 7.6 | -4.26 | 99.9 |
| 1998 | az + flusilazole | 9.6 | -2.0 | 7.6 | -3.03 | 88.2 |
| 1998 | az + propiconazole | 10.0 | -2.4 | 7.6 | -1.31 | 99.4 |
| 1998 | az + flutriafol | 9.7 | -2.1 | 7.6 | -2.50 | 99.3 |
| 1999 | az + epoxiconazole | 9.4 | -1.6 | 7.7 | -1.78 | 98.3 |
| 1999 | az + flusilazole | 7.3 | 0.4 | 7.7 | 0.66 | 24.5 |
| 1999 | az + fluquinconazole | 8.6 | -0.9 | 7.7 | -2.43 | 53.4 |
| 1999 | az + tetraconazole | 7.1 | 0.6 | 7.7 | 0.32 | 93.3 |
| 1999 | az + metconazole | * | * | * | * | * |
| 2000 | tr + epoxiconazole | 9.4 | -0.5 | 8.8 | -5.04 | 45.3 |
| 2000 | az + epoxiconazole | 9.5 | -1.0 | 8.6 | -3.43 | 94.5 |
| 2000 | az + fluquinconazole | * | * | * | * | * |
| 2000 | az + tetraconazole | 8.9 | -0.3 | 8.6 | -15.00 | 81.4 |
| 2000 | az + metconazole | 9.0 | -0.4 | 8.6 | -11.27 | 53.3 |

* az = azoxystrobin
tr = trifloxystrobin

**Discussion**

Dose-response curves for azoles in mixture with a strobilurin were generally similar to those for azoles when used alone (Paveley, 2000). This indicates that the basic properties of azole fungicides, which have been elucidated and exploited over many years, are not altered when in mixture with a strobilurin fungicide. However, values of the parameter k, were higher in the present work, which indicates greater efficacy of fungicides. This is not surprising, since two fungicide applications were made, compared with one in the work of Paveley (2000), and mixtures of two fungicides were used, rather than a single fungicide. Another difference between the two studies is that the use of a two spray programme in the present study, which was done to demonstrate the benefits that could be obtained in commercial practice, precludes differentiation of protectant and eradicant activity of the fungicides.

The relative performance of azole fungicides was similar in mixture with azoxystrobin to the ranking when used alone (Paveley, 2000). Epoxiconazole was clearly the most effective azole against *S. tritici* in the earlier HGCA-funded work on appropriate fungicide doses, and this superiority was evident in mixture with azoxystrobin, even though the strobilurin fungicide made a substantial contribution to both disease control and yield. Under the conditions of most severe disease and largest yield response to fungicide, in 1998, epoxiconazole gave a greater yield when used at one quarter of the recommended rate than any other azole fungicide at full rate. The azole which came closest to epoxiconazole in disease control and effect on yield was fluquinconazole, but this was always second best. It can be concluded that, under high risk of *S. tritici*, epoxiconazole is likely to remain the most effective azole fungicide even when used in mixture with a robust dose of a strobilurin.

## APPENDIX 6

### Experiment 6. Synergy or antagonism between azoxystrobin and epoxiconazole

### Introduction

The strong eradicant and protectant activity of triazoles against several pathogens, notably Septoria tritici, offers flexibility in application timing. Leaves are often colonised by this pathogen as they emerge, and a triazole applied later will arrest development of this latent infection. In contrast, a strobilurin fungicide , when used alone, has little eradicant activity. For example, at ADAS Rosemaund in 1997, Amistar (azoxystrobin) was applied as an experimental treatment to cv. Consort at full rate (1.0 l/ha) at GS 30, GS 31, GS 32 and GS 39. Disease control was poorer than that from a standard two-spray full dose Opus (epoxiconazole) at GS 32 + GS 39 programme (Jones & Bryson, 1998). Clearly, under high disease risk, Amistar needs to be used in mixture with a triazole to ensure satisfactory disease control and yield protection.

If mixture with a triazole is needed, this raises the questions of whether any other benefits from Amistar are compromised in such a mixture. If there could be antagonistic effects between azoxystrobin and a triazole fungicide, it is important to be able to quantify any such effects, so that the benefits of a mixture in terms of disease control could be weighed against the loss of any physiological benefits.

### Methods

Experiments were established at ADAS Rosemaund, Herefordshire, in each of 1997/98, 1998/99 and 1999/2000, on a stoneless silty clay loam of the Bromyard series. The previous crops were oilseed rape, beans and oats respectively. In each experiment, azoxystrobin (as Amistar, 250 g a.i./litre) and epoxiconazole (as Opus; 125 g a.i./litre) were each evaluated alone at a series of rates, and in mixtures with various proportions of the two fungicides. Treatments are detailed in Table 6.1. Fungicides were applied in 225 litre/ha water, using an MDM Oxford Precision Sprayer fitted with 03-F110 nozzles. The experiments were on cv. Riband in the first two years, and on cv. Consort in 1999/2000; both cultivars are susceptible to the target pathogen, *Septoria tritici* (Anonymous, 1999). The experimental design was a randomised block with three replicates of each treatment. Plot sizes were in the range 36-48 $m^2$.

Each treatment was applied on two occasions, at GS 31-32 and GS 39 (Tottman & Broad, 1987). Other agronomic inputs followed good commercial practice.

Table 6.1. Fungicide treatments

| | Rate of azoxystrobin (litre product/ha) | Rate of epoxiconazole (litre product/ha) |
|---|---|---|
| 1 | 0.00 | 0.00 |
| 2 | 0.00 | 0.00 |
| 3 | 0.00 | 0.25 |
| 4 | 0.00 | 0.375 |
| 5 | 0.00 | 0.50 |
| 6 | 0.00 | 0.75 |
| 7 | 0.00 | 1.00 |
| 8* | 0.00 | 2.00 |
| 9 | 0.25 | 0.00 |
| 10 | 0.375 | 0.00 |
| 11 | 0.50 | 0.00 |
| 12 | 0.75 | 0.00 |
| 13 | 1.00 | 0.00 |
| 14* | 2.00 | 0.00 |
| 15 | 0.25 | 0.25 |
| 16* | 0.375 | 0.375 |
| 17 | 0.50 | 0.50 |
| 18 | 0.75 | 0.75 |
| 19 | 1.00 | 1.00 |
| 20 | 0.50 | 0.25 |
| 21 | 0.75 | 0.375 |
| 22 | 1.00 | 0.50 |
| 23* | 2.00 | 1.00 |
| 24 | 0.25 | 0.50 |
| 25 | 0.375 | 0.75 |
| 26 | 0.50 | 1.00 |
| 27* | 1.00 | 2.00 |

* Treatment included in 1999/2000 only

Foliar diseases were assessed, as visual estimates of the percentage leaf area infected by each disease on each leaf layer, on 10 tillers taken at random from each plot approximately 21 and 35 days after the date of the second fungicide application. Percentage green leaf area was also estimated.

Grain yield was measured from each whole plot using a plot combine harvester. Grain was analysed for moisture content and specific weight using GAC 2000 grain analysis computer (Dickey-John Corporation).

The additive dose model was used to test for synergy or antagonism (Streibig and Kudsk, 1993). For each experiment, fitted curves were calculated for the dose-response for each product alone, and for mixtures with ratios of 1:2, 1:1 and 2:1 of the two fungicides, for disease and for yield, using the exponential function $y = a + be^k$ (Paveley, 2000). An example is shown in Figure 6.1. Within this model, parameter a is the lower asymptote which represents the lowest level of disease achievable, and is a measure of the efficacy of the fungicide. Parameter b is the difference between the untreated AUDPC and the lower asymptote, which represents the range of disease control which could potentially be achieved by the fungicide. Parameter k is a measure of the curvature of the line.

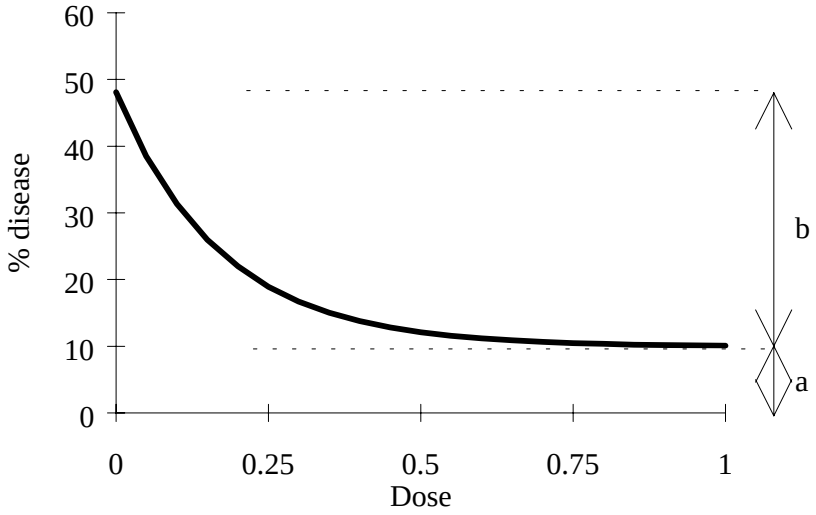


Figure 6.1. Example dose - response curve, showing parameters a and b.

In the figures below, actual data points are shown, and the solid line is the fitted curve.

From the calculated the dose-response curves, a particular level of disease control or yield enhancement on the 'shoulder' of the dose-response curves was selected. From this, the amount of each product alone, or of each ratio of the two fungicides, required to achieve the selected level of yield enhancement was determined. These were plotted such that, if there were no positive or negative interactions between the fungicides, there should be a straight line relationship between the amounts of the various mixtures required to give this level of performance. If the points for a mixture lie below the line, this is an indication of synergy, i.e. less fungicide is needed to achieve a given level of performance in a mixture than when using each fungicide alone. If the points for mixtures lie above the line, this is an indication of antagonism.

**Results**

*Septoria tritici* was severe in the 1997/98 experiment, and moderately severe in the later two years. The only other diseases recorded were traces of mildew and brown rust in 1997/98, both at less than 1% leaf area affected.

In 1997/98, dose-response curves of mixtures, for disease and for yield, were similar to those for epoxiconazole alone, but the curves for azoxystrobin alone had lower k values and did not appear to be approaching the asymptote at the highest dose tested (Figures 6.2 and 6.3). In 1998/99 and in 1999/2000, with less severe disease, curves for the two single active ingredients and for mixtures were broadly similar (Figures 6.4 - 6.7).

The tests for synergy or antagonism showed that, in 1997/98, the mixtures gave better disease control and higher yields than would be predicted from the performance of the single products (Figures 6.8 - 6.10). In 1998/99 and 1999/2000, results were more variable, but there was no indication of the consistent pattern seen in 1997/98.

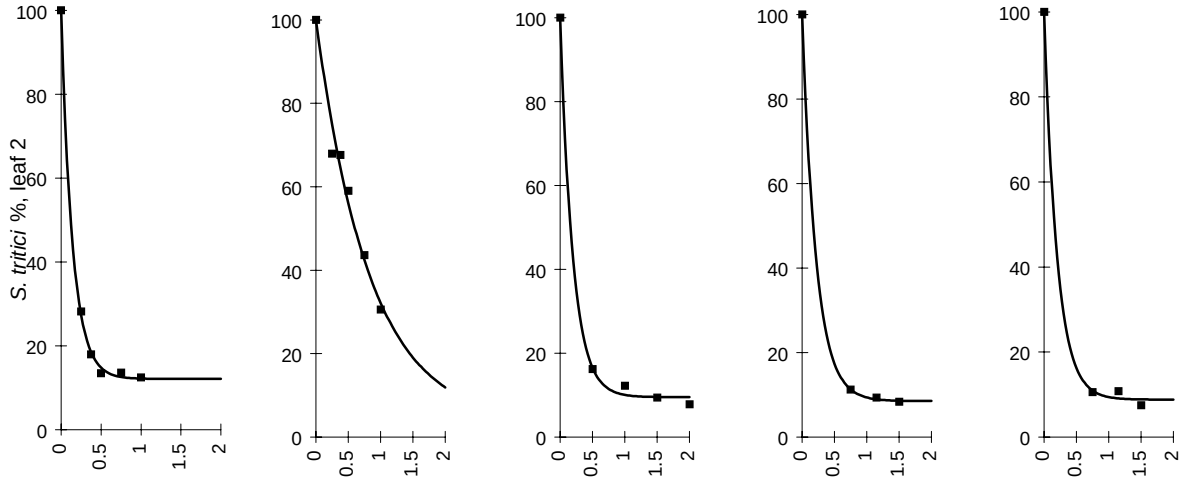


Figure 6.2. Dose-response curves for *S. tritici* control on leaf 2 at GS 75, 1997/98. From left to right: epoxiconazole alone; azoxystrobin alone; 1:1 mixture; 1:2 mixture; 2:1 mixture.

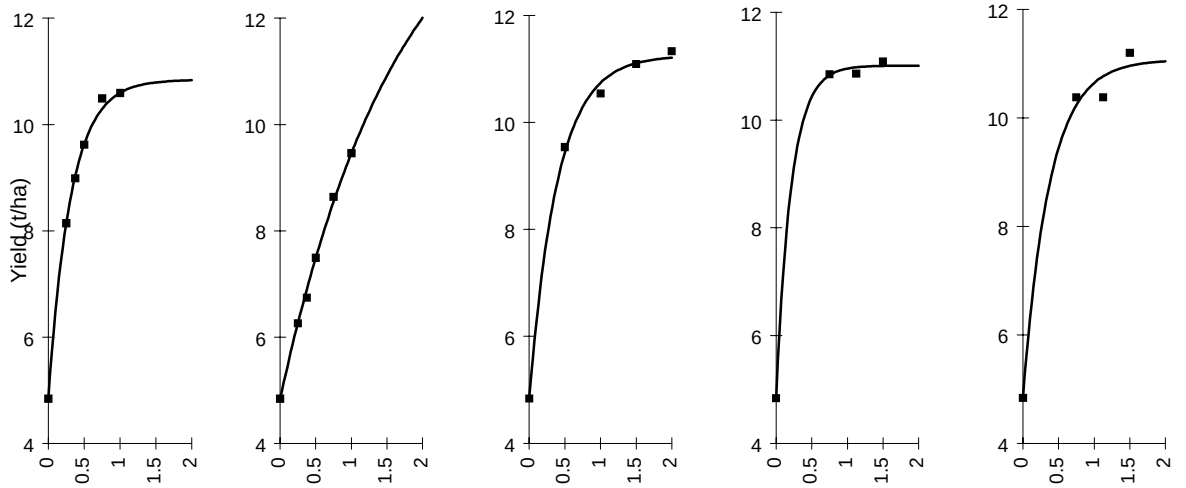


Figure 6.3. Dose-response curves for yield, 1997/98. From left to right: epoxiconazole alone; azoxystrobin alone; 1:1 mixture; 1:2 mixture; 2:1 mixture.

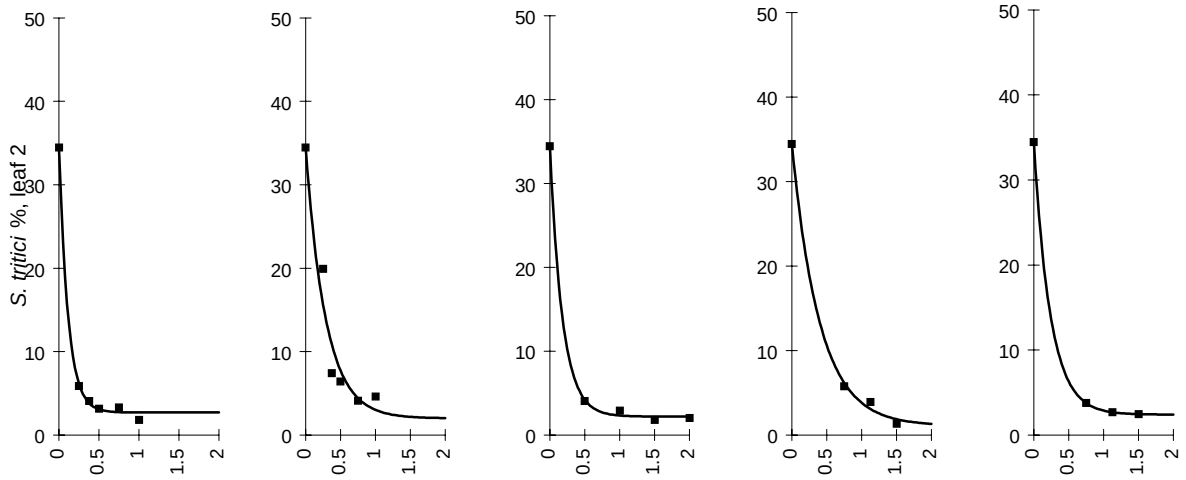


Figure 6.4. Dose-response curves for *S. tritici* control on leaf 1 at GS 75, 1998/99. From left to right: epoxiconazole alone; azoxystrobin alone; 1:1 mixture; 1:2 mixture; 2:1 mixture.

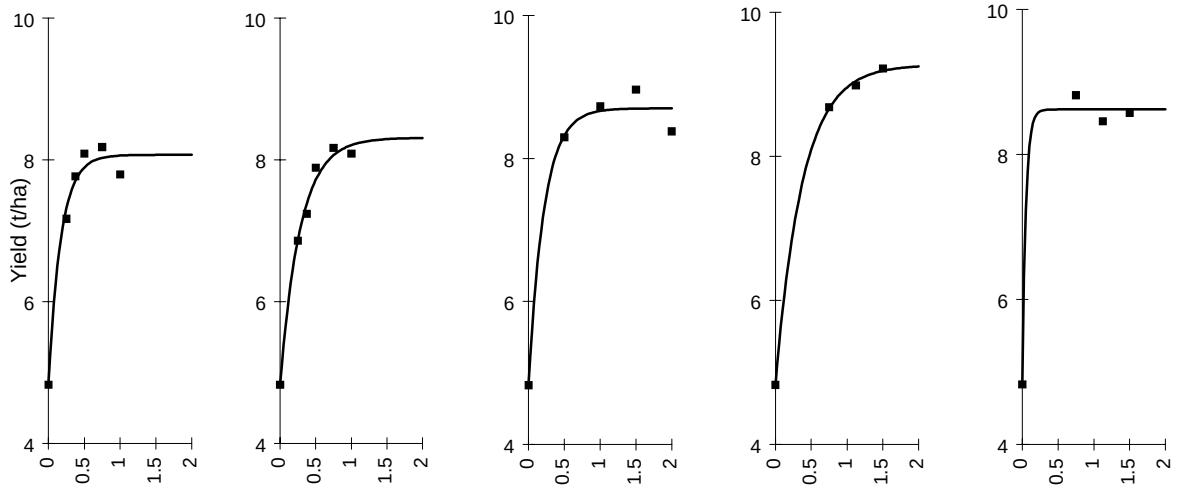


Figure 6.5. Dose-response curves for yield, 1998/99. From left to right: epoxiconazole alone; azoxystrobin alone; 1:1 mixture; 1:2 mixture; 2:1 mixture.

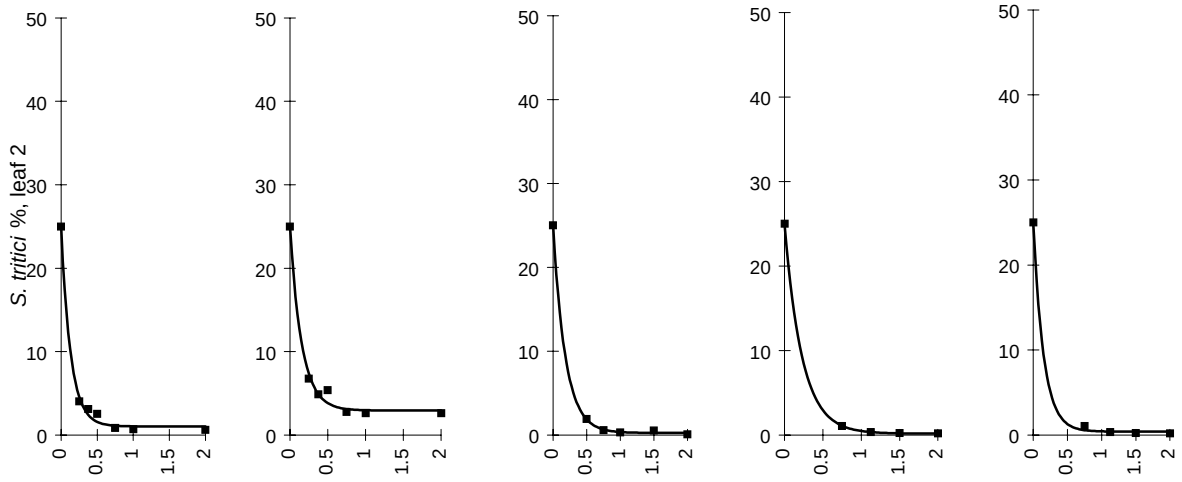


Figure 6.6. Dose-response curves for *S. tritici* control on leaf 2 at GS 75, 1999/2000. From left to right: epoxiconazole alone; azoxystrobin alone; 1:1 mixture; 1:2 mixture; 2:1 mixture.

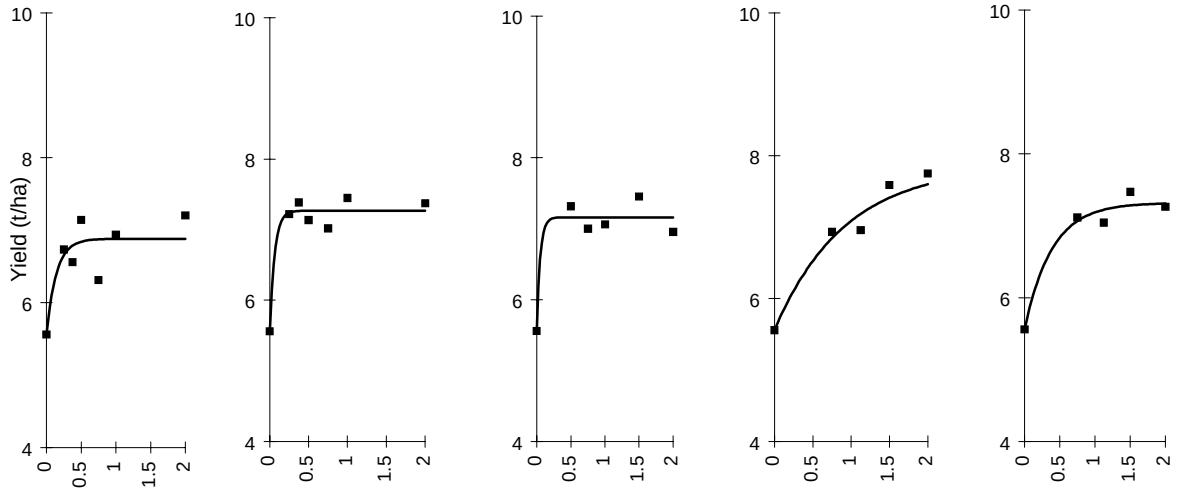


Figure 6.7. Dose-response curves for yield, 1999/2000. From left to right: epoxiconazole alone; azoxystrobin alone; 1:1 mixture; 1:2 mixture; 2:1 mixture.

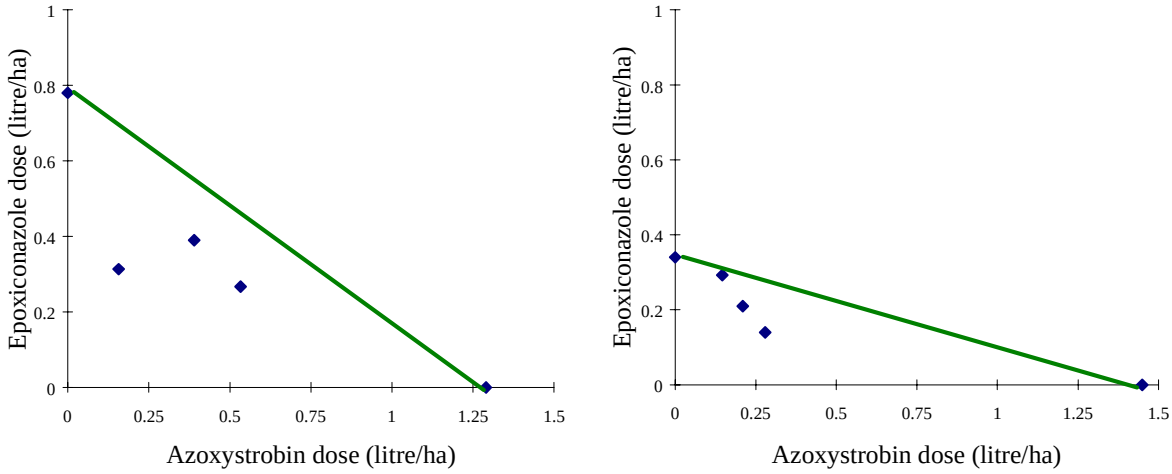


Figure 6.8. Test for synergy or antagonism between Amistar and Opus for yield (left) and *Septoria tritici* control on leaf 2 (right), 1997/98. The solid lines represent the dose of any mixture required to give a yield increase of 5.5 t/ha or a reduction in disease of 80%, assuming no synergy or antagonism between the fungicides.

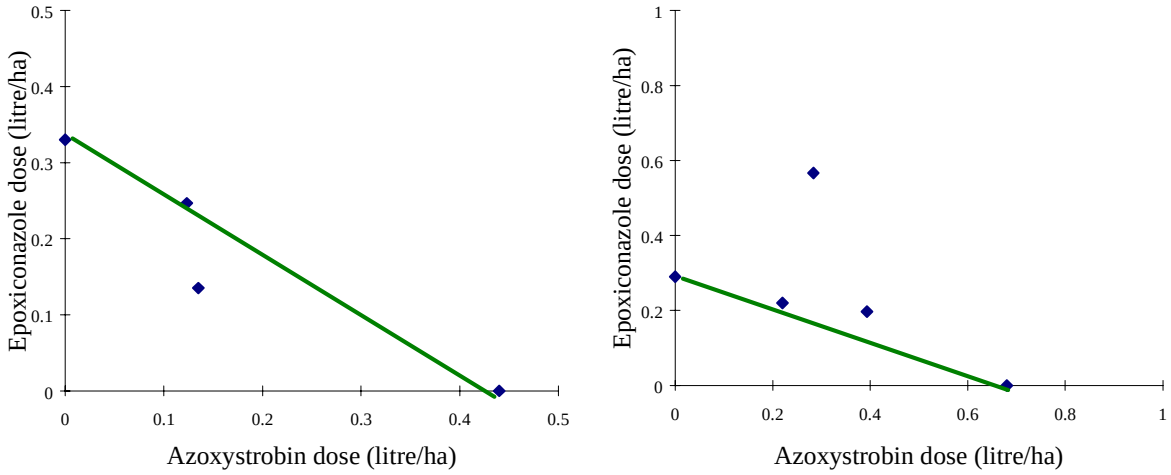


Figure 6.9. Test for synergy or antagonism between Amistar and Opus for yield (left) and *Septoria tritici* control on leaf 1 (right), 1998/99. The solid lines represent the dose of any mixture required to give a yield increase of 2.75 t/ha or a reduction in disease of 29.5%, assuming no synergy or antagonism between the fungicides.

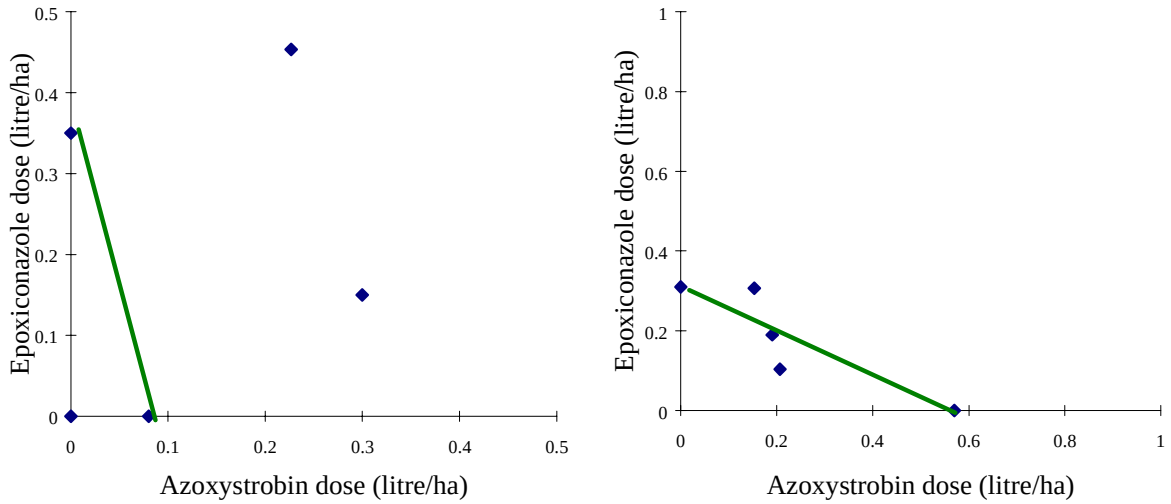


Figure 6.10. Test for synergy or antagonism between Amistar and Opus for yield (left) and *Septoria tritici* control on leaf 2 (right), 1999/2000. The solid lines represent the dose of any mixture required to give a yield increase of 1.2 t/ha or a reduction in disease of 21.5%, assuming no synergy or antagonism between the fungicides.

## Discussion

The results from the three years of this experiment show two distinct patterns. In 1997/98, disease was severe and there were very large yield responses to fungicide, with a maximum yield increase of 7.38 t/ha. Under these conditions, azoxystrobin showed much flatter dose-response curves than epoxiconazole, and all mixtures behaved similarly to epoxiconazole alone. This is probably an indication of the need for the strong eradicant activity of epoxiconazole under such favourable conditions for the pathogen, and the performance of azoxystrobin alone was poorer because of the lack of eradicant activity of this fungicide. In 1998/99 and 1999/2000, disease severity was much lower, and yield responses to fungicides were smaller. The largest yield increases from any treatment in these years were 4.39 t/ha in 1998/99 and 2.19 t/ha in 1999/2000. Each fungicide alone gave good disease control, with similar dose-response curves for the single fungicides and for mixtures.

The test for synergy or antagonism in 1997/98 indicated that there may be synergy between the fungicides. In contrast, under lower disease severity, there were no such indications in the following two years. This suggests that the result in 1997/98 may be a reflection of the beneficial effect given by mixture of the strobilurin with a triazole for control of severe disease, where the protectant activity of the strobilurin was complemented by the eradicant activity of the triazole. If mixture with a triazole compromised any non-fungicidal benefits from Amistar, this was more than outweighed by the superior disease control and the yield increase which resulted from this. In the later two years, each fungicide alone gave good disease control, so it is not surprising that it was not possible to show any specific benefit from mixtures. However, at the time the fungicides were applied, it was not apparent that disease would become more severe in 1997/98 than in the other years, so this work strongly supports the use of mixtures of a strobilurin with a good triazole fungicide to give optimum disease control and yield benefit.

## REFERENCES


Anonymous, 1998. *Cereal variety handbook*. National Institute of Agricultural Botany, Cambridge.

Anonymous, 1999. *Cereal variety handbook*. National Institute of Agricultural Botany, Cambridge.

Austin RB, Blackwell RD, 1980. Edge and neighbour effects in cereal yield trials. *Journal of Agricultural Science* **90**, 731-734.

Bertelsen JR, De Neergaaed E, and Smedegaard-Petersen V, 2001. Fungicidal effects of azoxystrobin and epoxiconazole on phyllosphere fungi, senescence and yield of winter wheat. *Plant Pathology* **50**, 190-205

Brocklehurst PA, 1977. Factors controlling grain weight in wheat. *Nature*, **266**, 348-9

Bryson RJ, Paveley ND, Clark WS, Sylvester-Bradley R, Scott RK, 1997. Use of in-field measurements of green leaf area and incident radiation to estimate the effects of yellow rust epidemics on the yield of winter wheat. *European Journal of Agronomy* **7**, 53-62.

Foulkes MJ, Scott RK, 1998. *Exploitation of Varieties for UK Cereal Production. Volume 2: Varietal responses to drought*. Project Report No. 174. Home-Grown Cereals Authority, London.

Glaab J and Kaiser WM, 1999. Increased nitrate reductase activity in leaf tissue after application of the fungicide kresoxim-methyl. *Planta*, **207**, 442-448.

Grossman K, Kwiatkowski J and Casper G, 1999. Regulation of phytohormone levels, leaf senescence and transpiration by the strobilurin kresoxim-methyl in wheat (*Triticum aestivum*). *Journal of Plant Physiology*, **154**, 805-808.

Grossman K, and Retzlaff G, 1997. Bioregulatory effects of the fungicidal strobilurin kresoxim-methyl in wheat (*Triticum aestivum*). *Pesticide Science*, **50**, 11-20

Jenkyn JF, Bateman GL, Gutteridge RJ and Edwards SG, 2000. Effects of foliar sprays of azoxystrobin on take-all in wheat. *Annals of Applied Biology*, **137**, 99-106

Jones DR, 2000. *Properties of new fungicides for winter wheat and winter barley*. Project Report No. 223. Home-Grown Cereals Authority, London.

Jones DR and Bryson RJ, 1998. *Physiological effects of strobilurins and plant activators in relation to yield of winter wheat*. HGCA Report No. 164

Köhle H, Grossman K, Retzlaff G, Saur R and Akers A, 1977. The yield physiology of kresoxim-methyl. *The Agronomist*, **3/97**, 10-14

Macbeth JE, 1996. *The prediction of mature grain weight in winter wheat (*Triticum aestivum, *L.)* PhD Thesis, Harper Adams Agricultural College, Shropshire.

Paveley ND, 2000. *Appropriate fungicide doses on winter wheat: research information for growers on the control of powdery mildew, brown rust and* Septoria nodorum. Project Report No. 214. Home-Grown Cereals Authority, London.

Sinclair TR, and Muchow, RC, 1999. Radiation use efficiency. *Advances in Agronomy* **65**, 215-265.

Spink JH, Whaley J, Semere T, Wade A, Sparkes D, Foulkes MJ, 2000. *Prediction of optimum plant population in winter wheat*. Project Report No. 234, Home-Grown Cereals Authority, London.

Streibig JC, Kudsk P, 1993. *Herbicide bioassays*. Boca Raton, Florida, USA: CRC Press Inc.

Sylvester-Bradley R, Stokes DT, Scott RK, Clare RW, 1998. Canopy management in winter wheat. *Proceedings of the sixth HGCA R & D conference on cereals and oilseeds*, pp. 5.1-5.10. Home-Grown Cereals Authority, London.

Thomas TA, 1977. An automated procedure for the determination of soluble carbohydrates in herbage. *Journal of Food and Agriculture* **28**, 639-642.

Thorne GN, Wood DW, 1987. The fate of carbon in dying tillers of winter wheat. *Journal of Agricultural Science* **108**, 515-522.

Tottman D, Broad H, 1987. The decimal code for the growth stages of cereals with illustrations. *Annals of Applied Biology* **110**, 441-454.