HGCA

PROJECT REPORT No. 164

PHSIOLOGICAL EFFECTS OF STROBILURINS AND PLANT ACTIVATORS IN RELATION TO YIELD OF WINTER WHEAT

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PHSIOLOGICAL EFFECTS OF STROBILURINS AND PLANT ACTIVATORS IN RELATION TO YIELD OF WINTER WHEAT

by

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CONTENTS

SUMMARY

Three experiments were undertaken in a one-year pilot study in 1997 to investigate whether new fungicides, particularly strobilurins, had effects on growth and yield of winter wheat additional to those associated with disease control. The strobilurin fungicides azoxystrobin (as Amistar) and kresoxim-methyl (co-formulated with epoxiconazole as BAS494) were evaluated in two spray programmes at GS 32 and GS 39, in comparison with the azole fungicide epoxiconazole (as Opus). Additional programmes of Amistar and Landmark were designed to exploit the perceived strengths of each product. In addition, there was a treatment with the plant activator benzothiadiazole (as A9180) at GS 30, followed by Opus at GS 39.

Severe *Septoria tritici* on cv. Consort was controlled well by Opus and BAS494, but Amistar was less effective and suffered earlier loss of canopy due to disease. All these fungicides also gave good control of yellow rust on cv. Brigadier. There was no evidence that benzothiadiazole could substitute for a conventional fungicide at GS 32 where either of these diseases was severe. Compared with Opus alone, the mixture with kresoxim-methyl gave small additional increases in green canopy duration, although disease control was no better. On the resistant cv. Pastiche, disease incidence was negligible, but fungicides delayed leaf senescence. BAS494 treatment resulted in increased chlorophyll content of the lower leaves compared with Opus, and showed decreased light transmission through the leaves, which suggests that photosynthetic rates could have been higher, although this was not determined.

Control of *S. tritici* resulted in yield increases of up to 6.06 t/ha. Yields from BAS494 were significantly higher than those from Amistar, with Opus intermediate. There were no differences between fungicides in yield response to yellow rust control, with increases in the range 2.04-2.58 t/ha. On cv. Pastiche, disease levels were very low, but all fungicide treatments delayed canopy loss and increased yield by up to 1.03 t/ha. The largest increases were from BAS494, followed by Amistar.

This pilot study showed that strobilurins, particularly kresoxim-methyl, increased green canopy persistence and chlorophyll content. Further investigation is required to determine the magnitude and consistency of these effects, and to show whether photosynthetic activity is increased. There were also small yield benefits over azole fungicides. The lack of eradicant activity of azoxystrobin against *S. tritici* on a susceptible cultivar was demonstrated and, where there is a high risk of disease, this fungicide will need to be mixed with an azole fungicide if other benefits are to be expressed.

INTRODUCTION

After more than a decade in which the UK cereal fungicide market was dominated by azole and morpholine fungicides, an important new fungicide group, the strobilurin analogues, was introduced in 1997. The two new active ingredients which are already available are kresoxim-methyl and azoxystrobin. Other strobilurin analogues and other new areas of chemistry are also under development by several companies. Another interesting new approach to disease control is the activator of host resistance, benzothiadiazole, under development by Novartis.

The potential importance of new fungicides for UK cereal growers has been widely recognised, and the HGCA commissioned a study of their biological properties (Project 0027/01/95), which commenced in the 1996 harvest year. The objective of that study is to understand the protectant and eradicant properties of new fungicides against each of the major pathogens of winter wheat and barley. At some winter wheat sites in 1996, formulated mixtures containing the strobilurin fungicide kresoxim-methyl gave periods of green canopy retention in excess of that achieved by the best commercial standard. The increase in green canopy duration was particularly pronounced for the lower stem leaves (eventual leaves 3 and 4), and could not be attributed directly to disease control. In some instances, kresoxim-methyl treatments also gave greater yield increases than conventional treatments, particularly from applications at GS 32 or GS 33, i.e. earlier than the 'normal' optimum timing of GS 39. This led to speculation that these new fungicides were having physiological effects on the crop which differ from those of current azole and morpholine fungicides.

In order to understand how the new fungicides have such a persistent effect on the crop canopy, and what effect this has on yield, this one-year pilot investigation was undertaken to determine which aspects of crop growth and development are affected and to provide the framework for more detailed projects in subsequent years.

OBJECTIVE

To determine whether the effects of new fungicides on green canopy retention are due solely to fungicidal activity or whether there are additional effects on the crop in the absence of disease. To investigate whether the activity of some fungicides in prolonging green leaf life has a beneficial effect in terms of yield, and to quantify the effects of treatments on leaf greenness in terms of chlorophyll content and activity.

MATERIALS AND METHODS

To investigate the benefits of new fungicides in the presence and absence of disease, sites and cultivars were chosen to give one crop with severe disease and one with very little disease. The severe disease crop was of cv. Consort (susceptible to *Septoria tritici*) at ADAS Rosemaund, Hereford & Worcester, and the low disease crop was of cv. Pastiche at ADAS Boxworth, Cambridgeshire. In addition to these experiments funded by the HGCA, an additional experiment was undertaken at ADAS Boxworth on cv. Brigadier (susceptible to yellow rust). This was undertaken as part of a MAFF-funded studentship. It had the same treatments and the same schedule of disease assessments as the adjacent experiment on cv. Pastiche, Disease and yield data from this experiment are included in this report.

Treatments and application rates are listed in Table 1.

Table 1. Treatments

	Fungicide	Product	Rate c.p./ha	Timing
1	Untreated			
2	Epoxiconazole	Opus	1.0 litre	GS 32 + 39
3	Benzothiadiazole	A9180	0.06 kg	GS 30
	Epoxiconazole	Opus	1.0 litre	GS 39
4	Azoxystrobin	Amistar	1.0 litre	GS 32 + 39
5	Azoxystrobin	Amistar	0.25 or 1.0 litre*	GS 30 + 31 + 32 + 39
6	Kresoxim methyl & epoxiconazole	BAS494+	1.0 litre	GS 31 + 39
7	Kresoxim methyl & epoxiconazole	BAS494+	1.0 litre	GS 32 + 39

* 0.25 litre/ha at Boxworth, 1.0 litre/ha at Rosemaund

+ Now marketed as Landmark

In selecting these treatments, the need to use each fungicide to its best advantage was a fundamental constraint, which precluded a balanced design in terms of fungicides and timings. The design selected allowed a comparison of an azole (Opus) with each strobilurin at standard timings (GS 32 + 39; Treatments 2, 4 & 7), but also included an additional treatment of each strobilurin which it was thought would optimise its performance. The other treatment was selected to determine whether use of the plant activator, benzothiadiazole, applied at GS 30, could act as a substitute for a conventional azole spray at GS 32 (cf Treatments 2 & 3).

Each experiment was a randomised block with three replicates of seven treatments. Plot sizes were 48 m^2 at Boxworth and 96 m^2 at Rosemaund; one half of each plot was used for destructive sampling for growth analysis and the other half for disease assessments and yield determination. Ten indicator plants across each experiment were tagged, so that leaf emergence dates could be estimated.

Foliar diseases were assessed as percentage leaf area infected on each leaf layer on 10 tillers per plot from all plots at 10/11 day intervals from GS 31 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 the graphs of disease progress in each treatment (e.g. Figure 1), as the area under the line showing disease development for that treatment.

Two of each sample of 10 tillers used for foliar disease assessment were then selected at random, and leaf length and width measured. From these, leaf area was calculated, using a form factor (Bryson *et al.*, 1997). 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), which provides a measure of green canopy size during the period in which photosynthesis is contributing primarily to grain filling rather than to canopy structure.

Growth analysis was undertaken at selected growth stages (GS 31, GS 33, GS 37-39, GS 59-65, GS 75, GS 83-87 and pre-harvest. The following were recorded, at all relevant growth stages except where indicated otherwise:

Shoot number
Projected green area of leaves, stems and ears
Total dry weight of leaves, stems and ears
Soluble stem carbohydrates at GS 59-65 and GS 83-87
Nitrogen content of grain (converted to grain protein by a factor of 4.9)
Thousand grain weight
Harvest index

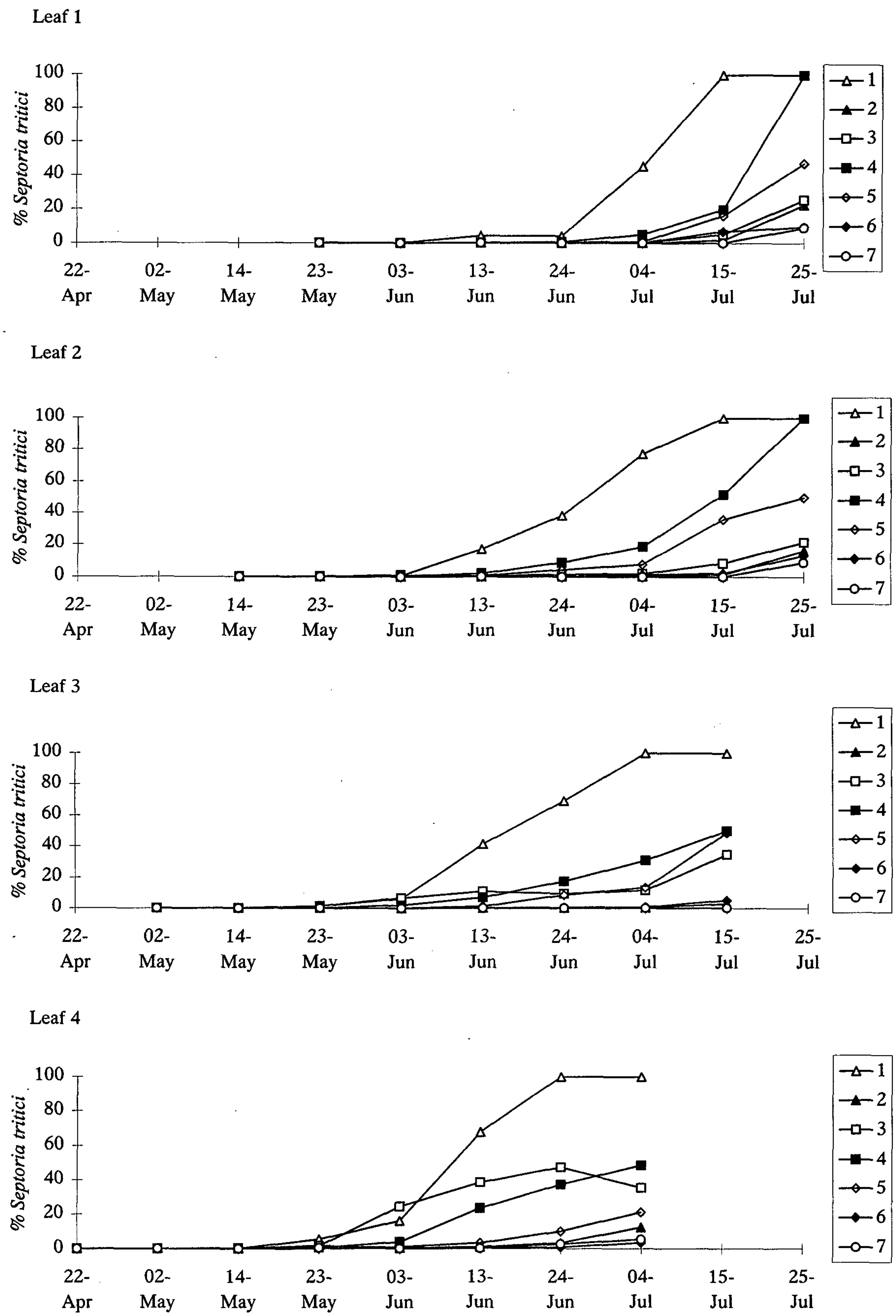
To investigate effects of treatments on chlorophyll content, it was determined in the top three leaves of untreated plots and 2 fungicide treatments on cv. Pastiche at Boxworth at GS 39 Bruinsma, 1963; Lichtenthaler & Wellburn, 1983). Since the assessment of chlorophyll concentration by conventional extraction is time-consuming and requires destructive sampling, some preliminary information was obtained on the possibility that chlorophyll concentration can be estimated rapidly. This was done using a SPAD meter (Minolta corp.), which provides a rapid, non-dstructive assessment of protein content. SPAD readings were taken at the same time as the samples for chlorophyll extraction.

Another technique under development for rapid assessment of crop function is spectral reflectance. This provides objective measurements of the greenness of leaves, and offers the potential for rapid assessment of chlorophyll content and indirect indications of photosynthetic activity. A detailed investigation of this technique was outside the scope of this project, but some information was obtained, as a preliminary to more detailed investigation in future projects. A spectroradiometer (LI-COR inc.) was used at Boxworth on cv. Pastiche at GS 61 to determine whether fungicide treatment had any effect on the amount of light transmitted through leaves.

RESULTS

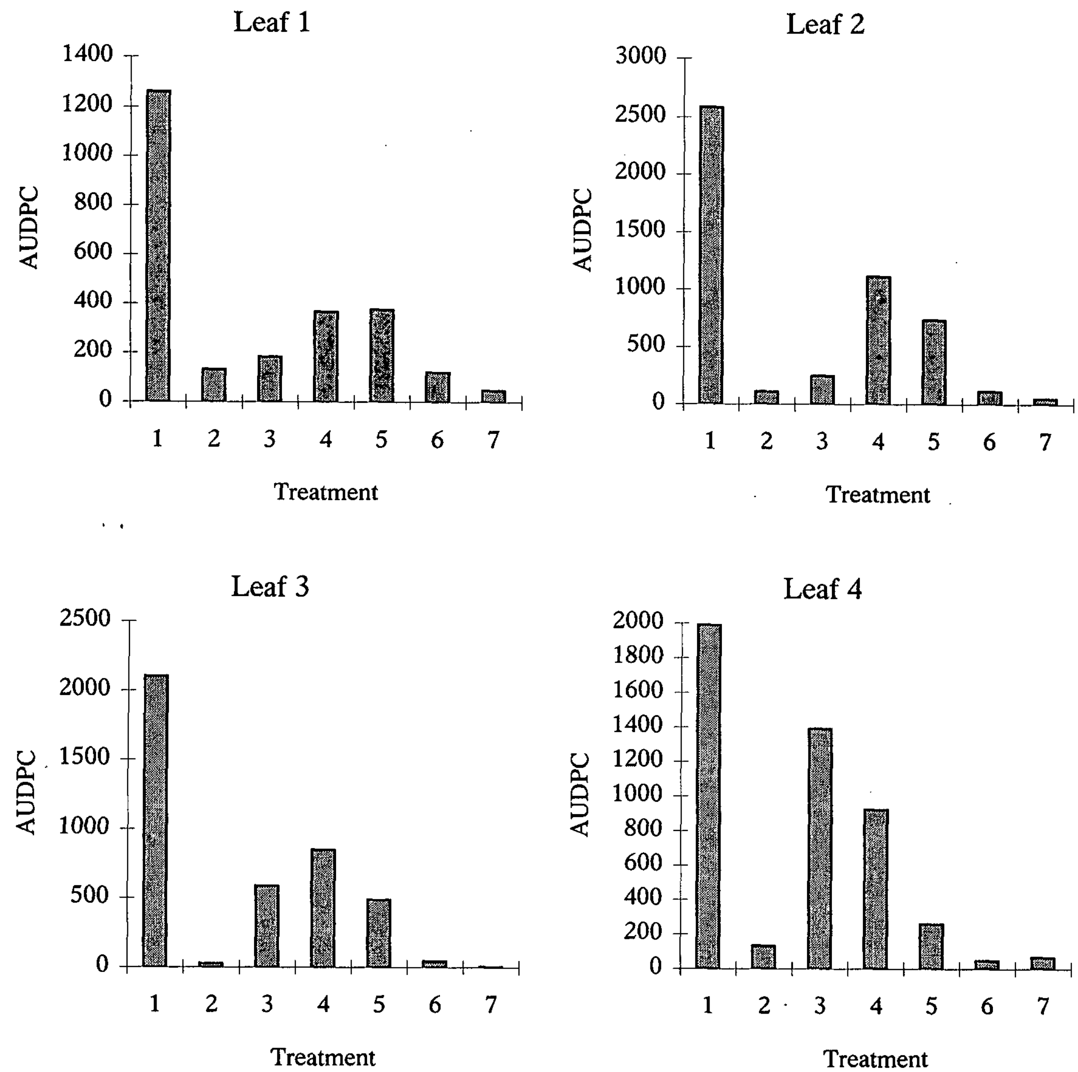
Disease control

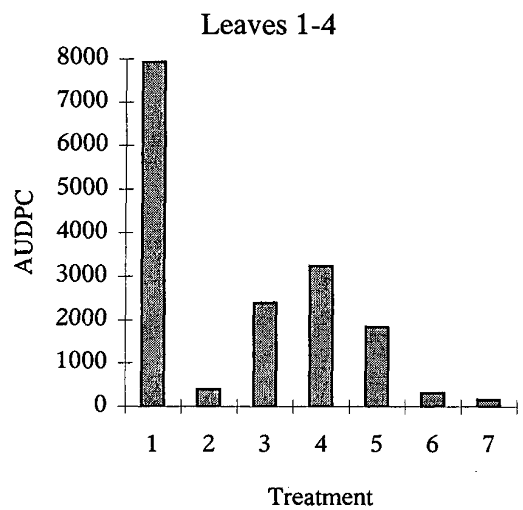
At Rosemaund, there was a severe *Septoria tritici* epidemic on cv. Consort. The only other foliar disease recorded was mildew, at negligible levels. Disease progress on the four stem leaves is shown in Figure 1, and AUDPCs are given in Table 2 and shown in Figure 2. All fungicide treatments gave good disease control in comparison with the untreated plots. The two BAS494 treatments and Opus gave almost complete control on each leaf layer until a small amount of disease appeared by the time of final assessment on each leaf layer. The A9180 followed by Opus treatment had no effect on *S. tritici* on leaf 4 until June, and was less effective than the best treatments on leaf 3, but gave good disease control on the top 2 leaves. The Amistar treatments were less effective than BAS494 and Opus programmes, with a clear difference in disease severity between the 2 and 4 spray programmes of Amistar.



1 Untreated
2 Opus GS32 & GS39
3 A9180 GS30; Opus GS39
4 Amistar GS32 & GS39
5 Amistar GS30, GS31, GS32 & GS39
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 1. Rosemaund, cv. Consort. Disease progress on each leaf layer.





Treatments

1 Untreated
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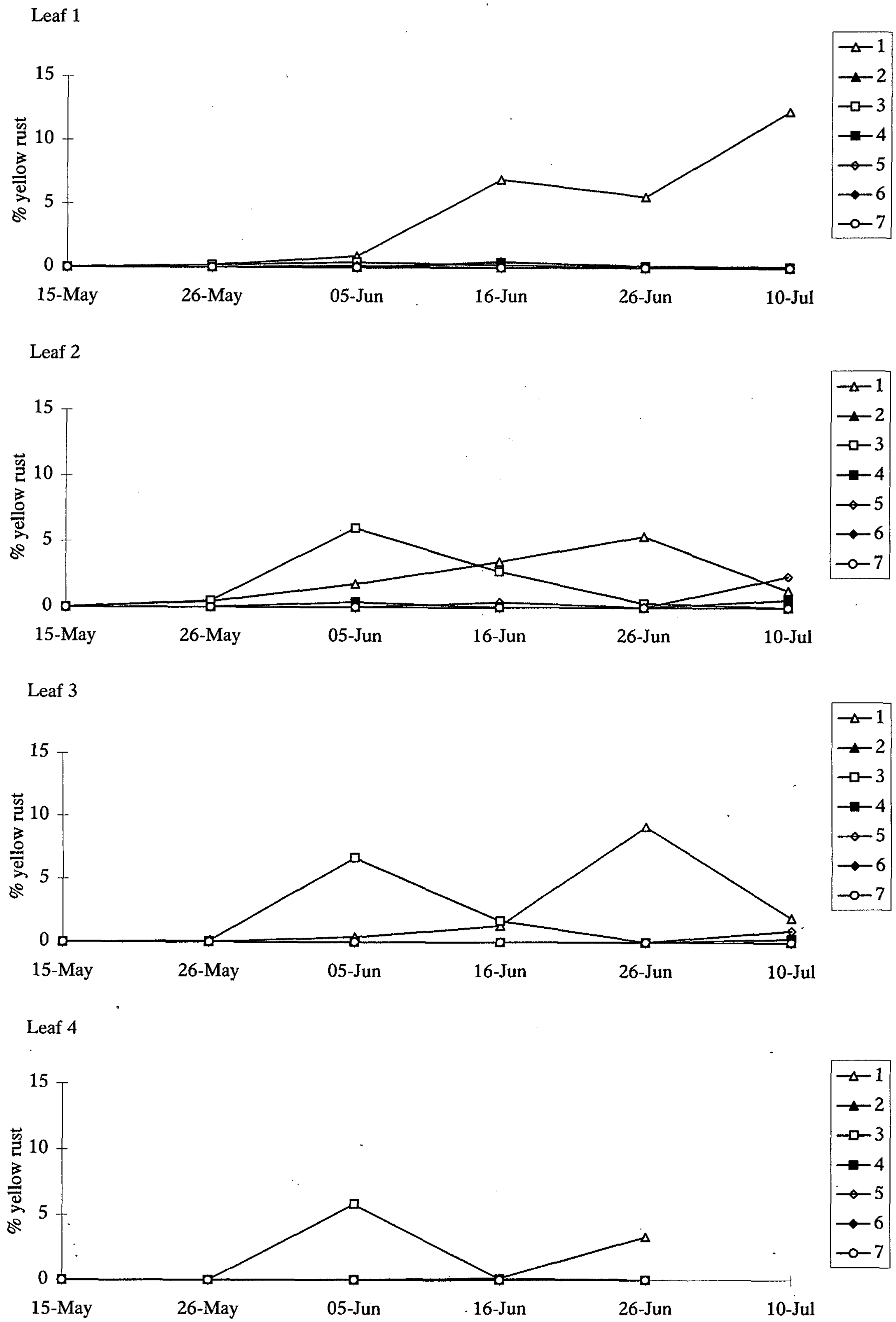
5 Amistar GS30, GS31, GS32 & GS39
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 2. Rosemaund, cv. Consort. Areas under disease progress curves (AUDPC)



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3 A9180 GS30; Opus GS39
4 Amistar GS32 & GS39
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6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 3. Boxworth, cv. Brigadier. Disease progress on each leaf layer.

Table 2. *Septoria tritici* areas under disease progress curves (AUDPC), Rosemaund, cv. Consort

Treatment		AUDPC L1	AUDPC L2	AUDPC L3	AUDPC L4	AUDPC L1-4
1	Untreated	1259	2579	2105	1987	7931
2	Opus GS 32&39	130	107	27	131	394
3	A9180 & Opus	183	243	588	1390	2404
4	Amistar GS 32&39	365	1110	850	924	2148
5	Amistar x4	373	728	488	258	1848
6	BAS494 GS 31&39	120	109	41	44	313
7	BAS494 GS 32&39	47	47	5	65	163
SED (12 df)		106.1	100.0	129.0	143.5	316.0
P		<0.001	<0.001	<0.001	<0.001	<0.001

At Boxworth, disease was negligible on cv. Pastiche, with only 1-2% *Septoria tritici* on lower leaves.

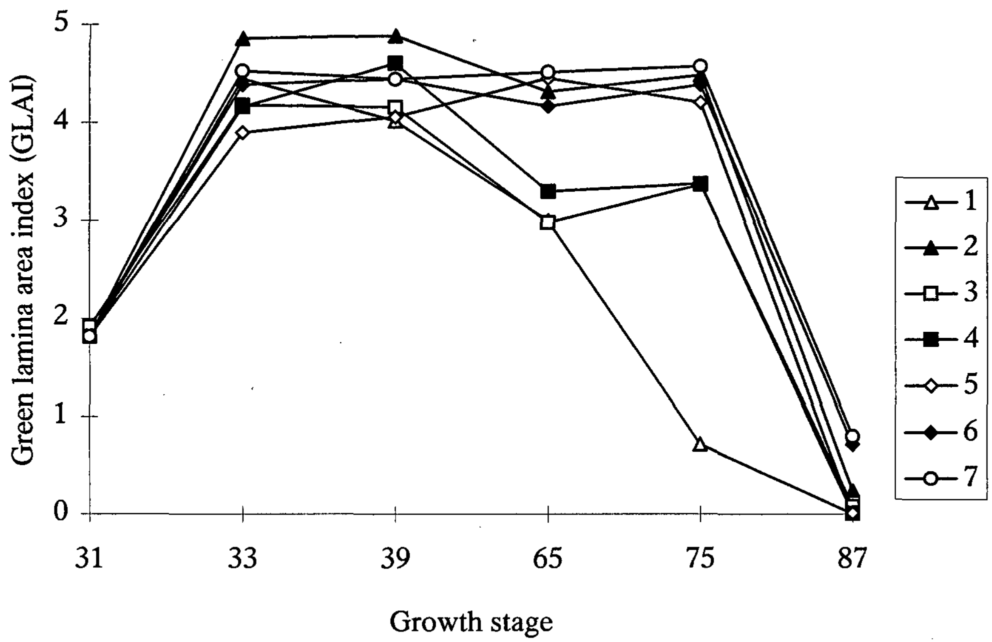
On cv. Brigadier at Boxworth, yellow rust was the main disease. All fungicide treatments gave almost complete control on leaf 1 (Figure 3). On the other leaves, there was some yellow rust in the A9180 followed by Opus treatment, but this was brought under control during June. Yellow rust also developed on the top 3 leaves, though at low levels, in the Amistar treatments.

Canopy size

Data on total canopy size were obtained from the growth analyses for cvs Consort and Pastiche; growth analysis was not done on cv. Brigadier. On cv. Consort, differences between treatments were not statistically significant at either GS 33 or GS 39 (Figures 4 and 5). At GS 65, canopy size, as either green lamina area index (GLAI) or total green area index (GAI) was significantly lower ($P<0.05$) in the untreated control, A9180 followed by Opus and Amistar (x2) than in other treatments. By GS 75, these 2 fungicide treatments still had smaller canopies than other fungicide treatments, but much larger than in the untreated controls. The only treatments with significant green lamina remaining at GS 87 were the BAS494 treatments. The untreated control reached GAI 4.8 at GS 39 but declined thereafter. The A9180 followed by Opus and Amistar (x2) treatments had maximum canopy sizes of less than GAI 6.0, whereas other treatments had maximum GAI of over 6.8, and the largest canopy size in any fungicide treatment was GAI 7.4 in the Opus programme at GS 75.

On cv. Pastiche, the BAS494 (GS 32 & 39) programme had the largest canopy at GS 37, GS 59 and GS 83, although the differences were statistically significant only at GS 83 (Figures 6 and 7). The untreated control had the smallest canopy at GS 75 (not statistically significant) and GS 83. The mean canopy size at GS 59 was 5.8, and canopy size had declined in all treatments by GS 75.

There were no significant differences between treatments in final shoot numbers on either cultivar, which were in the range 500-600/m^2 on cv. Consort and were approximately 600/m^2 on cv. Pastiche.



1 Untreated
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4 Amistar GS32 & GS39
5 Amistar GS30, GS31, GS32 & GS39
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 4. Rosemaund, cv. Consort, green lamina area index

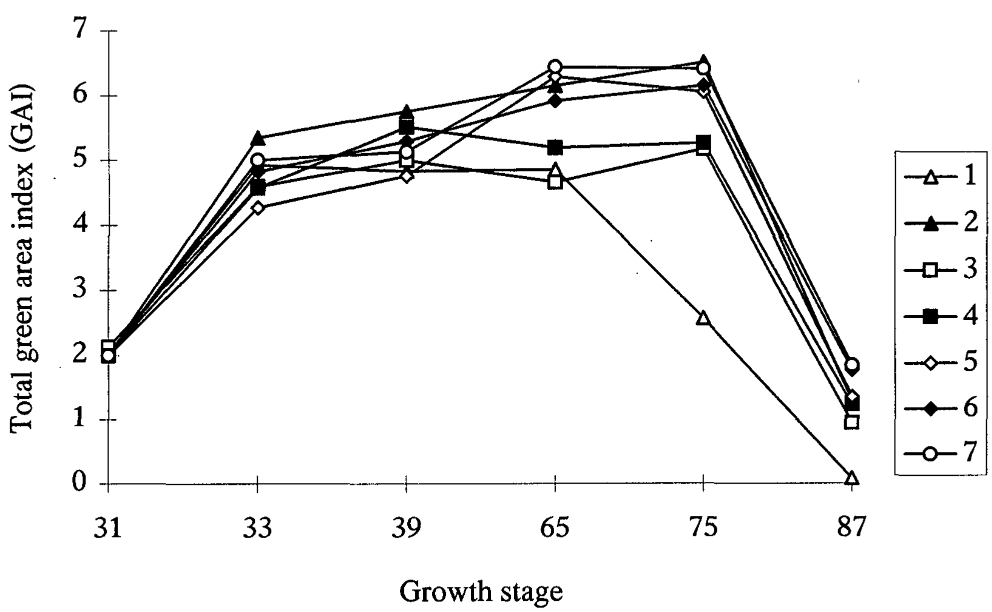
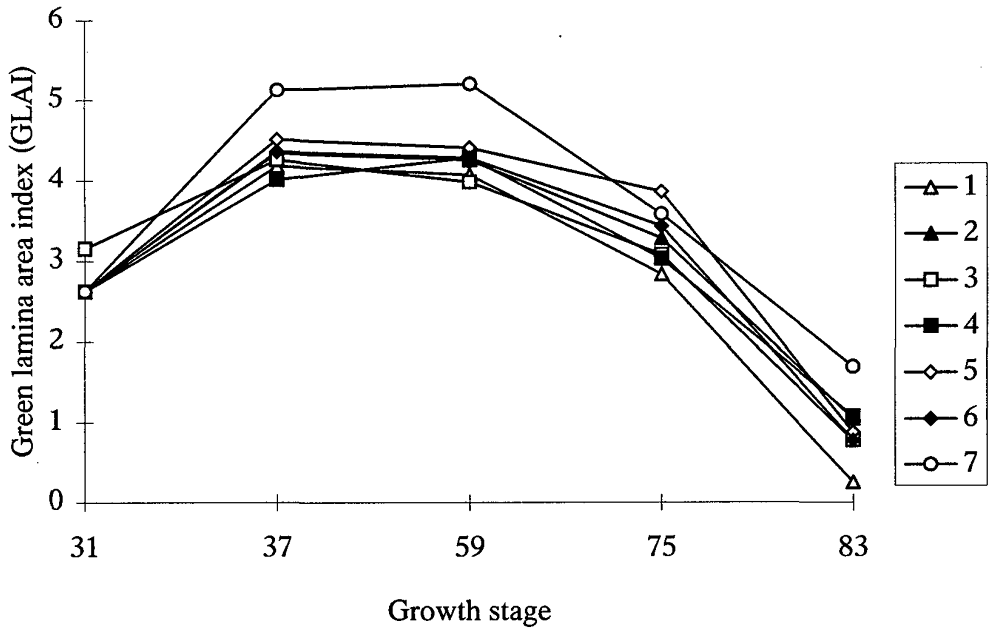


Figure 5. Rosemaund, cv. Consort, total green area index



1 Untreated
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6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 6. Boxworth, cv. Pastiche, green lamina area index

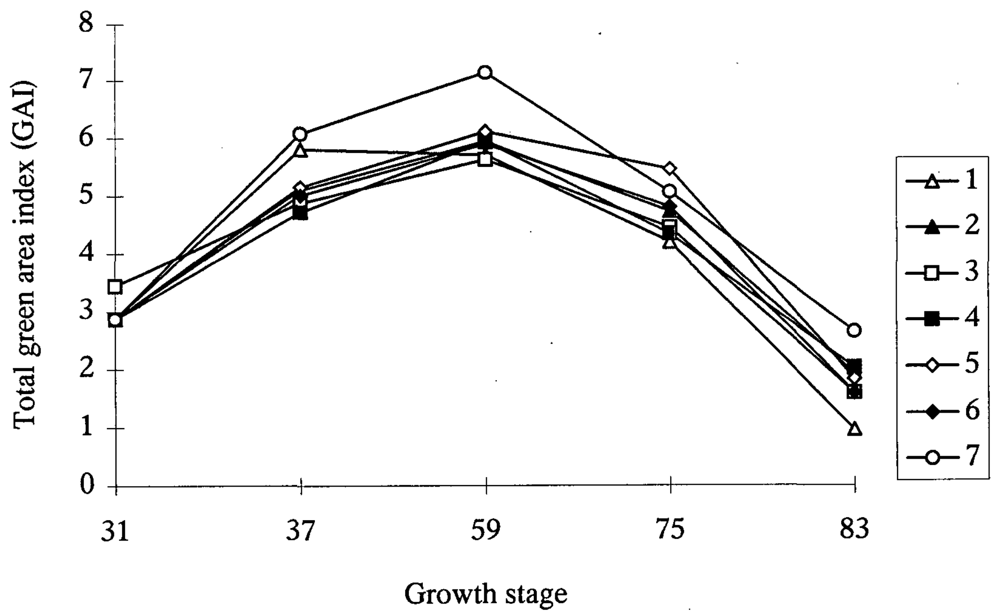


Figure 7. Boxworth, cv. Pastiche, total green area index

On cv. Consort, all fungicide treatments showed marked increases in percentage green leaf retention on the top 3 leaves (Figure 8). On leaves 1 and 2, the BAS494 treatments had the greatest green leaf area at the final assessment, but the Opus programme and A9180 followed by Opus were only slightly less effective. Amistar was less effective, with green canopy loss on the top two leaves commencing 21 days earlier than in the other fungicide treatments. Very little green leaf remained in Amistar treatments on 25 July at which time there was still over 50% green leaf area in other fungicide treatments on these leaf layers. On leaf 3, the pattern was similar except that A9180 followed by Opus was comparable with Amistar (x4).

At Boxworth, all fungicide treatments increased green leaf retention of cv. Pastiche although there was very little disease (Figure 9). Differences between the fungicide treatments were very small on the top 2 leaves but, on leaf 4, the BAS494 treatments and Amistar (x4) maintained some green leaf up to 10 days longer than other treatments.

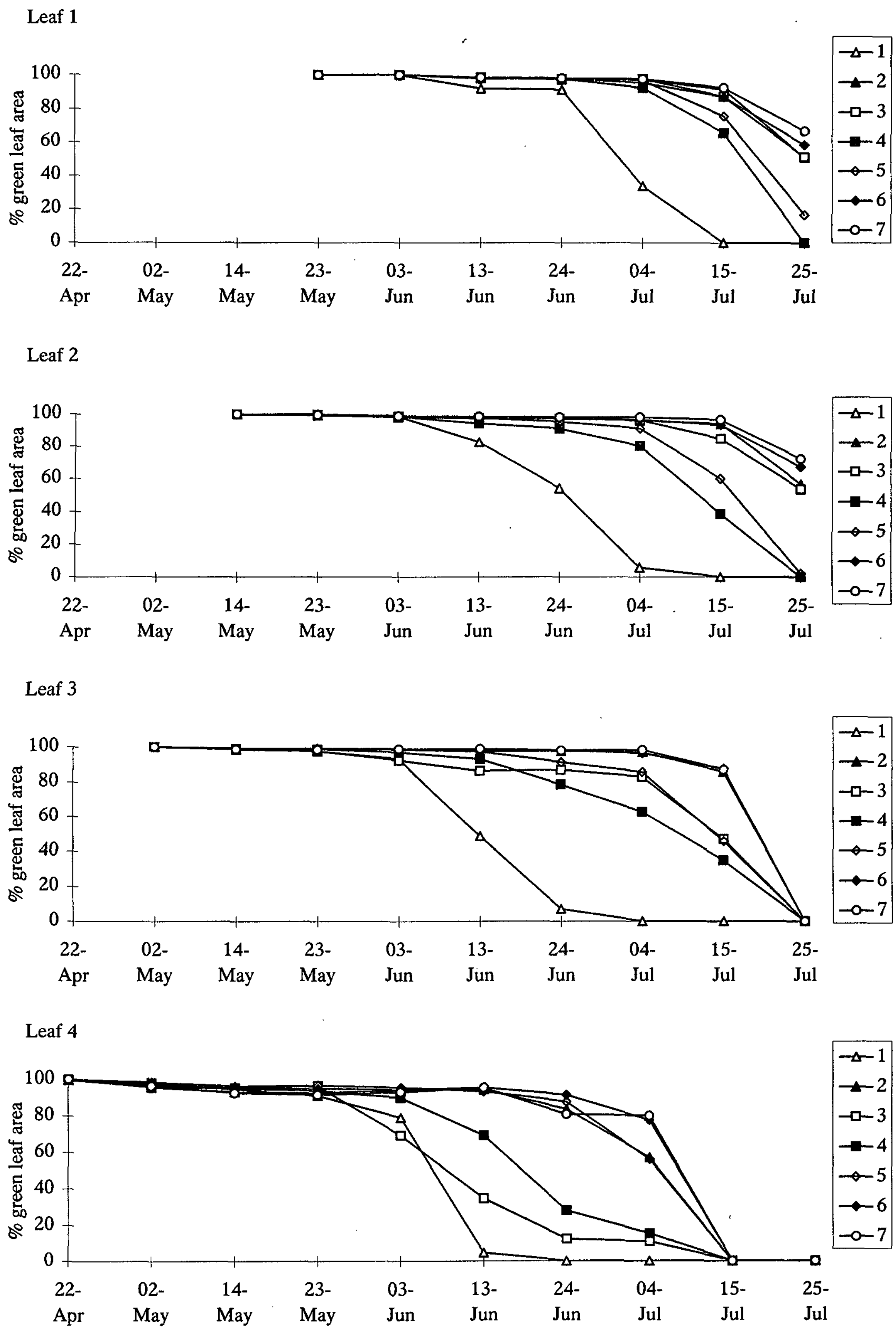
On cv. Brigadier, there was a clear effect of fungicides in maintaining green area of leaves 1-3, with the main difference between treatments being that Amistar treatments started to lose green area earlier than other treatments (Figure 10). There were larger differences between treatments on leaf 4, with BAS494 (GS 32 & 39) maintaining more green area than other treatments in late June and July.

The relationship between disease and percentage green area for cv. Consort is illustrated in Figure 11, in the final assessment before leaf senescence on leaves 2 and 4. On leaf 4 on 4 July, there was little difference in disease control between the BAS494 treatments and the Opus treatment, but there was approximately 20% greater green area in the BAS494 treatments. There was a similar, though smaller, effect on leaf 2 on 25 July.

For calculation of healthy area duration from GS 39 (HAD39), the growth analysis did not provide sufficient data points, so this was calculated from the leaf area measurements which were done as part of each foliar disease assessment. On cv. Consort, all fungicide treatments significantly increased HAD39 on each of the upper 4 leaves (Table 3; Figure 12). There were no significant differences between fungicides on leaf 1 but, on leaves 2 and 3, BAS494 at GS 31 and GS 39 had greater HAD39 values than either Amistar programme or A9180 followed by Opus. On leaf 4, BAS494 at GS 31 and GS 39 had a significantly greater HAD39 than any other treatment.

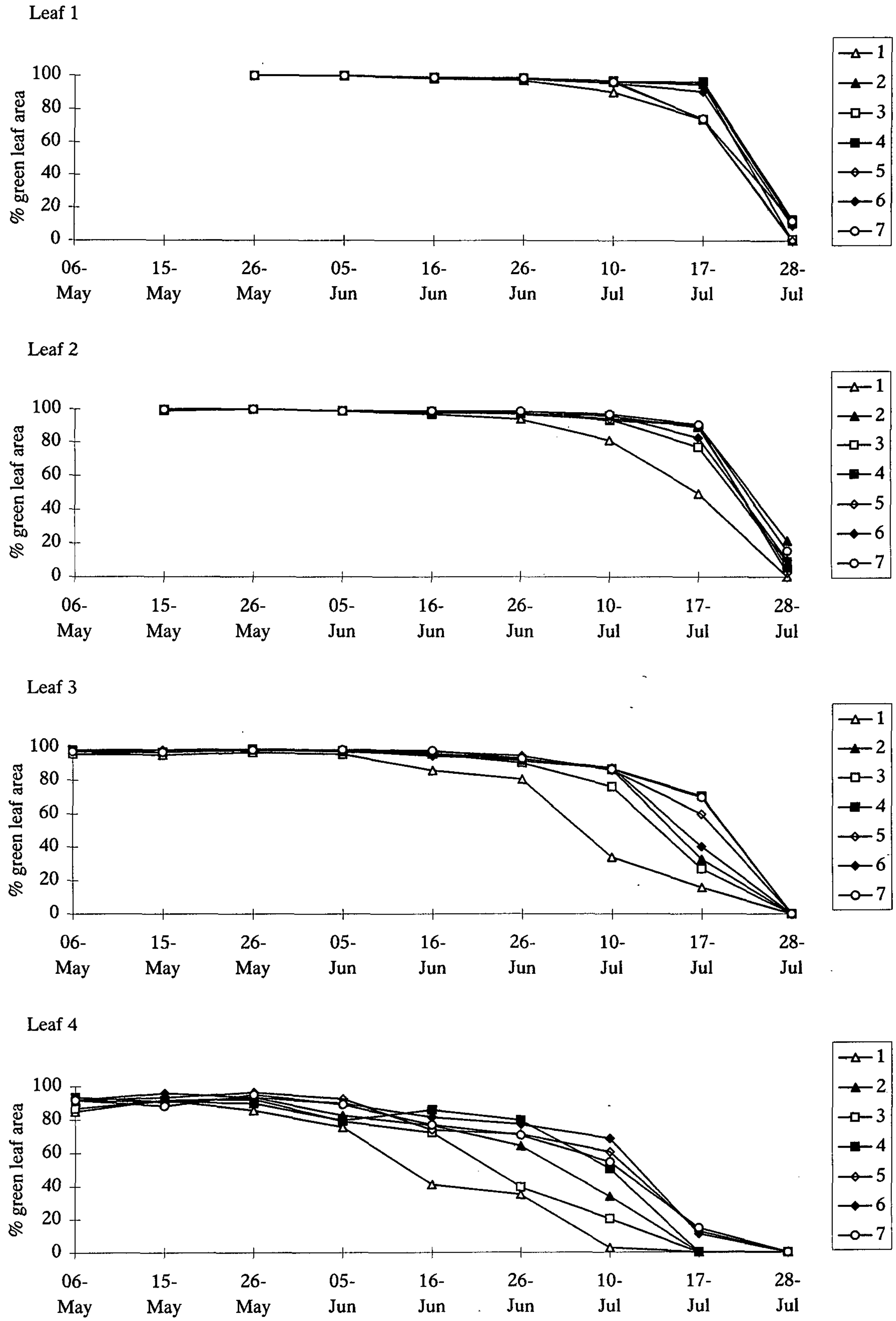
Table 3. Healthy area duration from GS 39 (HAD39), Rosemaund, cv. Consort

Treatment		HAD39 L1	HAD39 L2	HAD39 L3	HAD39 L4	HAD39 L1-4
1	Untreated	59.3	42.0	24.6	14.4	140.4
2	Opus GS 32&39	93.2	82.8	70.8	47.5	294.2
3	A9180 & Opus	87.0	76.3	53.4	20.4	237.1
4	Amistar GS 32&39	83.1	68.9	58.2	29.1	239.3
5	Amistar x4	85.4	72.5	60.1	45.6	263.6
6	BAS494 GS 31&39	88.3	86.9	75.7	57.6	308.6
7	BAS494 GS 32&39	84.2	78.8	68.3	48.0	279.3
SED (12 df)		5.98	4.47	5.81	3.22	17.73
P		0.003	<0.001	<0.001	<0.001	<0.001



1 Untreated
2 Opus GS32 & GS39
3 A9180 GS30; Opus GS39
4 Amistar GS32 & GS39
5 Amistar GS30, GS31, GS32 & GS39
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 8. ADAS Rosemaund 1997, cv. Consort. Green leaf area (%) of each leaf layer.



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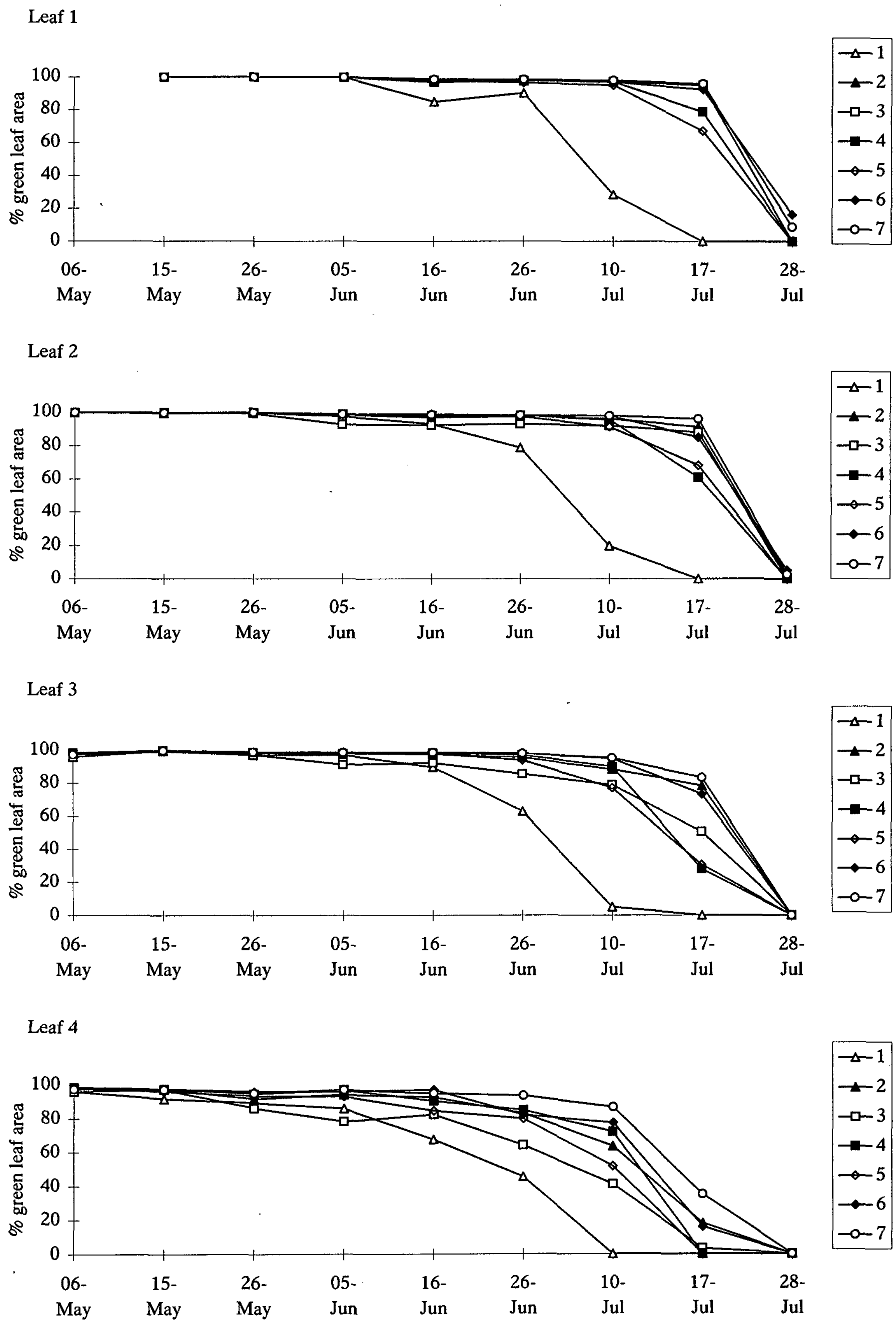
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 9. Boxworth, cv. Pastiche. Green leaf area (%) of each leaf layer.



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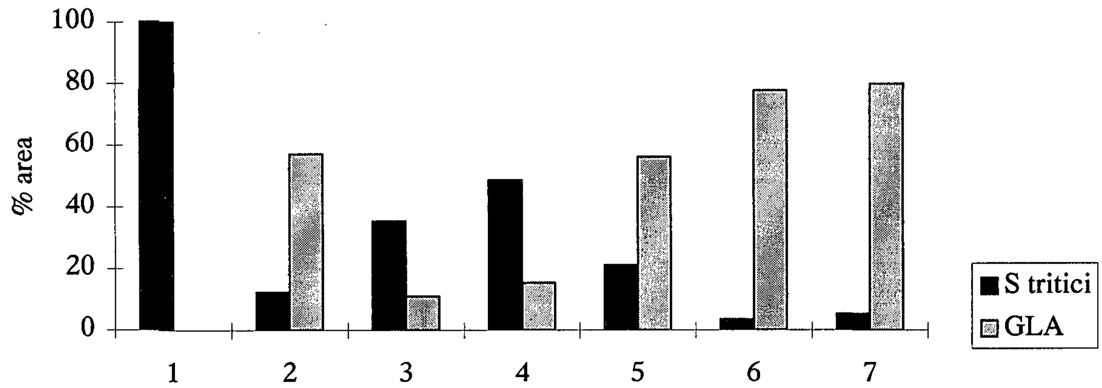
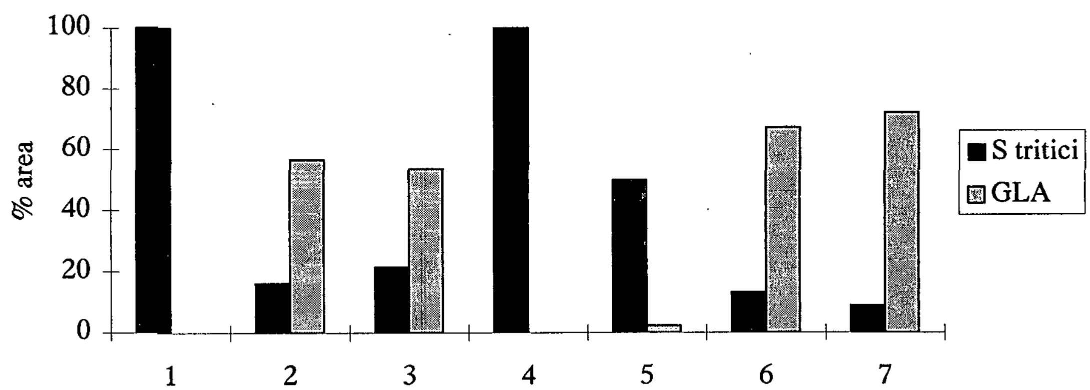
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 10. Boxworth, cv. Brigadier. Green leaf area (%) of each leaf layer.

Leaf 4, 4 July



Leaf 2, 25 July



Treatments

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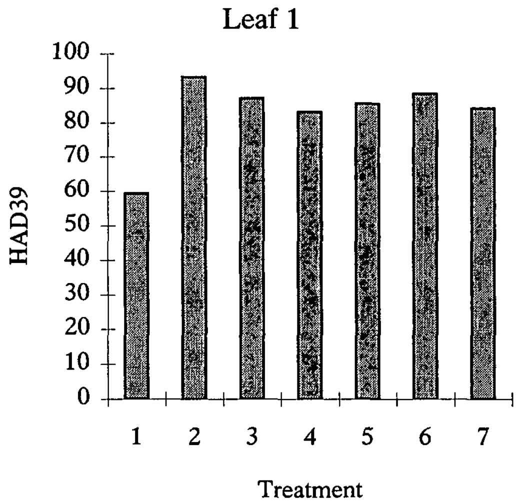
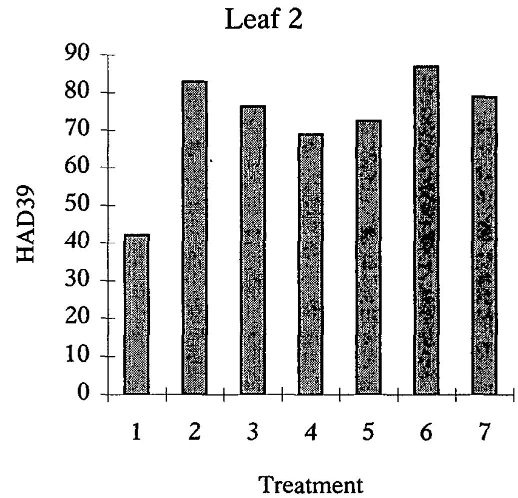
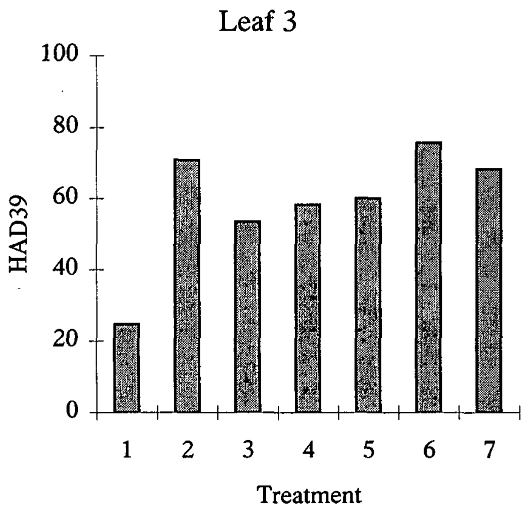
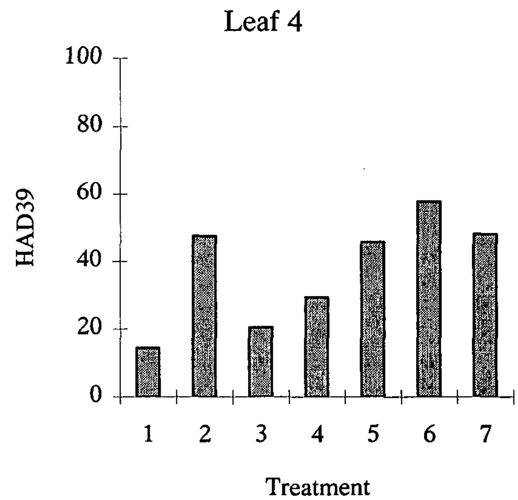
5 Amistar GS30, GS31, GS32 & GS39
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

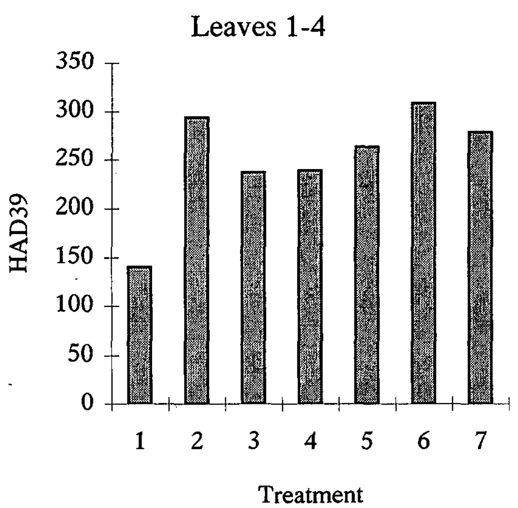
Figure 11. Rosemaund, cv. Consort. Effect of treatments on disease and green leaf area.











Treatments

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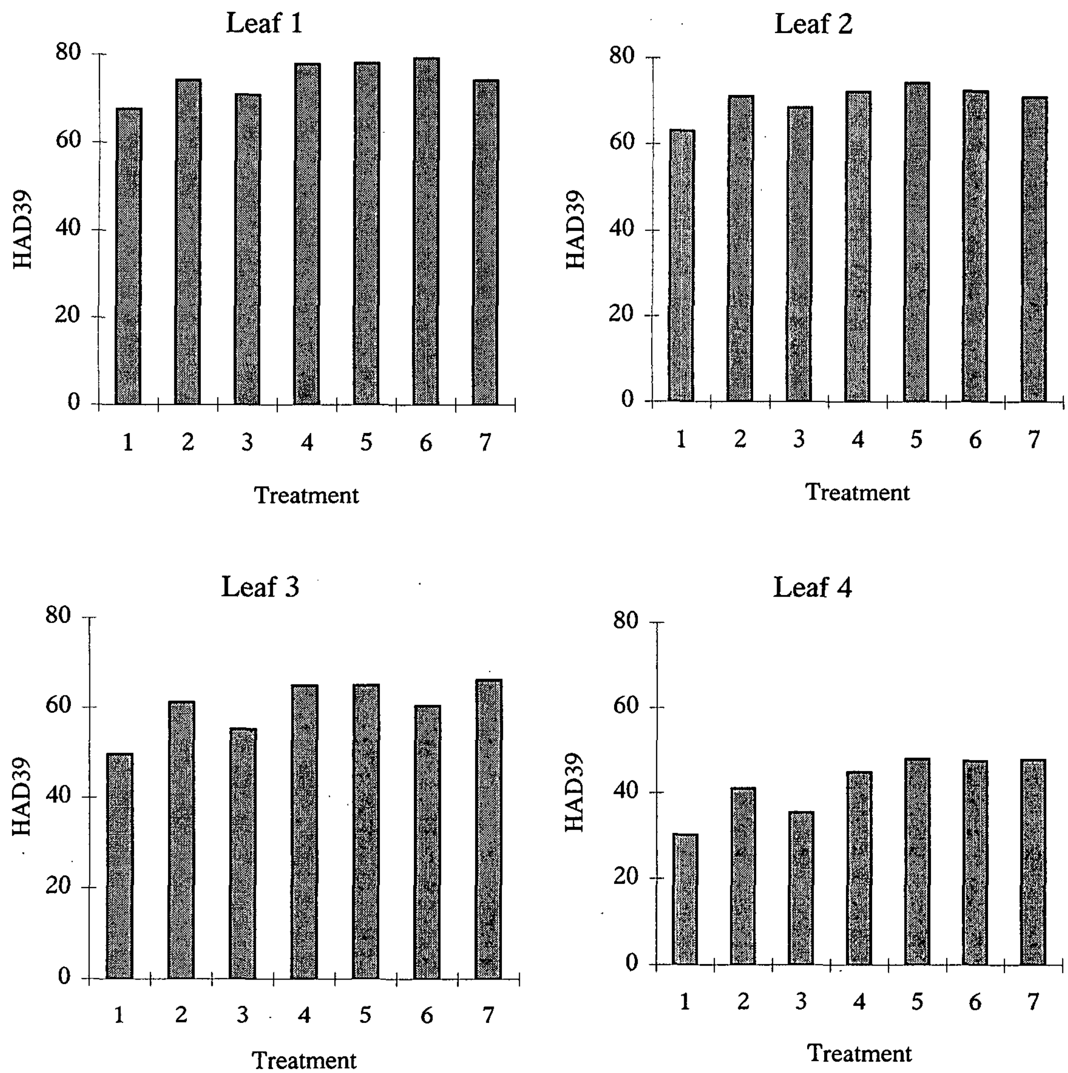
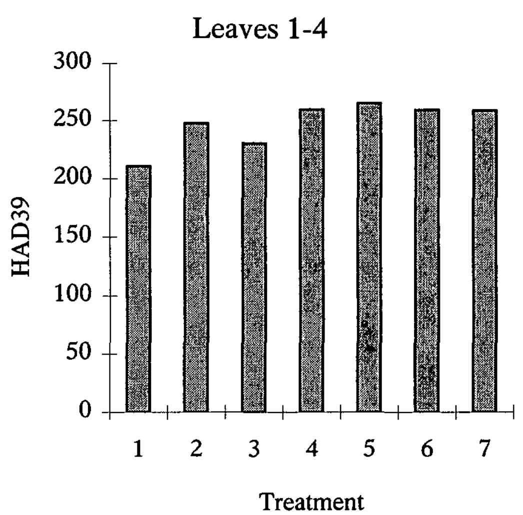
4 Amistar GS32 & GS39
5 Amistar GS30, GS31, GS32 & GS39
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 12. Rosemaund, cv. Consort. Healthy area duration from GS39 (HAD39)





Treatments

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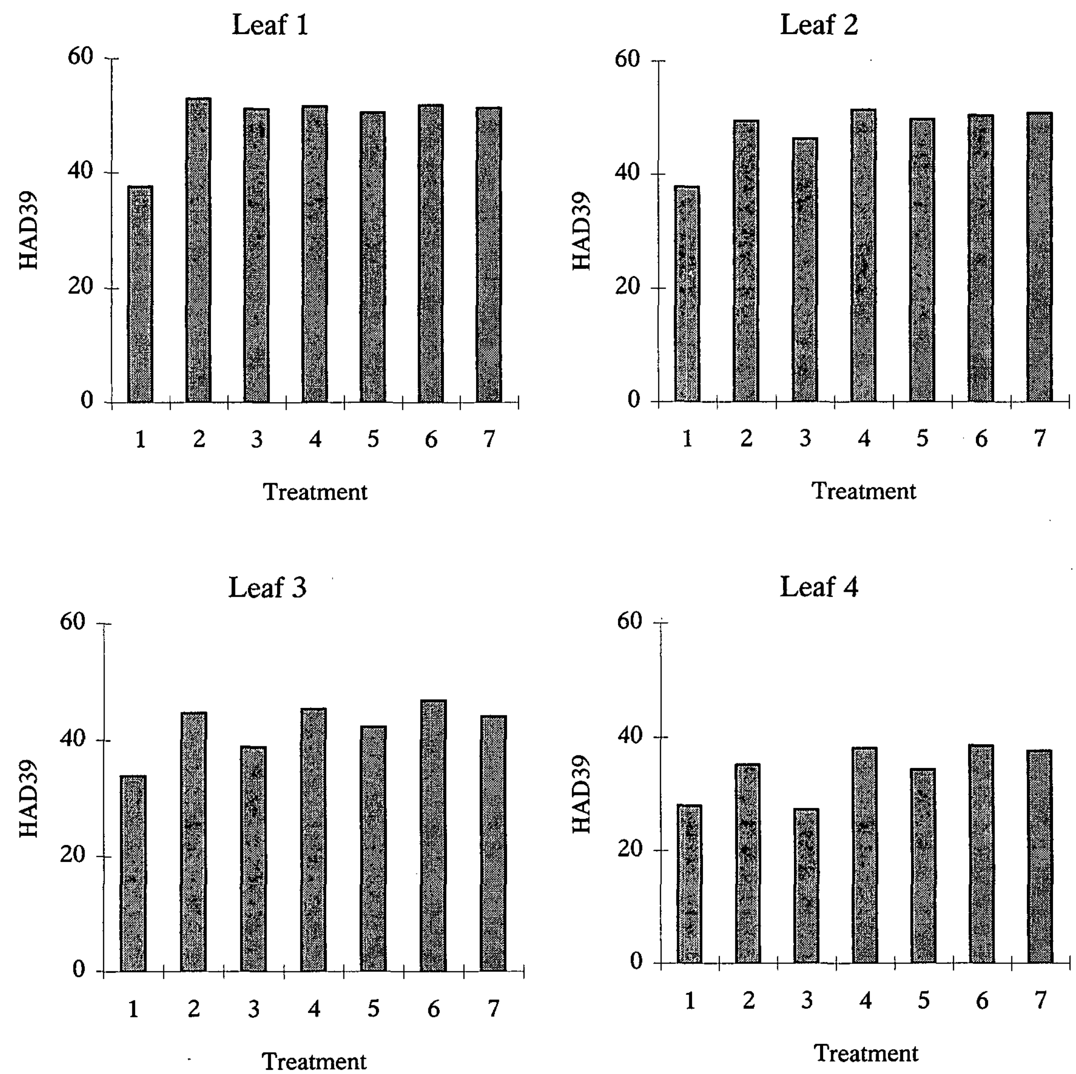
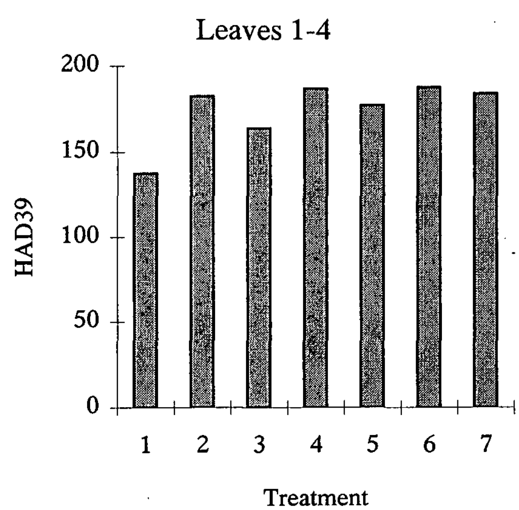
7 BAS494 GS32 & GS39

Figure 13. Boxworth, cv. Pastiche. Healthy area duration from GS39 (HAD39)





Treatments

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3 A9180 GS30; Opus GS39
4 Amistar GS32 & GS39
5 Amistar GS30, GS31, GS32 & GS39
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 14. Boxworth, cv. Brigadier. Healthy area duration from GS39 (HAD39)

On cv. Pastiche, all fungicides appeared to increase HAD39 on the top 2 leaves, though this was not statistically significant (Table 4, Figure 13). On leaves 3 and 4, all fungicides except A9108 followed by Opus significantly increased HAD39.

Table 4. Healthy area duration from GS 39 (HAD39), Boxworth, cv. Pastiche

Treatment		HAD39 L1	HAD39 L2	HAD39 L3	HAD39 L4	HAD39 L1-4
1	Untreated	67.6	63.1	49.5	30.2	210.4
2	Opus GS 32&39	74.1	71.1	61.1	40.9	247.2
3	A9180 & Opus	70.9	68.5	55.1	35.4	229.9
4	Amistar GS 32&39	77.8	72.2	64.8	44.8	259.6
5	Amistar x4	78.0	74.2	64.9	47.8	264.9
6	BAS494 GS 31&39	79.2	72.4	60.3	47.4	259.3
7	BAS494 GS 32&39	74.1	71.0	66.0	47.7	258.8
SED (12 df)		5.84	4.03	2.90	4.59	11.58
P		0.443	0.226	<0.001	0.012	0.004

On cv. Brigadier, all fungicides increased HAD39 on all leaf layers, except for A9180 followed by Opus on leaves 3 and 4 (Table 5, Figure 14). There were no significant differences between Opus and strobilurin treatments on any leaf layer or in the total HAD39 value for all 4 leaf layers.

Table 5. Healthy area duration from GS 39 (HAD39), Boxworth, cv. Brigadier

Treatment		HAD39 L1	HAD39 L2	HAD39 L3	HAD39 L4	HAD39 L1-4
1	Untreated	37.6	37.8	33.9	28.0	137.2
2	Opus GS 32&39	52.9	49.4	44.7	35.2	182.1
3	A9180 & Opus	51.1	46.2	38.9	27.3	163.5
4	Amistar GS 32&39	51.6	51.4	45.4	38.1	186.4
5	Amistar x4	50.5	49.7	42.3	34.4	176.9
6	BAS494 GS 31&39	51.8	50.4	46.8	38.5	187.4
7	BAS494 GS 32&39	51.3	50.8	44.1	37.6	183.7
SED (12 df)		2.24	2.17	2.58	2.30	5.30
P		<0.001	<0.001	<0.001	0.001	<0.001

Chlorophyll content and light transmission

Figure 15 gives the total chlorophyll concentration (μg/cm2) of leaves 1-3, in the untreated, Opus and BAS494 (GS 32 & 39) programmes at GS 39. The amount of chlorophyll in any of the untreated leaf layers was less than either of the two fungicide treatments. Chlorophyll concentrations were higher in each leaf layer in the BAS494 treatment compared with the Opus treatment with leaf 3 showing the greatest difference.

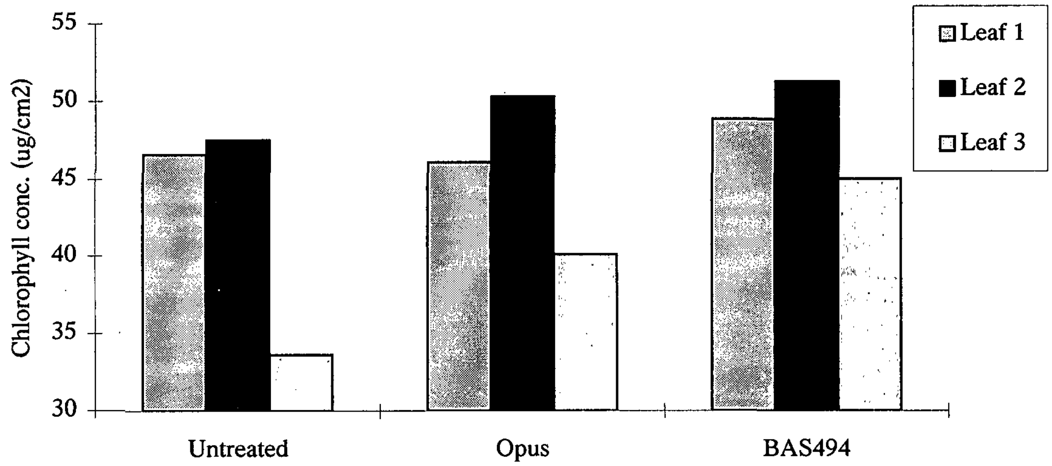


Figure 15. Chlorophyll concentration (ug/cm2) of Treatments 1, 2 and 7 at GS39

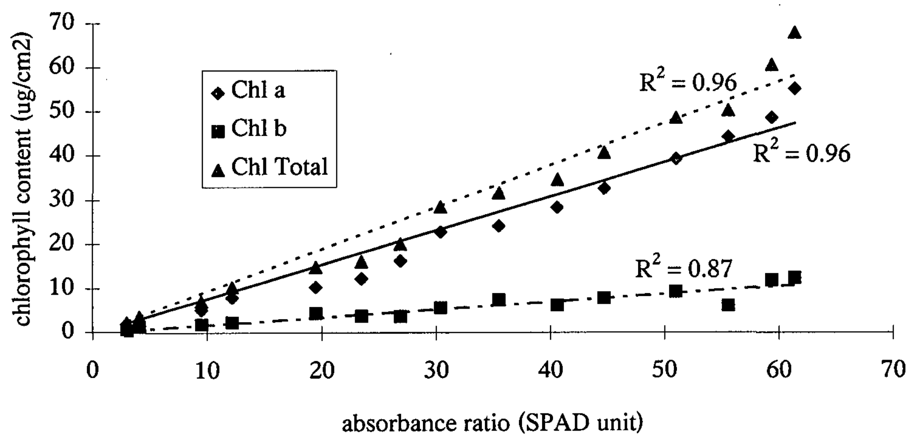


Figure 16. Chlorophyll (Chl) concentration (ug/cm2) of wheat leaves plotted against SPAD readings for Chl. a, Chl. b and total Chl.

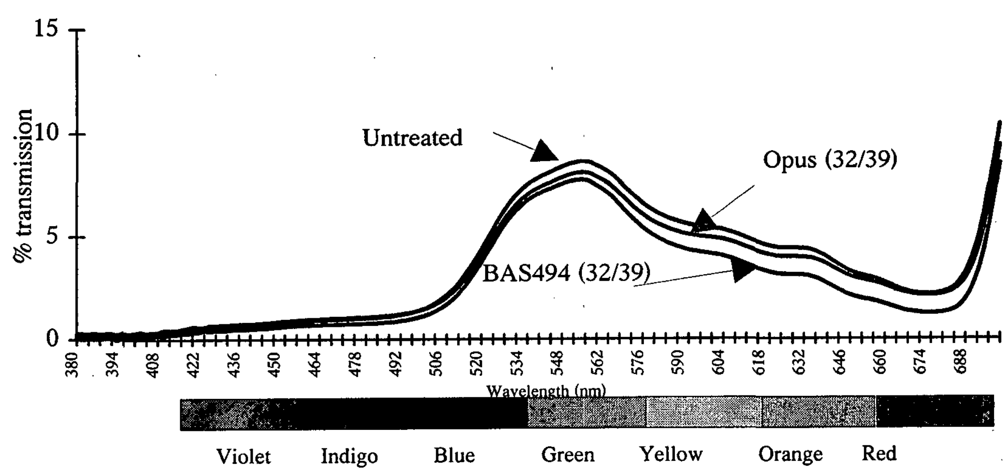


Figure 17. Percent spectral transmission in the visible spectrum at GS61 of Leaf 3 of Treatments 1, 2 and 7, cv. Pastiche

The comparison of SPAD data with chlorophyll extractions showed that there were good correlations between the SPAD measurements and the actual concentrations of both chlorophyll a and chlorophyll b (Figure 16).

Using the LI-COR spectroradiometer transmission spectral signatures of individual leaves from the untreated, Opus and BAS494 (GS 32 & 39) programmes on Pastiche were obtained (Figure 17). Chlorophyll absorbs 70 - 90% of light in the blue (*c.* 450 nm) to red (*c.* 670 nm) region of the visible spectrum, but less in the green region (*c.* 550 nm). Throughout the visible spectrum, both fungicide treatments transmitted less light than the untreated, with consistently less transmission in the BAS494 treatment than the Opus treatment.

Water-soluble carbohydrates

On cv. Consort, there were no significant differences between treatments in water-soluble carbohydrates in the stems at GS 65 (Table 6). By GS 87, these reserves had been fully depleted in the untreated plots, whereas fungicide treatments had detectable reserves which were, in all cases, significantly different from the untreated controls. There was significantly less in the A9180 followed by Opus and Amistar (x2) treatments than in BAS494 (GS 32 & 39).

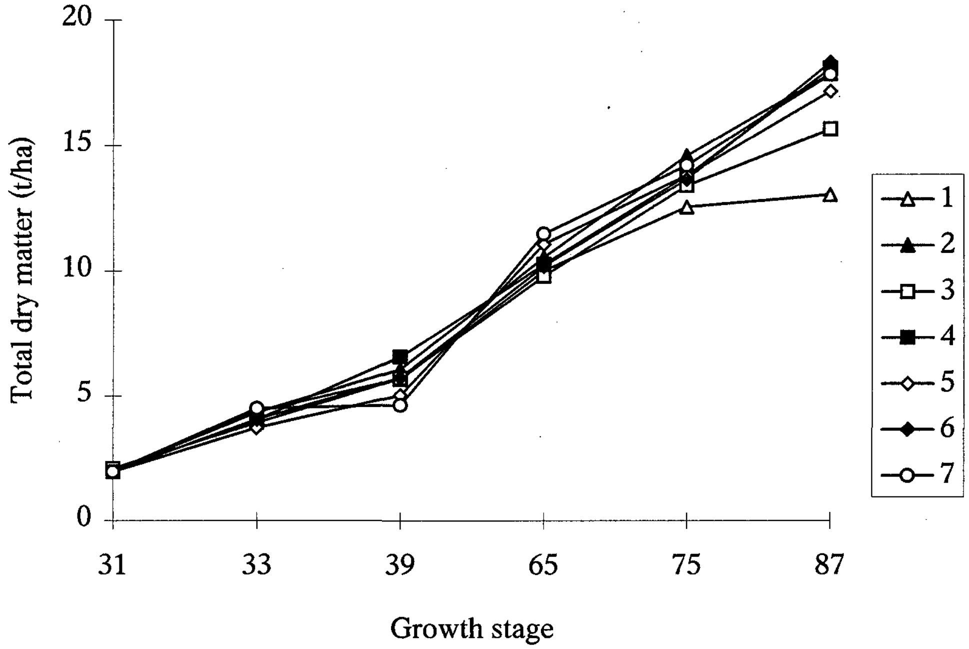
Water-soluble carbohydrate reserves were greater in cv. Pastiche than in cv. Consort. At GS 59, BAS494 (GS 32 & 39) had significantly greater reserves than the untreated control, A9180 followed by Opus and Amistar (x2). This difference was still evident at GS 83.

Table 6. Water-soluble carbohydrate (WSC) reserves in stems, cvs Consort and Pastiche

Treatment		WSC (t/ha) cv. Consort GS 65	WSC (t/ha) cv. Consort GS 87	WSC (t/ha) cv. Pastiche GS 59	WSC (t/ha) cv. Pastiche GS 83
1	Untreated	1.63	0.00	3.25	0.78
2	Opus GS 32&39	1.92	0.17	3.31	0.81
3	A9180 & Opus	1.68	0.12	3.21	0.91
4	Amistar GS 32&39	1.74	0.11	3.51	1.16
5	Amistar x4	2.20	0.18	3.58	1.09
6	BAS494 GS 31&39	1.87	0.19	3.41	1.13
7	BAS494 GS 32&39	1.85	0.22	3.79	1.21
SED (12 df)		0.361	0.038	0.146	0.113
P		0.765	0.002	0.022	0.010

Total crop biomass

Differences between treatments on cv. Consort did not become statistically significant until GS 83, although trends were becoming evident at GS 75 (Figure 18). At GS 83, the untreated control had 2.6 t/ha less dry matter than A9180 followed by Opus, and other treatments had between 4.1 and 5.3 t/ha greater dry matter than the untreated control. BAS494 (GS 31 & 39) had the highest total biomass, 18.3 t/ha.



1 Untreated
2 Opus GS32 & GS39
3 A9180 GS30; Opus GS39
4 Amistar GS32 & GS39
5 Amistar GS30, GS31, GS32 & GS39
6 BAS494 GS31 & GS39
7 BAS494 GS32 & GS39

Figure 18. Rosemaund, cv. Consort, total biomass

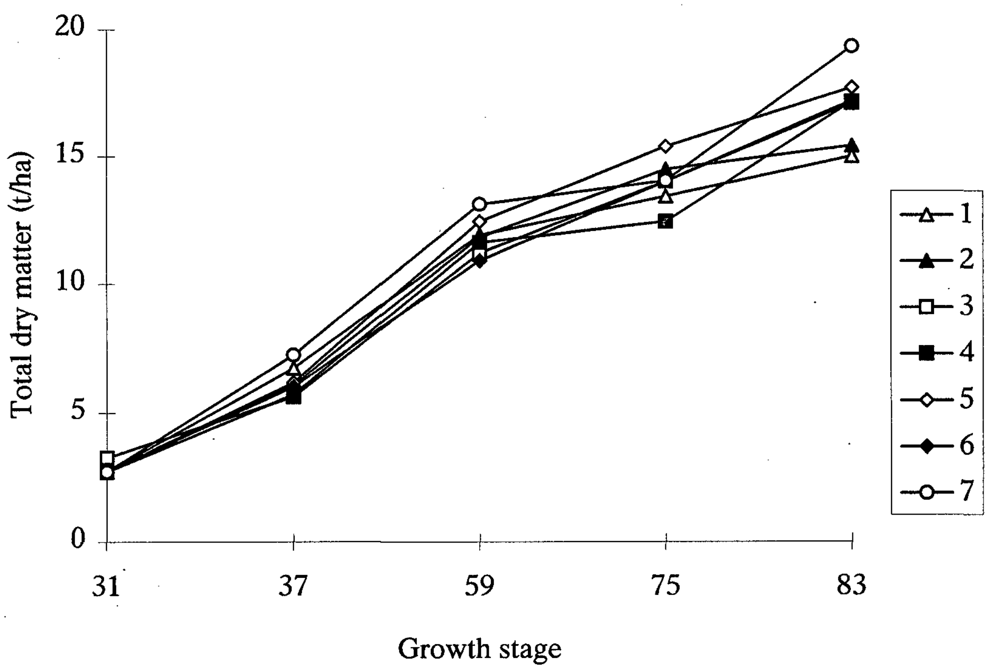


Figure 19. Boxworth, cv. Pastiche, total biomass

Differences between treatments were not significant at any stage on cv. Pastiche (Figure 19). The highest dry matter at GS 83 was recorded in BAS494 (GS 32 & 39) and the lowest in the untreated control and the Opus programme.

Yield

All treatments gave large yield increases on cv. Consort, in the range 4.29-6.06 t/ha above the untreated yield of 3.75 t/ha (Table 7, Figure 20). The highest yields were from BAS494, with the programme starting at GS 31 having the greater yield, significantly greater than all treatments except the other BAS494 programme and Opus.

Yield increases were much smaller on cv. Pastiche, in the range 0.42-1.03 t/ha, but all were significantly above than the untreated yield of 6.68 t/ha (Table 8, Figure 21). The BAS494 programmes had significantly higher yields than the Opus programme.

On cv. Brigadier, all treatments increased yield significantly over the untreated yield of 5.63 t/ha, but there were no significant differences between fungicide treatments, with increases in the range 2.04-2.58 t/ha (Table 9, Figure 22).

Harvest index

All fungicides increased harvest index significantly on all cultivars, with the exception of A9180 followed by Opus on cv. Pastiche (Tables 7-9). On cv. Consort, harvest indices in BAS494 treatments were higher than in all other fungicide treatments, with the exception of Amistar (x4). On cv. Pastiche, there were no significant differences between fungicide treatments, other than the lower index from A9180 followed by Opus. On cv. Brigadier, BAS494 (GS 32 & 39) had a significantly higher harvest index than any other fungicide treatment but there were no differences between other fungicide treatments.

Specific weight and thousand grain weight

On cv. Consort, all treatments gave large increases in both specific weight and thousand grain weight (Table 7). The thousand grain weights of Amistar treatments were lower than those of other fungicides, a difference which was statistically significant for BAS494 (GS 32 & 39). The only significant difference between fungicide treatments in specific weight was that BAS494 (GS 32 & 39) had higher specific weight than Amistar (x2).

Thousand grain weight of cv. Pastiche was increased significantly by the BAS494 treatments, but not by others (Table 8). There were no significant differences in specific weight.

On cv. Brigadier, there were large increases in both specific weight and thousand grain weight from all fungicide treatments (Table 9). The only significant difference between fungicides was that BAS494 (GS 32 & 39) and Amistar (x2) had higher thousand grain weight than A9180 followed by Opus.

Grain protein

On cvs Consort and Brigadier, there was large, statistically significant reductions in grain protein from all fungicide programmes (Tables 7 & 9). There were no significant effects of fungicide on protein on cv. Pastiche (Table 8).

Table 7. Yield, grain quality and harvest index, Rosemaund, cv. Consort

Treatment		Yield (t/ha)	Specific weight (kg/hl)	Thousand grain weight (g)	Grain protein %	Harvest index
1	Untreated	3.75	61.1	30.4	12.6	43.0
2	Opus GS 32&39	8.88	69.5	46.4	11.3	53.0
3	A9180 & Opus	8.04	69.0	45.5	10.9	53.8
4	Amistar GS 32&39	8.13	68.3	43.1	11.1	53.3
5	Amistar x4	8.48	69.0	43.2	10.9	54.6
6	BAS494 GS 31&39	9.81	69.6	46.8	11.3	58.1
7	BAS494 GS 32&39	9.22	70.1	47.6	11.1	56.0
SED (12 df)		0.494	0.71	1.95	0.33	1.76
P		<0.001	<0.001	<0.001	<0.001	<0.001

Table 8. Yield, grain quality and harvest index, Boxworth, cv. Pastiche

Treatment		Yield (t/ha)	Specific weight (kg/hl)	Thousand grain weight (g)	Grain protein %	Harvest index
1	Untreated	6.68	78.8	48.2	12.7	48.0
2	Opus GS 32&39	7.10	78.5	49.9	13.2	49.0
3	A9180 & Opus	7.21	78.6	47.1	13.0	47.7
4	Amistar GS 32&39	7.33	79.1	50.3	12.8	50.3
5	Amistar x4	7.27	78.5	49.7	13.0	50.3
6	BAS494 GS 31&39	7.71	79.0	51.5	13.0	49.3
7	BAS494 GS 32&39	7.63	79.0	52.4	12.8	49.7
SED (12 df)		0.178	0.41	1.45	0.19	0.38
P		<0.001	0.551	0.046	0.272	<0.001

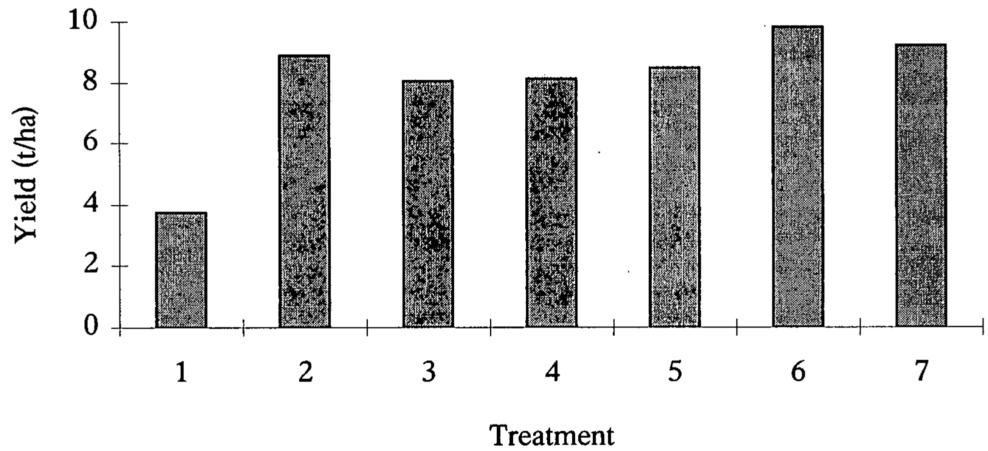


Figure 20. Rosemaund, cv. Consort, effect of treatments on yield.

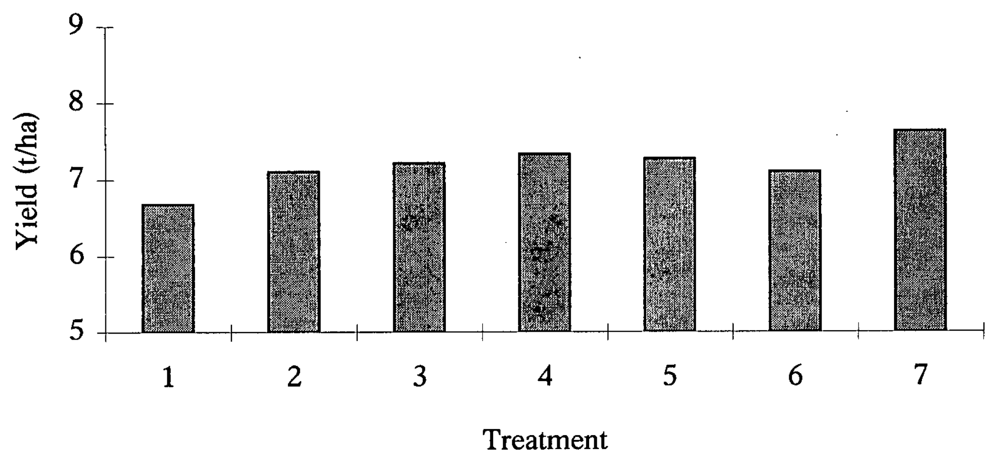


Figure 21. Boxworth, cv. Pastiche, effect of treatments on yield.

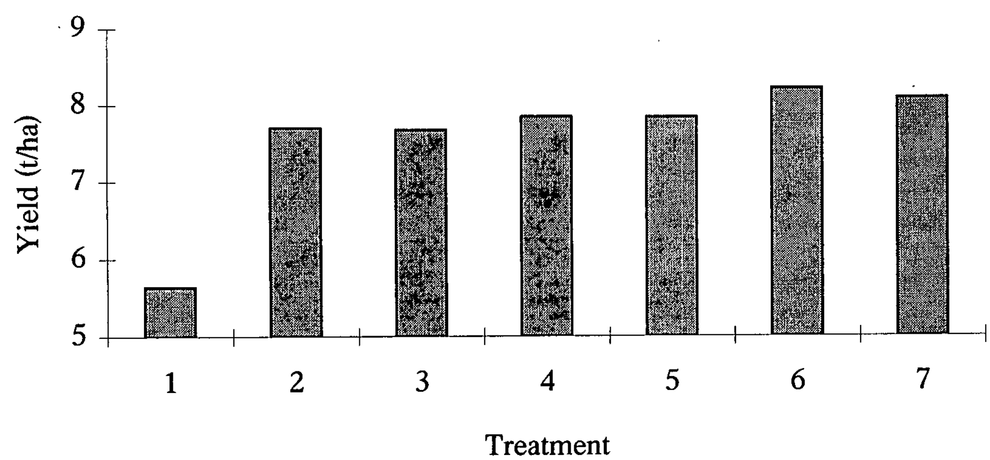


Figure 22. Boxworth, cv. Brigadier, effect of treatments on yield.

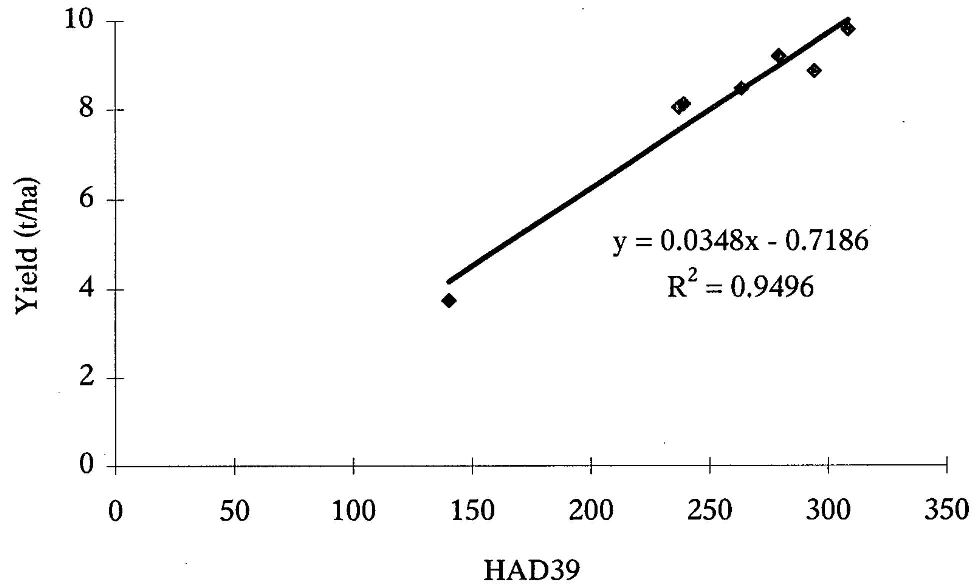


Figure 23. Rosemaund, cv. Consort, relationship between canopy duration and yield.

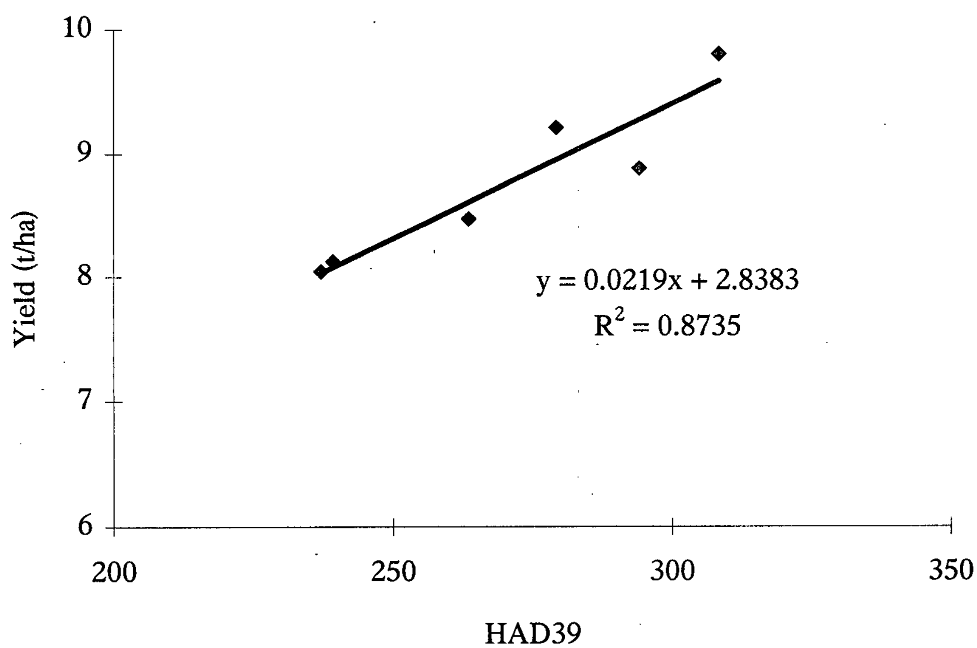


Figure 24. Rosemaund, cv. Consort, relationship between canopy duration and yield, omitting untreated control.

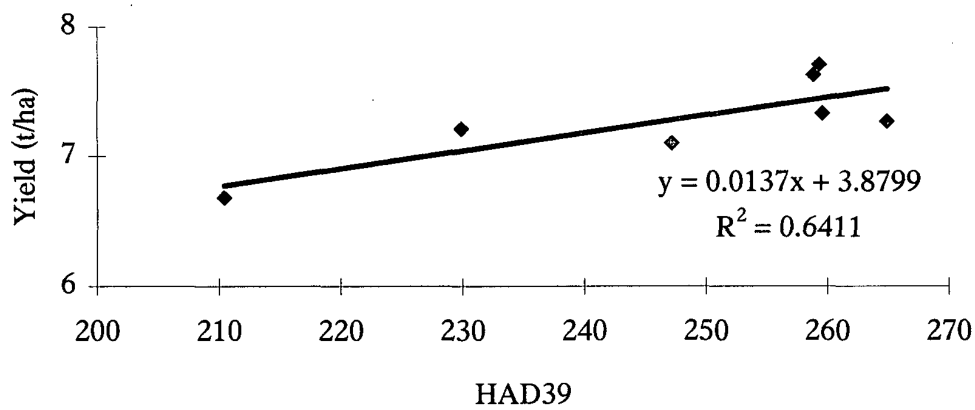


Figure 25. Boxworth, cv. Pastiche, relationship between canopy duration and yield.

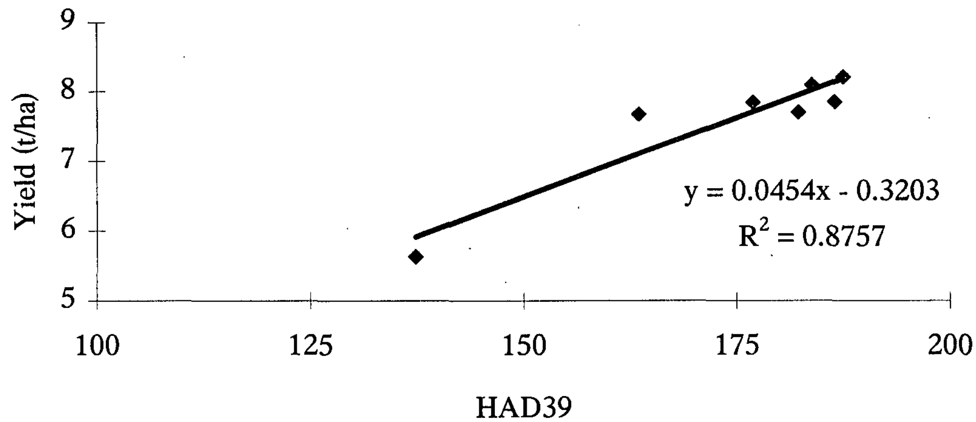


Figure 26. Boxworth, cv. Brigadier, relationship between canopy duration and yield.

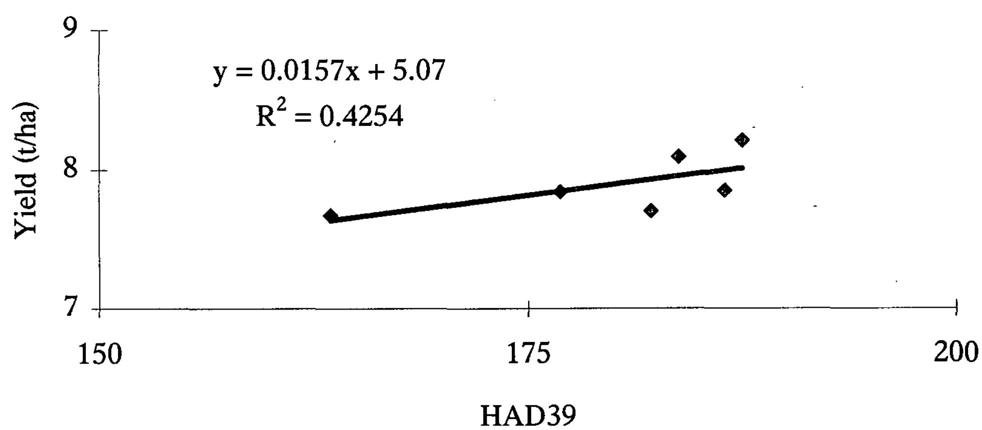


Figure 27. Boxworth, cv. Brigadier, relationship between canopy duration and yield, omitting untreated control.

Table 9. Yield, grain quality and harvest index, Boxworth, cv. Brigadier

Treatment		Yield (t/ha)	Specific weight (kg/hl)	Thousand grain weight (g)	Grain protein %	Harvest index
1	Untreated	5.63	69.1	34.7	10.7	46.0
2	Opus GS 32&39	7.70	76.1	45.4	10.1	52.0
3	A9180 & Opus	7.67	75.9	43.1	10.4	51.7
4	Amistar GS 32&39	7.85	76.2	46.2	10.1	52.3
5	Amistar x4	7.84	75.5	44.5	10.0	51.3
6	BAS494 GS 31&39	8.21	76.4	45.5	10.2	52.3
7	BAS494 GS 32&39	8.09	76.4	47.1	10.1	53.7
SED (12 df)		0.309	0.44	1.44	0.10	0.61
P		<0.001	<0.001	<0.001	<0.001	<0.001

Relationship between canopy size and yield.

To determine whether the effects of fungicides on yield are caused solely or largely through increases in green canopy size or duration, regressions were done for each cultivar of yield of each treatment on HAD39 for that treatment (Bryson *et al.*, 1997). On cv. Consort, there was a highly significant regression ($r^2 = 0.95$) (Figure 23). Because the untreated control exerts a large influence on the regression, it was repeated without the untreated control (Figure 24). This regression accounted for a slightly lower proportion of the variance ($r^2 = 0.87$). On cv. Brigadier, the regression which included the untreated control also accounted for a large proportion of the variance ($r^2 = 0.88$), but omission of the untreated control reduced the percentage of variance accounted for to 43% (Figures 26 & 27). On cv. Pastiche, a regression including the untreated accounted for 64.1% of the variance (Figure 25).

DISCUSSION

This project was a one-year pilot study, designed primarily to show whether strobilurin fungicides have effects on crop growth, yield and quality which are not related to disease control. With only three field experiments in one year, the results from this project should be regarded as indicators of areas worthy of further investigation in the new three year HGCA-funded project (0026/01/97), rather than definitive answers.

Previous findings on the persistence of disease control and green leaf retention from kresoxim-methyl plus epoxiconazole were confirmed on cvs Consort and Brigadier. There were indications that the kresoxim-methyl co-formulation (BAS494) was slightly more effective than epoxiconazole alone (Opus) for *S. tritici* control but, since Opus alone gave very good disease control, there was little scope for the mixture to demonstrate any superiority. Azoxystrobin (Amistar) controlled both *S. tritici* and yellow rust, but it was clearly less effective than Opus against *S. tritici*, even in the programme of 4 full rate sprays which commenced at GS 30, i.e. before the final 4 leaves started to emerge. The protectant activity of Amistar was clearly insufficient to compensate for lack of the eradicant activity of azole fungicides. Amistar was also slightly less effective than Opus against yellow rust, in that there was a little late development of yellow rust in both Amistar treatments on cv. Brigadier, whereas there was none in the Opus and BAS494 treatments.

The plant activator benzothiadiazole (A9180) is regarded primarily as a mildew fungicide (Ruess *et al.*, 1996). At Rosemaund, cv. Consort was used for this experiment rather than cv. Riband to increase the likelihood of gathering some information on mildew control, but the *S. tritici* epidemic was so severe that mildew was of negligible importance. There was no evidence that A9180 had any activity against either *S. tritici* or yellow rust. It was clear that, where there was a severe epidemic of either *S. tritici* or yellow rust, A9180 could not substitute for the GS 32 application of an azole fungicide.

Where there was severe disease, effects of treatments on canopy were largely in line with what would be expected from the disease control. However, there were indications on all 3 cultivars that BAS494 treatments had greater green area than Opus, although disease control was comparable. The differences were not large, but were consistent and need confirmation. On cv. Pastiche, in the absence of severe disease, Amistar treatments maintained greater green area on leaves 3 and 4 as these leaves started to senesce, but Amistar was less effective than Opus in maintaining green canopy where disease did develop, particularly when subjected to severe *S. tritici* on cv. Consort.

The work at Boxworth on chlorophyll content indicated BAS494 maintained a higher chlorophyll content in the leaves than Opus. This effect was most marked in older leaves, and was presumably caused by kresoxim-methyl. The lower light transmission through leaves treated with BAS494 suggests that there is greater light absorption in lower green leaves following kresoxim-methyl plus epoxiconazole treatment compared with epoxiconazole alone. However, further work is needed to show if greater chlorophyll content and light absorption by lower leaves is associated with increased photosynthesis. If there are beneficial effects of kresoxim-methyl (and any other fungicide) on photosynthesis, this could have implications for the optimum canopy structure of the crops. Increased photosynthetic potential in lower stem leaves would have little overall effect on crop growth if there is a dense canopy but, in a more open canopy (say, GAI 5-6 or less), maintaining greater photosynthesis in lower leaves could be beneficial.

The calibration of the SPAD meter readings with actual chlorophyll content of leaves showed a good relationship on one cultivar at one site. However, further work is required with different site/season/sultivar combinations to establish whether use of a SPAD meter will provide a rapid, non-destructive method of estimating chlorophyll content.

Strobilurin treatments did give small increases in stem water-soluble carbohydrates (WSC) on cv. Pastiche, which may have contributed to the yield effects of these treatments. Although there were no significant effects of treatments on cv. Consort at GS 65, the WSC levels were relatively low in all treatments. This was consistent with other cultivars at Rosemaund in 1997, and was associated with prolonged dull weather around ear emergence and anthesis. It is possible that, in a season more favourable for WSC reserves to be laid down, treatments which have early effects on disease control could allow greater stem reserves to be accumulated which could act as a buffer against later loss of canopy associated with disease.

There were small but consistent yield advantages from the BAS494 treatments compared with Opus on all cultivars, even though differences between the fungicides were small. Comparing the GS 32 & 39 programmes of these two products, the smallest benefit from addition of kresoxim-methyl was on cv. Consort, where *S. tritici* was very severe. On all 3 cultivars, there were indications that BAS494 at GS 31 & 39 gave higher yields than the programmes which commenced at GS 32, although this was not statistically significant. This did not appear to be related to differences in disease or canopy size or duration. and needs further study to determine whether it is a real effect. Amistar was inferior to Opus on cv. Consort, consistent with its poorer control of severe *S. tritici*, but Amistar gave slightly higher yields than Opus (though lower than BAS494) in the absence of disease on cv. Pastiche, and also on cv. Brigadier, despite being less effective against yellow rust.

The relationship between green canopy and yield was particularly close for cv. Consort, whether or not the untreated control was included in the regression. The poorer relationships on cvs Pastiche and Brigadier (when the undue influence of the untreated control was removed) suggest that, on these cultivars, there may be factors other than canopy size and duration which affect yield. In this context, it is worth noting that, on both these cultivars, the yields for the BAS494 treatments lie above the regression line, whereas those for Amistar lie on or below. This is far from being conclusive evidence that kresoxim-methyl is having effects on crop yield other than those associated with disease control, but is clearly worth investigating further.

These regressions, particularly on cv. Consort, provide further evidence that HAD39 is a useful measure of green canopy and therefore of photosynthetic activity leading to yield accumulation, even though it is recognised that some assumptions are made; for example, that all green canopy is equally useful, regardless of position on the plant.

The large positive effects of treatments on specific weight and thousand grain weight on crops with severe disease in untreated controls are what would be expected. Similarly, the reduction in grain protein from fungicide treatment on cvs Consort and Brigadier is clearly a dilution effect which would be expected where there are large increases in yield. In the absence of severe disease, on cv. Pastiche, there was no evidence of any effect of treatments on protein content.

This one year pilot study has provided evidence of greater canopy persistence from kresoxim-methyl than would be expected from disease control alone, and has also shown that it can increase leaf chlorophyll content and light absorption. These provisional conclusions will be tested as part of the successor project (0026/01/97) which commences in 1998, since it is important to understand how consistent these benefits are across sites and seasons, and on cultivars which differ in susceptibility to disease and responsiveness to fungicides. There was some evidence that azoxystrobin had similar effects on canopy retention where disease levels were low but, under conditions of severe disease, its limitation as a fungicide compared with epoxiconazole prevented any other potential benefits from being expressed. It is clear, from this study and from other work in 1997, that Amistar should be mixed with an azole fungicide under conditions where severe disease is anticipated. However, it is important to understand whether there are other beneficial effects of Amistar under conditions of less severe disease, and whether these effects could be affected adversely by mixture with an azole fungicide. This will also be investigated in the successor project.

ACKNOWLEDGEMENTS

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REFERENCES

Bruinsma, J. (1963). The quantitative analysis of chlorophylls *a* and *b* in plant extracts. *Photochemistry and Photobiology*, **2**, 241-249.

Bryson, R. J., Paveley, N. D., Clark, W. S., Sylvester-Bradley, R. & Scott, R. K. (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.

Lichtenthaler, H.K. & Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochemical Society Transactions*, **11**, 591-592.

Ruess, W., Mueller, K., Knauf-Beiter, G., Kunz, W. & Staub, T. (1996). Plant activator CGA245704: an innovative approach for disease control in cereals and tobacco. *Brighton Crop Protection Conference - Pests & Diseases 1996*, 53-60.