

SESSION 9B

INTERACTIONS BETWEEN PEST AND DISEASE CONTROL AND CROP PHYSIOLOGY

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Improving and exploiting self-defence against wheat diseases

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ABSTRACT

Disease escape and tolerance can contribute valuable levels of self-defence against *Septoria tritici* in winter wheat. Epidemic progress on the yield forming leaves was delayed by up to one latent period by disease escape, which reduces the efficiency of pathogen dispersal. Disease tolerance, which reduces yield loss per unit reduction of green canopy (due to disease), differed by 30% between the most and least tolerant varieties. Currently, breeding programmes do not actively select for disease escape or tolerance. The combined benefits of escape, resistance and tolerance are not accounted for adequately in disease management decisions.

INTRODUCTION

Key targets for wheat breeding in the UK are yield, quality and standing power. Disease resistance ranks no higher than fourth in breeders priorities (Brown, 2002). This strategy has delivered improvements to wheat yields of approximately 0.1 t ha⁻¹ per annum. However, this improvement is detected only in treated crops. Untreated yields have changed little, if at all, over the past two decades. Growers are thus dependant on substantial fungicide inputs to achieve the grain yield potential of modern cultivars.

Progress toward sustainable wheat production could be achieved if crops were more robust to disease. Improving and exploiting all the potential self-defence capabilities of plants might achieve this. Three self-defence mechanisms act in sequence to reduce crop loss. Escape inhibits spore transfer to the upper canopy, resistance reduces the capacity of spores that arrive on the upper leaves to infect and cause symptoms, and tolerance reduces the impact of disease on yield. Disease escape and tolerance have not been widely recognised, or selected in plant breeding, largely because they are not readily visible and have been difficult to quantify. The work reported here aims to quantify the potential value of these novel mechanisms of protection against *Septoria tritici* (anamorph of *Mycosphaerella graminicola*), and to identify traits and genes that confer them.

METHODS

Escape

Over seven site/seasons, four near isogenic lines of the septoria susceptible cultivar Mercia, which varied only for genes conferring different magnitudes of dwarfing (*rht*, *Rht2*, *Rht3* and *Rht10*), were grown in field experiments located in England. Disease severity was measured weekly in all experiments. The magnitude of each epidemic was quantified by the area under the disease progress curve (AUDPC) over the yield forming leaf layers (the top three stem leaves).

Tolerance

Over three crop seasons twenty-one varieties of winter wheat, variously susceptible to *Septoria tritici*, were grown at two sites in England. Disease area index and green area index (dimensionless planar area, of disease and green leaf respectively, expressed per unit of ground area that they cover) were measured weekly. An extended regression analysis was used to detect differences between varieties in the slope of the fitted straight lines of yield on both area under the disease area index curve (AUDAI) and healthy area duration, which is the integral of green area index (HAD, *sensu* Waggoner & Berger, 1987). Mixed linear models were fitted using a residual maximum likelihood (REML) method (Patterson & Thompson, 1971).

RESULTS & DISCUSSION

Escape

The number of spores moved vertically by rain-splash declines exponentially with distance (Shaw 1987: 1991). Therefore, due to the long latent period of *S. tritici* (210 day degrees), and the relatively short wheat phyllochron (~ 110 day degrees), yield-forming leaves can sometimes grow away from inoculum located on rosette leaves. For dwarf lines, however, the rate of stem extension is slowed, so that the distance between rosette and upper canopy leaves is small (Figure 1). Compared to the commercial variety (*rht*), reductions in plant height were of the order 20% (*Rht2*), 50% (*Rht3*) and 60% (*Rht10*). As a consequence, dwarfing caused earlier development of epidemics (Figure 2). The maximum displacement of epidemics (*i.e.*, between *rht* and *Rht10*) was of the order of one latent period. This effect is similar in magnitude to that observed for a well-timed application of triazole fungicide (*cf.* Paveley *et al.*, 2000). The *Rht2* dwarfing gene reduced height to an agronomically acceptable level, whilst maintaining most of the escape benefit of the taller *rht* line.

The effect of dwarfing on the relative magnitude of epidemics on the final 3 leaf layers was found to be consistent across sites and seasons. This suggests that there is no interaction between the environment and disease escape conferred by crop height. Thus, whilst disease pressure (the amount of viable inoculum) differs substantially across sites/seasons, the benefits from disease escape will be robust. Hence the economic optimum, or 'appropriate', dose of fungicide should be reduced by a predictable amount on cultivars with good escape characteristics.

Disease escape is entirely dependant on increasing the distance between inoculum and the yield forming leaves. However, this does not imply reliance upon tall crops to achieve the benefits from escape. Other canopy traits have been implicated to increase the distance between diseased leaves and the final three leaf layers. For example, Avalon, a relatively short cultivar, expresses disease escape due to features of leaf morphology (Lovell *et al.*, 1997). A doubled-haploid mapping population has been developed from a cross between parents that vary greatly

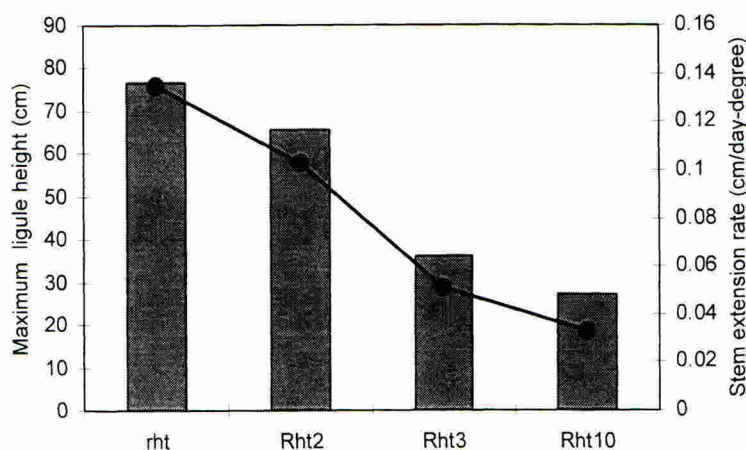


Figure 1. Flag leaf ligule height (bars) and rate of stem extension (line), for near isogenic lines of cv. Mercia varying for dwarfing genes, grown at Long Ashton, 2000 harvest.

for canopy traits believed to confer escape, such as crop height, stem extension rate, internodal length (inter-leaf distance), leaf length and leaf insertion angle. This population is being used to associate canopy traits, and the genes that control them, with disease escape.

Tolerance

Evidence for tolerance, measured as the response of yield to disease induced changes in HAD, was found across a comparison of 21 commercial cultivars. These differences were large enough to be of agronomic importance. A difference of approximately 30% was found in the sensitivity of yield to green area loss between the most and least tolerant cultivars. Tolerance was not detected directly through measurements of disease. However, symptom measurements do not provide accurate quantification of the impact of disease on green canopy area loss, and hence on light capture, and are thus less useful for describing yield loss relationships (Madden & Nutter 1995; Bryson *et al.*, 1997).

A plot of tolerance (measured as the slope of the relationship between yield and HAD) indicated that tolerance was associated weakly with low attainable yield (Figure 3). Gaunt (1981) suggested that, to demonstrate a variety as tolerant, the yield potential should be equivalent to non-tolerant varieties. This requirement could be stated more appropriately as a breeding objective. Tolerance present within modern lines does not occur as a result of specific selection of associated traits. During the 1980s to the mid-1990s, European wheat prices, and the relatively low costs of fungicides, reduced the commercial incentive to breed

varieties with good disease escape, resistance and tolerance. In addition, the UK variety trials system, which has placed great emphasis on treated yield, has acted to focus plant breeders to the achievement of greater attainable yield. Hence, breeding efforts might have inadvertently selected out tolerance traits.

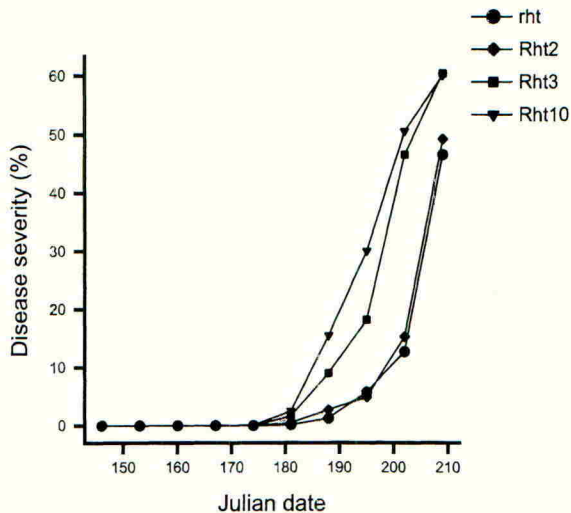


Figure 2. Disease progress on the flag leaf of near isogenic lines of cv. Mercia, varying for dwarfing genes that control internode length and stem extension rate, grown at High Mowthorpe 1999.

The analysis presented here focussed on recent commercial cultivars. Studies using a wider range of germplasm may help to elucidate tolerance mechanisms and identify tolerance traits that are compatible with productivity. For example:

- (i) Recent studies of the physiological changes, over the past three decades, in UK cultivars (Shearman, 2001) are being used to develop mechanistic hypotheses to explain variation in disease tolerance apparent over this period,
- (ii) In the spring wheat variety Miriam, the rate of carbon fixation per unit of chlorophyll was greater in diseased than in healthy plants (Zuckerman *et al.*, 1997).
- (iii) Field studies, using sequential measurements of crop growth and light interception to estimate radiation use efficiency, showed high RUE for the varieties identified as intolerant here e.g., Brigadier. In contrast, the tolerant varieties e.g., Mercia were shown to have lower RUE (Foulkes *et al.*, 1998). Empirical evidence suggests that this difference in RUE may be associated with the 1BL/1RS chromosome translocation (*ibid*), introduced to commercial cultivars in the mid-1980s. Indeed

three of the four cultivars shown to be tolerant in this study did not contain 1BL/1RS, whereas all the intolerant cultivars are known to carry this translocation. An association of intolerance with high RUE is plausible since each unit of green lamina area lost to disease would have been more productive than in a low RUE cultivar.

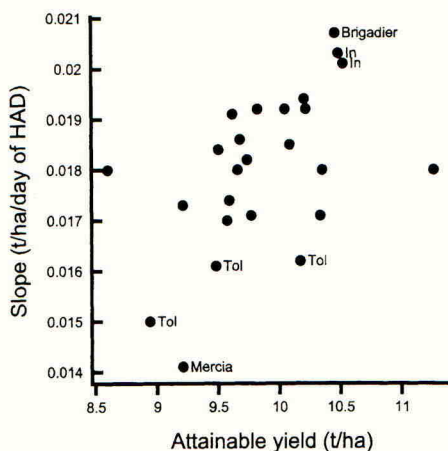


Figure 3. Disease tolerance (slope of the relationship between HAD and yield) against attainable yield (expressed as the mean fungicide treated yield). The most and least tolerant cultivars are identified. Cultivars not marked Tol (tolerant) or In (intolerant) do not differ for tolerance from the population mean.

CONCLUSION

Opportunities exist to complement conventional disease resistance with escape and tolerance mechanisms. Advances in breeding technology are improving the prospects for introducing additional self-defence traits. The greater challenge is likely to be in developing analytical frameworks, which allow breeders to balance trade-offs in traits that might be beneficial to some aspects of production and deleterious to others. For example, to reconcile disease escape and lodging risk. Progress towards such a framework would facilitate the more rational approach to crop design envisaged by Donald (1968).

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REFERENCES

- Brown J K M (2002). Yield penalties of disease resistance in crops. *Current Opinion in Plant Biology* **5**, 339-344.
- Bryson R J; Paveley N D; Clark N D; 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.
- Donald C M (1968). The breeding of crop ideotypes. *Euphytica* **17**, 385-403.
- Foulkes M J; Scott R K; Sylvester-Bradley R; Pickett A; (1998). *Variety typing trials and NIAB additional character assessments*. Home-Grown Cereals Authority Final Project Report No. 174, Volume V. HGCA, London.
- Gaunt R E (1981). Disease tolerance - an indicator of thresholds. *Phytopathology* **71**, 915-916.
- Lovell D J; Parker S R; Hunter T; Royle D J; Coker R R (1997). Influence of crop growth and canopy structure on the risk of epidemics by *Mycosphaerella graminicola* (*Septoria tritici*) in winter wheat. *Plant Pathology* **47**, 126-138.
- Madden L V; Nutter F W (1995). Modelling crop losses at the field scale. *Canadian Journal of Plant Pathology* **17**, 124-137.
- Patterson H D; Thompson R (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika* **58**, 545-554.
- Paveley N D; Lockley D; Vaughan T B; Thomas J; Schmidt K (2000). Predicting effective fungicide doses through observation of leaf emergence. *Plant Pathology* **49**, 748-766.
- Shaw M W (1987). Assessment of upward movement of rainsplash using a fluorescent tracer method and its application to the epidemiology of cereal pathogens. *Plant Pathology* **36**, 201-213.
- Shaw M W (1991). Variation in the height to which tracer is moved by splash during natural summer rain in the UK. *Agricultural and Forest Meteorology* **55**, 1-14.
- Shearman V J (2001). *Changes in the yield limiting processes associated with the genetic improvement of wheat*. PhD thesis, University of Nottingham, 238pp.
- Waggoner P E; Berger R D; (1987). Defoliation disease and growth. *Phytopathology* **77**, 393-398.
- Zuckerman E; Eshel A; Eyal Z (1997). Physiological aspects related to tolerance of spring wheat cultivars to *Septoria tritici* blotch. *Phytopathology* **87**, 60-65.

The influence of crop physiology on the development and impact of summer aphid infestations on wheat

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ABSTRACT

The potential impact of crop physiology on aphid reproduction and on the damage caused by summer infestations of aphids to wheat crops is discussed. Four field experiments assessed the implications of crop physiological parameters on decision making for cereal aphid control. Low stem soluble carbohydrate reserves increased the yield loss caused by aphids. Late nitrogen application which prolonged the grain filling period increased the peak numbers of aphids, but also decreased the impact of aphid feeding, so that the overall impact was neutral. Aphids increased more quickly in thinner canopies and, since yield loss was more closely correlated with aphid numbers per tiller than with numbers per unit area, were more damaging in such situations. Wet weather prevented aphid reproduction and tests to assess the impact of the prolongation of grain filling following the use of strobilurin fungicides were inconclusive.

INTRODUCTION

Direct yield loss to cereal crops caused by cereal aphids in the summer is mainly due to the grain aphid (*Sitobion avenae*) in the UK although the rose-grain aphid (*Metopolophium dirhodum*), may occasionally be damaging. In warmer climates the Russian wheat aphid (*Diuraphis noxia*) and the greenbug (*Schizaphis graminum*) are more important. Various authors have reported variations in aphid population dynamics associated with crop physiological parameters. The impact of aphid numbers on yield has also been found to be affected by crop physiology. Further studies have been conducted to understand how crop physiology during the grain filling period may impact on decision making for aphid control.

Crop physiology v aphid numbers

Many authors have reported a positive impact on aphid numbers from the use of nitrogen (Dixon, 1987). However, the resultant increase in canopy density may be deleterious to *S. avenae*, especially in cold conditions when temperature reduction may reduce growth rate (Honk, 1985), and the increased humidity may favour the spread of fungus disease (Duffield *et al.*, 1997). Stress can reduce canopy size, favouring aphid multiplication due to higher temperature, and increase the proportion of the plant's mass allocated to seeds, which also favours aphid increase through improved phloem sap quality. Plant breeding to increase the proportion of biomass in the seeds may have incidentally improved the performance of aphids on crops (Honk, 1990; 1991). The introduction of modern fungicide programmes has extended the grain filling period. Late aphid infestations now tend to occur more frequently, peaking during the latter part of the grain filling period. Such late infestation can still cause significant yield loss (Oakley & Walters 1994, Oakley *et al.*, 2002).

Crop physiology v impact of aphids

Rautapää (1966) correlated yield loss caused by aphid infestations with the number of aphid days recorded between particular growth stages. An aphid day is defined as one aphid feeding on one plant for 24 hours (Gerloff & Ortman, 1971; Kieckhefer *et al.*, 1995). Wratten (1978) introduced an aphid index, assigning a lower value to early instars, as a modification of the aphid day system, and found some improvement in yield relationships as a result (Lee *et al.*, 1981). However, the need to identify all aphids to instar to calculate the index makes this modification for impractical for large scale studies. The effect of aphid feeding on plants is similar to that caused by drought stress (Cabrera *et al.*, 1995). Oakley *et al.*, (2002) examined the rate of yield loss caused by infestations of *S. avenae* during the grain filling stages on a range of crops between 1994 and 1996 and found considerable variation in the rate of loss per aphid day. The variation between sites was from 0.8 to 2.9 kg ha⁻¹ yield loss per aphid day. Pilot experiments were conducted to establish whether crop agronomy influenced this variation and needed to be taken into account in determining the need for aphid control.

MATERIALS AND METHODS

High v low stem reserves and canopy persistence.

In one pilot experiment conducted at Terrington in Cambridgeshire in 1997, crop shades were used to investigate the effect of the ratio between the available soluble stem carbohydrate (source) and numbers of grain sites (sink). A source limited crop was produced by shading between GS31 and 39 and a sink limited crop by shading between GS 39 and 55 (Beed *et al.*, 1999).

The influence of soluble stem carbohydrate reserves and photosynthesis during the grain filling period was investigated at three sites in 1997. Sites were located at Bridgets in Hampshire, Boxworth in Cambridgeshire and High Mowthorpe in North Yorkshire. The experiments compared two varieties, Rialto with high soluble stem carbohydrate reserves and Hereward with lower levels, with and without a late urea application aimed at extending the period of active photosynthesis after anthesis. Crops were sown in October and a spray of cypermethrin at 25 g ai (Toppel 10 at 0.25 litre) ha⁻¹ was applied in November to prevent overwinter infestation with aphids. The urea was applied in late June at 130 kg of 46% urea prills in 350 litres water ha⁻¹. Three differential aphid treatments were applied to the plots to test the sensitivity of crops to aphids. Aphid cultures were added to the relevant treatments in mid-May at 15 pots per plot. Pirimicarb was applied at 140 g ai pirimicarb (280 g Aphox) for full rate and 46.7 g ai for 1/3 rate both in 200 litres water ha⁻¹. Treatments applied were:

- a) Hereward; plus foliar urea; plus aphids; unsprayed.
- b) Hereward; plus foliar urea; plus aphids; plus pirimicarb at 1/3 rate at GS61.
- c) Hereward; plus foliar urea; no aphids; plus full rate pirimicarb at GS61 & GS73.
- d) Hereward; no foliar urea; plus aphids; unsprayed.
- e) Hereward; no foliar urea; plus aphids; plus pirimicarb at 1/3 rate at GS61.
- f) Hereward; no foliar urea; no aphids; plus full rate pirimicarb at GS61 & GS73.
- g) Rialto; plus foliar urea; plus aphids; unsprayed.
- h) Rialto; plus foliar urea; plus aphids; plus pirimicarb at 1/3 rate at GS61.
- i) Rialto; plus foliar urea; no aphids; plus full rate pirimicarb at GS61 & GS73.

- j) Rialto; no foliar urea; plus aphids unsprayed.
- k) Rialto; no foliar urea; plus aphids; plus pirimicarb at 1/3 rate at GS61.
- l) Rialto; no foliar urea; no aphids; plus full rate pirimicarb at GS61 & GS73.

Aphid numbers were counted weekly on all plots weekly from 23 May to 17 July. Fertile tiller counts were done in mid-June and sub-samples analysed for soluble stem carbohydrate levels. The plots were harvested on 12 August when the central 2.43 m width was cut from each plot. A separate regression analysis was carried out on the individual plot results for the variety x urea treatments at each site.

Plant populations.

More open canopies are thought to favour aphid multiplication, which could have implications for canopy managed crops. To test possible implications experiments were conducted at the same three sites as in 1997 in 1998 using the cv. Reaper. Plots were sown with four different seed rates and these were challenged by four different aphid burdens established by the use of different aphid introduction rates and aphicide treatments as full rate pirimicarb. Treatments used were:

- a) 125 seeds m⁻²; no aphids added, sprayed with pirimicarb at GS 61 and once more if and when aphid numbers exceed 2 per tiller on any subsequent weekly count
- b) 125 seeds m⁻², 3 pots of aphid culture per plot added, no aphicides
- c) 125 seeds m⁻², 15 pots of aphid culture per plot added, no aphicides
- d) 250 seeds m⁻²; no aphids added, sprayed with pirimicarb at GS 61 and once more if and when aphid numbers exceed 2 per tiller on any subsequent weekly count
- e) 250 seeds m⁻², 3 pots of aphid culture per plot added, no aphicides
- f) 250 seeds m⁻², 15 pots of aphid culture per plot added, no aphicides
- g) 375 seeds m⁻²; no aphids added, sprayed with pirimicarb at GS 61 and once more if and when aphid numbers exceed 2 per tiller on any subsequent weekly count
- h) 375 seeds m⁻², 3 pots of aphid culture per plot added, no aphicides
- i) 375 seeds m⁻², 15 pots of aphid culture per plot added, no aphicides
- j) 500 seeds m⁻²; no aphids added, sprayed with pirimicarb at GS 61 and once more if and when aphid numbers exceed 2 per tiller on any subsequent weekly count
- k) 500 seeds m⁻², 3 pots of aphid culture per plot added, no aphicides
- l) 500 seeds m⁻², 15 pots of aphid culture per plot added, no aphicides

Aphid numbers were counted weekly on all plots from 21 May to 16 July. Fertile tiller counts were done on 12 June and sub-samples analysed for soluble stem carbohydrate levels. The plots were harvested in August when the central 2.43 m width was cut from each plot.

Fungicide programmes.

The introduction of strobilurin fungicides, which prolong canopy life and increase yield, was thought liable to change the aphid susceptibility of crops as had urea application. The influence of fungicide programmes was tested at three sites in 1999 comparing three fungicide programmes. Programme A utilised conventional fungicides at GS 32, 39 and 59, programme B substituted a strobilurin fungicide at GS 32 and 39 and programme C used a strobilurin at all three timings.

Fungicide programmes were:

- A GS32 epoxiconazole as Opus (0.5 litre ha⁻¹); GS39 epoxiconazole (1 litre ha⁻¹); GS59 tebuconazole as Folicur (0.5 litre ha⁻¹).
- B GS32 epoxiconazole plus kresoxim-methyl as Landmark (0.5 litre ha⁻¹); GS39 epoxiconazole plus kresoxim-methyl (1.0 litre ha⁻¹); GS59 tebuconazole (0.5 litre ha⁻¹) + azoxystrobin as Amistar (0.5 litre ha⁻¹).
- C GS32 epoxiconazole plus kresoxim-methyl (0.5 litre ha⁻¹); GS39 epoxiconazole plus kresoxim-methyl (1.0 litre ha⁻¹); GS59 epoxiconazole plus kresoxim-methyl (1.0 litre ha⁻¹)

Treatments used were:

1. Fungicide programme A, no aphids added, pirimicarb at GS 61
2. Fungicide programme A, no aphids added
3. Fungicide programme A, 3 pots of aphids per plot
4. Fungicide programme A, 15 pots of aphids per plot
5. Fungicide programme B, no aphids added, pirimicarb at GS 61
6. Fungicide programme B, no aphids added
7. Fungicide programme B, 3 pots of aphids per plot
8. Fungicide programme B, 15 pots of aphids per plot
9. Fungicide programme C, no aphids added, pirimicarb at GS 61
10. Fungicide programme C, no aphids added
11. Fungicide programme C, 3 pots of aphids per plot
12. Fungicide programme C, 15 pots of aphids per plot

RESULTS

High v low stem reserves and canopy persistence.

At Terrington early shading of the crop improved aphid performance by reducing canopy size, later shading increased the significance of aphid infestations (Table 1).

Table 1. Regression analysis of yield loss against aphid burden for shaded crops at Terrington in 1997.

Treatment	slope (kg ha ⁻¹ yield loss per aphid day)	<i>P</i>	<i>R</i> ²	maximum aphid days on plots
Shaded 31-39	0.75 ± 0.41	0.088	19.4	789
Shaded 39-55	1.32 ± 0.59	0.042	21.1	605
No shading	0.49 ± 1.54	0.755	0.7	364

At Boxworth, the urea treatment caused some scorching, which counteracted its effect as a fertiliser and no yield response was obtained from the crop. Similar degrees of sensitivity were found between the four crop treatments. At Bridgets and High Mowthorpe, where the canopy life was extended by the use of urea, yield loss relationships were not changed in Rialto, but the low stem carbohydrate variety Hereward showed a differential response with urea decreasing the crops' sensitivity to aphid damage (Tables 2 & 3).

Table 2. Total aphid days (AD) and yield (tonnes ha⁻¹ @ 85% d.m.) in 1997 experiments

Treatment	Boxworth		Bridgets		High Mowthorpe	
	AD	Yield	AD	Yield	AD	Yield
Hereward						
a. urea, unsprayed	1340	6.82	447	8.14	285	6.18
b. urea, third rate	281	8.08	102	8.44	50	6.37
c. urea, twice	90	8.32	25	8.48	33	6.34
d. none, unsprayed	1607	6.78	336	7.55	220	6.16
e. none, third rate	383	8.07	105	8.17	43	6.42
f. none, twice	89	8.48	40	8.36	21	6.29
Rialto						
g. urea, unsprayed	1185	7.96	447	8.56	236	7.03
h. urea, third rate	296	8.90	102	8.94	69	7.22
i. urea, twice	91	9.13	30	9.10	19	7.14
j. none, unsprayed	1364	7.91	433	8.19	240	6.58
k. none, third rate	324	8.96	88	8.98	39	6.73
l. none, twice	98	9.22	34	8.93	30	6.86
SEM (33 df)	149.5	0.158	38.4	0.017	61.8	0.34
CV%	50.2	3.9	42.1	4.0	57.7	5.10
<i>P</i> Variety	0.411	<0.001	0.548	<0.001	0.860	<0.001
<i>P</i> Urea	0.269	0.718	0.381	0.016	0.364	0.045
<i>P</i> Aphids	<0.001	<0.001	<0.001	<0.001	<0.001	0.220

Table 3. Regression analysis of yield loss against aphid burden for variety:urea interactions in 1997.

Site and treatment	slope (kg ha ⁻¹ yield loss per aphid day)	<i>P</i>	R ²	maximum aphid days on plots
Boxworth				
Hereward + urea	1.00 ± 0.15	< 0.001	81.5	2211
Hereward - urea	1.05 ± 0.06	< 0.001	96.6	2179
Rialto + urea	1.08 ± 0.15	< 0.001	83.4	1646
Rialto - urea	0.99 ± 0.12	< 0.001	85.2	1677
Bridgets				
Hereward + urea	0.82 ± 0.48	0.301	10.6	657
Hereward - urea	2.51 ± 0.84	< 0.001	74.5	395
Rialto + urea	1.31 ± 0.70	0.013	47.9	556
Rialto - urea	1.82 ± 0.69	< 0.001	67.1	559
High Mowthorpe				
Hereward + urea	0.65 ± 1.26	0.615	2.6	356
Hereward - urea	1.64 ± 1.21	0.203	15.6	333
Rialto + urea	0.97 ± 0.84	0.277	11.7	418
Rialto - urea	0.61 ± 0.48	0.237	15.2	320

The soluble stem carbohydrate reserves at GS 61 ranged from 2.61 tonnes ha⁻¹ at 100% d.m. at Bridgets to 1.94 at High Mowthorpe for Hereward and 3.60 at Bridgets to 2.41 at High Mowthorpe for Rialto compared with published values of 2.72 and 3.49 respectively (Anon., 1997).

Plant populations.

Aphid numbers remained too low to affect yields at Boxworth or High Mowthorpe. At Bridgets, aphids increased to higher numbers in the plots with the lowest tiller population, and had a greater effect on yield (Table 4). Fertile tiller populations averaged 279, 309, 367 and 361 (± 10.5) tillers m⁻² for the four sowing rates.

Table 4. Accumulated aphid count per tiller and unit area and yield at Bridgets in 1998.

Treatment	Total aphid days		Yield (tonnes ha ⁻¹ @ 85% d.m.)
	per tiller	per m ²	
125 seeds			
1 sprayed	143	40618	7.38
2 low rate introduction	1215	351900	7.37
3 high rate introduction	1500	406397	7.13
250 seeds			
4 sprayed	173	52641	8.47
5 low rate introduction	721	221853	8.33
6 high rate introduction	1005	309469	8.26
375 seeds			
7 sprayed	165	56717	8.79
8 low rate introduction	859	315051	8.52
9 high rate introduction	692	258551	8.66
500 seeds			
10 sprayed	113	39779	8.85
11 low rate introduction	376	128972	8.72
12 high rate introduction	815	311407	8.73
SEM (33 df)	152.8	52946	0.133
CV%	47	51	3.2
<i>P</i> seed rate	0.002	0.119	<0.001
<i>P</i> aphids	<0.001	<0.001	0.160
<i>P</i> interaction	0.067	0.235	0.891

The different plant populations did not affect the rate of yield loss per aphid day.

Fungicide programmes.

The strobilurin fungicides increased yields by an average of 0.69 tonnes per hectare at Boxworth and 0.51 tonne per hectare at Bridgets, but had no effect on yield at High Mowthorpe (Table 5). Aphid numbers were reduced by heavy rainfall in early June at all three sites. No effect of fungicide programme on aphid numbers was found and aphid numbers were too low for regression analysis to compare crop sensitivity.

Table 5. Yields from different fungicide programmes in 1999.

Fungicide	Yield (tonnes ha ⁻¹ @85% d.m.)		
	Boxworth	Bridgets	High Mowthorpe
Programme A	12.27	9.72	11.82
Programme B	12.92	10.18	12.05
Programme C	13.00	10.32	11.97
SEM (33 df)	0.069	0.112	0.092
<i>P</i>	<0.001	0.002	0.226
CV%	2.2	4.4	3.1

DISCUSSION

Reduced tiller population improved the performance of *S. avenae*, resulting in higher aphid numbers and yield loss, confirming the results of Honjk (1985). Optimisation of canopy structure is therefore likely to increase the incidence of problem aphid infestations.

Early nitrogen usage, which increases the density of canopies, is likely to inhibit aphid performance and reduce the incidence of damaging aphid infestations (Duffield *et al.*, 1997). Late nitrogen application to prolong the grain filling period was shown to also extend the period of aphid infestation, increasing the potential for yield loss, but at the same time to reduce the rate of loss in relation to aphid infestation, so that the overall impact may be neutral.

Strobilurin fungicides may have a similar effect to late nitrogen in prolonging the grain filling period, but wet weather prevented the assessment of these effects in the studies reported here. The effect may be similar to late nitrogen, giving a compensating reduction in the effect of infestation.

Overall the impact of aphids on wheat was similar to that of drought, as on barley (Cabrera *et al.*, 1995), so that the choice of varieties with high soluble stem carbohydrate reserves may mitigate damage. Droughted crops also have thinner canopies favouring aphid multiplication. The best overall advice for producing crops able to withstand higher aphid infestation would be to seek to increase drought tolerance and also to ensure that sources of carbohydrate are more than adequate to fill the available grain sink. Drought has been shown to increase the rate of yield loss caused by *S. graminium* from 0.51 to 1.17 kg ha⁻¹ yield loss per aphid day (Kindler *et al.*, 2002).

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REFERENCES

Anon. (1997) *1997 Cereals Variety Handbook*. NIAB, Cambridge.

- Beed F; Winder L; Marchesi A; Duffield S (1999). The effect of reducing growth in winter wheat on the population dynamics of the grain aphid *Sitobion avenae* (F.). *Agricultural and Forest Entomology*, **1**, 281-286.
- Cabrera H M; Argandoña V H; Zúñiga G E; Corcuera L J (1995). Effect of infestation by aphids on the water status of barley and insect development. *Phytochemistry*, **40**, 1083-1088.
- Dixon A F G (1987). Cereal aphids as an applied problem. *Agricultural Zoology Reviews*, **2**, 1-57.
- Duffield S J; Bryson R J; Young J E B; Sylvester-Bradley R; Scott R K (1997). The influence of nitrogen fertiliser on the population development of the cereal aphids *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Wlk.) on field grown winter wheat. *Annals of Applied Biology*, **130**, 13-26.
- Gerloff E D; Ortman E E (1971). Physiological changes in barley induced by greenbug feeding stress. *Crop Science*, **11**, 174-176.
- Honk A (1985). Plant density and abundance of cereal aphids (Hom., Aphididae). *Journal of Applied Entomology*, **100**, 309-316.
- Honk A (1990). Host plant energy allocation to and within ears, and abundance of cereal aphids. *Journal of Applied Entomology*, **110**, 68-72.
- Honk A (1991). Environmental stress, plant quality and abundance of cereal aphids (Hom. Aphididae) on winter wheat. *Journal of Applied Entomology*, **112**, 65-70.
- Kieckhefer RW; Gellner; J L; Riedell W (1995). Evaluation of the aphid-day standard as a predictor of yield loss caused by cereal aphids. *Agronomy Journal*, **87**, 785-788.
- Kindler SD; Elliott NC; Giles KL; Royer, TA; Fuentes-Granados, R; Tao, F (2002). Effect of greenbugs (Homoptera: Aphididae) on yield loss in winter wheat. *Journal of Economic Entomology*, **95**, 89-95.
- Lee C; Stevens D J; Stokes S; Wratten S D (1981). Duration of cereal aphid populations and the effects on wheat yield and bread-making quality. *Annals of Applied Biology*, **98**, 169-178.
- Oakley J N; Walters K F A (1994). A field evaluation of different criteria for determining the need to treat winter wheat against the grain aphid *Sitobion avenae* and the rose-grain aphid *Metopolophium dirhodum*. *Annals of Applied Biology*, **124**, 195-211.
- Oakley J N; Ellis S A; Watling M A; Walters K F A; Young J E B (2002). Responses of cereal aphid populations to reduced rate aphicide applications in large field plots of winter wheat. *Agricultural and Forest Entomology*. in press.
- Rautapää J (1966). The effect of the English grain aphid *Macrosiphum avenae* (F.) (Hom. Aphididae) on the yield and quality of wheat. *Annales agriculturae Fenniae*, **5**, 334-341.
- Wratten S D (1978). Effects of feeding position of the aphids *Sitobion avenae* and *Metopolophium dirhodum* on wheat yield and quality. *Annals of Applied Biology*, **84**, 11-20.

Effects of light leaf spot (*Pyrenopeziza brassicae*) infection on canopy size and yield of winter oilseed rape (*Brassica napus*)

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*Plumpton College, Ditchling Road, Nr. Lewes, East Sussex BN7 3AE, UK***ABSTRACT**

Mechanisms of yield loss from light leaf spot (LLS, *Pyrenopeziza brassicae*) and effects of tebuconazole fungicide regimes were examined in winter oilseed rape at Rothamsted in 1997/98. There was no evidence that light leaf spot decreased plant populations, although disease-related winter kill can decrease yield. The best light leaf spot control was with routine or autumn/spring applications of tebuconazole, with responses greater for cv. Bristol (susceptible) than cv. Capitol (resistant). Cv. Capitol had a greater pod green area index (GAI, estimated from light interception measurements) than cv. Bristol and routine tebuconazole treatments produced largest GAIs for both cultivars. Cv. Capitol yielded more than cv. Bristol and routine treatments increased yield most (by 1.29 and 1.93 t ha⁻¹, respectively). Other treatments increased yield, with best responses for treatments including a spring fungicide application. Yield was positively related to pod GAI in June, indicating that the main mechanism of yield loss from LLS was reduction in numbers of pods and canopy GAI (which reduced capacity to capture light).

INTRODUCTION

Light leaf spot (LLS, *Pyrenopeziza brassicae*) can cause yield losses in winter oilseed rape of over 1 t ha⁻¹ (Hardwick *et al.*, 1991) and costs UK producers more than £30M per annum (Fitt *et al.*, 1997). Mechanisms of yield loss are not clear; yield formation in oilseed rape is poorly understood and the LLS epidemic cycle within the crop is complex. Epidemics start in autumn with infection of emerging crops by wind-dispersed ascospores released from apothecia on debris from the previous season. At this stage, spore dispersal and infection can occur over considerable distances. Subsequently, disease is spread by splash dispersal of asexual conidia from the white spore masses that develop on infected leaves, between neighbouring plants and vertically up the canopy. Initially, the disease is usually localised as foci in crops and

may cause over-winter loss of plants. The crop can usually compensate for low plant populations by increased branching, although yield may be lost if large disease patches develop. Reduction in green area index (GAI) and photosynthetic capacity of leaves by leaf spots may limit ability of the vegetative canopy to provide sufficient assimilate to produce reproductive structures. The growing points of flowers and branches may also be infected and subsequent development halted. Although oilseed rape is able to compensate effectively for widely differing pod densities, with similar yields possible between 4 – 12,000 pods m⁻² (Lunn *et al.*, 2001), limitation of pod numbers and green area during pod filling is a potential yield loss mechanism.

During stem extension and flowering, new ascospores released from dead leaf material and continued splash dispersal of conidia contribute to development of LLS epidemics on stems and pods. Reduced photosynthetic capacity of pods due to LLS infection, abortion and shrivelling of seeds and premature pod shatter cause yield loss, since almost all seed matter is produced in a 6 week period in June and July by pod hull and branch photosynthesis. The triazole fungicide tebuconazole is used to control light leaf spot but also has plant growth-regulating effects, reducing height and lodging (Lunn *et al.*, 2002) and enhancing yield. This work was done on winter oilseed rape at Rothamsted in 1997/98 to assess the mechanism of yield loss due to the disease and to investigate disease control and PGR effects of tebuconazole.

MATERIALS AND METHODS

The cultivars Bristol and Capitol, with LLS resistance ratings of 2 (susceptible) and 8 (resistant), respectively (Anon., 1997), were grown at Rothamsted in 1997/98. Plots were drilled on 29 August 1997 at 120 seeds m⁻². To ensure LLS infection, stem debris from plots harvested in July 1997 was scattered over plots on 22 October. Standard treatments of fertiliser, insecticides, herbicides and desiccants were applied to all plots. Tebuconazole treatments were applied at various dates (Table 1). Plant numbers m⁻² were assessed in November and January. Disease was assessed at monthly intervals through the season; records of percentage leaf, stem and pod area affected by light leaf spot were taken. On leaves and pods, assessments of white spore masses were made after incubation at 5-10°C for 2-5 days (Fitt *et al.*, 1998). Assessments of phoma stem canker, downy mildew and alternaria were also made.

Light interception was measured in early June, after flowering. Incident photosynthetically-active light radiation (I , $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) above the canopy was measured with a Sunscan ceptometer (Delta T, Burwell, Cambs.). A simultaneous reading of light transmitted by the pod canopy (T) was taken with a ceptometer at the base of the pods. The percentage of the incident light intercepted (i.e. absorbed and reflected) by the canopy was calculated as $(I-T)*100/I$. Light extinction through a canopy approximates Beer's Law, from which the equation $(1-F) = e^{-k\text{GAI}}$ can be derived; F is the fraction of light intercepted (i.e. $(I-T)/I$), k is the extinction coefficient and GAI is the green area index (area of green material per square metre of ground). Assuming $k = 0.66$, the green area indices (GAI) of different pod canopies were estimated.

Areas of healthy and diseased canopy were then calculated from GAI and percentage LLS values. The experiment was harvested on 22-24 July 1998 and yield (at 10% moisture) determined.

Table 1. Dates and rates of application of tebuconazole to winter oilseed rape in an experiment at Rothamsted in 1997/98

Code	Treatment	Application date
U	Untreated	N/A
R	Routine (monthly full rate)	23 Oct, 21 Nov, 14 Dec, 23 Jan, 25 Feb, 20 March, 22 April
O	October full rate	23 October
N	November full rate	21 November
D	December full rate	14 December
OM	October half / March half rate	23 November + 20 March
NM	November half / March half rate	21 November + 20 March
DM	December half / March half rate	14 December + 20 March
M	March full rate	20 March
F	Full rate at flowering	22 April

RESULTS AND DISCUSSION

Plant and disease development

Plant population assessments showed no differences between treated and untreated plots (data not shown); establishment was *c.* 50% with a population of *c.* 60 plants m⁻² in November and January. However, plant death after January, when the LLS epidemic developed further, may have contributed to yield loss. Light leaf spot development in untreated plots started in November and reached a maximum (40% leaf area affected) at the end of January (Figure 1). There was more LLS on Bristol than Capitol. Only routine sprays prevented development of leaf lesions (until May). October and November full rate sprays of tebuconazole delayed appearance of disease and reduced leaf area affected in Bristol. The December spray reduced the area affected. Half rate applications also delayed the development of LLS but did not decrease severity much. The spring half rate spray reduced area of LLS affected leaves only in plots previously sprayed in December. By May, only the routine spray and December/March split application had decreased LLS on leaves. On Capitol, there was less disease and fewer treatment differences. October full rate application delayed the epidemic, although final LLS levels were similar to those of the control. November and December sprays delayed the epidemic. Only routine and December/March sprays reduced final LLS levels in May. For the pod phase of the disease, it was difficult to demonstrate significant differences since standard errors were large. For Bristol, routine sprays delayed development of the pod phase of the disease. In other treatments, *c.* 15-40% of the pod area was affected, with indications that spring sprays reduced the pod area affected. On Capitol, there was less light leaf spot on pods than on Bristol, but no treatment effects. For other diseases assessed, there were no differences between treatments (data not shown).

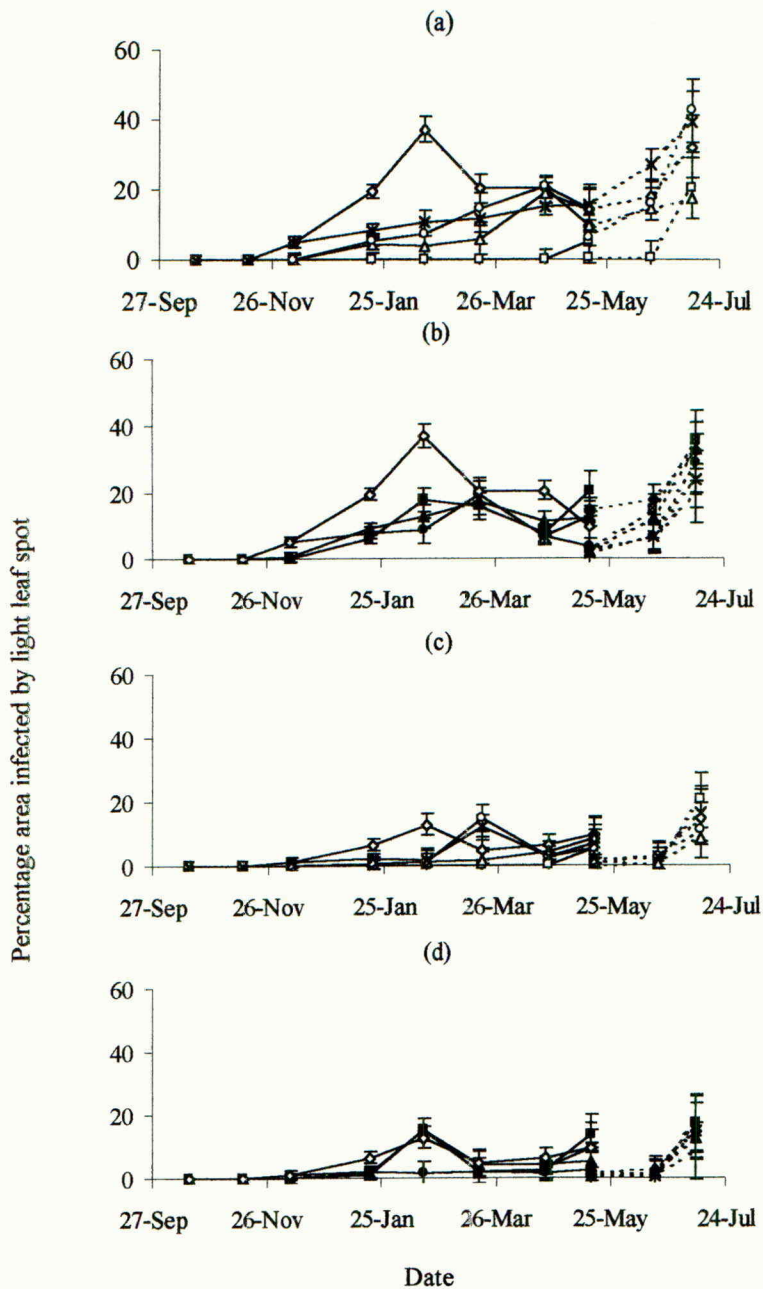


Figure 1. Effects of fungicide treatments (\diamond untreated, \square routine, Δ October, \circ November, \times December, \blacksquare October/March, \bullet November/March, \blacktriangle December/March, $*$ March, $+$ April) on percentage area of leaves (—) and pods (----) affected by light leaf spot infection on cv. Bristol (a,b) or cv. Capitol (c,d); spring (a,c) or autumn treatments (b,d).

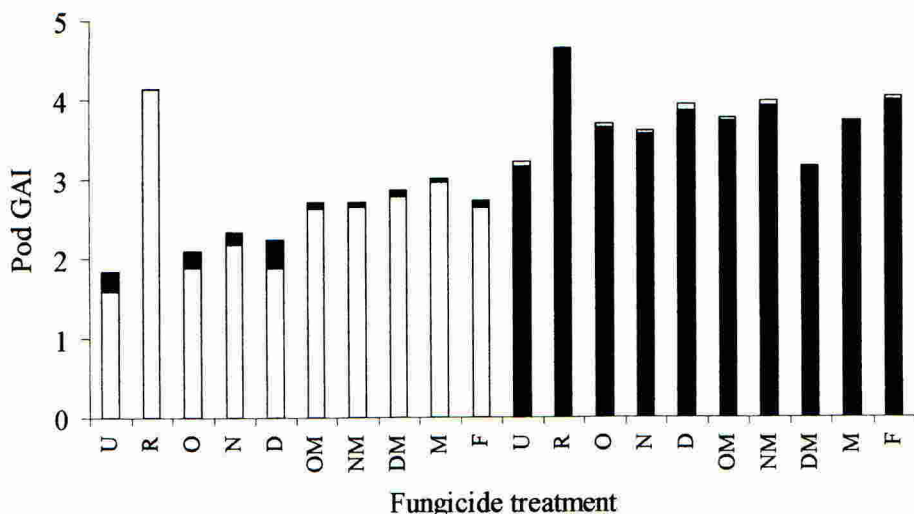


Figure 2: Effect of fungicide treatment on healthy (lower part of bar) and diseased (upper part) canopy green area index (GAI). Bristol: white lower and shaded upper bar, Capitol: shaded lower and white upper bar. Treatment codes shown in Table 1.

Canopy size, disease and light interception

Analysis of variance showed significant effects ($P < 0.001$) of cultivar and fungicide treatment on total canopy size (Figure 2), with a significant ($P = 0.047$) cultivar \times fungicide interaction. Capitol had a greater GAI than Bristol. Untreated Bristol and Capitol had the smallest canopies (c. 1.8 and 3.2 units, respectively), whilst the routine treatments produced the largest canopies for both (c. 4.1 and 4.6, respectively). On Capitol, fungicide treatments other than routine produced little increase in canopy size. On Bristol, autumn full rate sprays produced larger canopies than untreated plots, whilst treatment including spring sprays produced the largest canopies. Bristol had a greater proportion of canopy diseased than Capitol, except in the routine treatment. Treatments with spring applications of tebuconazole had less diseased canopy area than those with only autumn sprays. For Capitol, fungicide treatment had little effect on the proportion of canopy diseased.

Light interception results (data not shown) also showed differences ($P < 0.001$) due to cultivar and fungicide treatment, with a significant cultivar \times fungicide interaction ($P = 0.019$). Capitol intercepted more light than Bristol. Untreated Bristol intercepted least light (72%); routine spraying gave the highest light interception (92%), close to light interception by routine-sprayed Capitol (94%). Fungicide treatment did not affect light interception in Capitol, but light interception by Bristol was better in sprayed than unsprayed treatments and in treatments including spring applications than those with only autumn applications.

Yield, canopy size and disease control

Capitol yielded more than Bristol (Figure 3). Routine treatment gave the greatest yield increases for both Bristol and Capitol (+ 1.93 and + 1.29 t ha⁻¹ respectively).

Other spray treatments gave increases from 0.36 – 1.25 and 0.26 – 0.89 t ha⁻¹ for Bristol and Capitol, respectively. The greatest yield increases came from treatments that included full or half-rate applications in spring (March-April). Tebuconazole has plant growth-regulating effects, through inhibition of gibberellic acid synthesis, which resulted in stem shortening, reduction in lodging and alterations in canopy structure and light interception. PGR effects of tebuconazole can increase yield by >0.5 t ha⁻¹, when diseases have already been controlled (Lunn *et al.*, 2002). On average, disease control by autumn spraying increased yields by 0.41 and 0.37 t ha⁻¹ for Bristol and Capitol, respectively, and the spring application by 0.53 and 0.43 t ha⁻¹. The benefit in spring might be mostly due to PGR effects, especially on Capitol, with little effect on disease, but levels of pod disease were also reduced on Bristol. Some PGR effects were noted, with shortening of plants in plots that received full rate applications in March and April (data not shown). However, the PGR and disease effects cannot be fully separated in this experiment. Full control of disease by routine spraying yielded a further 0.99 and 0.49 t ha⁻¹ compared to the autumn/spring split applications for Bristol and Capitol, respectively, although this could also include some PGR effects.

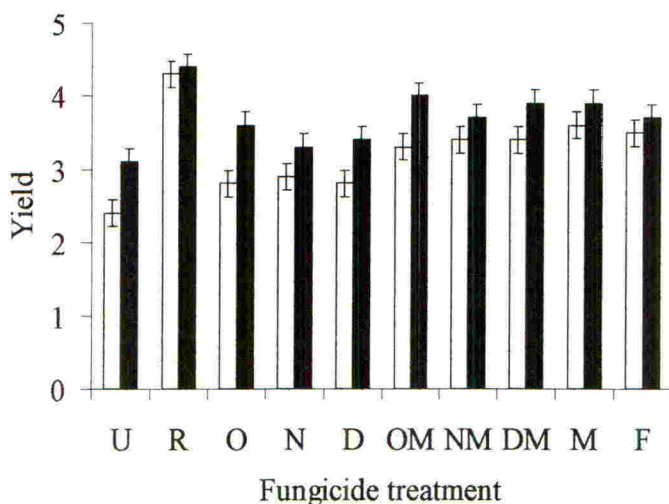


Figure 3. Effect of cultivar (□ Bristol, ■ Capitol) and fungicide treatment on combine harvest yield (t ha⁻¹ @ 90% DM) of winter oilseed rape at Rothamsted in 1997/98. Treatment codes in Table 1. Error bars show ± SEDs.

Regression analysis showed yield was positively related to canopy size (Figure 4) and radiation interception (data not shown), which were related to disease control strategy. Canopy size accounted for 60% of the variance in yield, according to the equation Yield (t ha⁻¹) = 0.49*(Pod canopy GAI in June) + 1.88. As well as affecting yield via reduced canopy size, LLS infection may also have reduced yield via reduced pod photosynthesis.

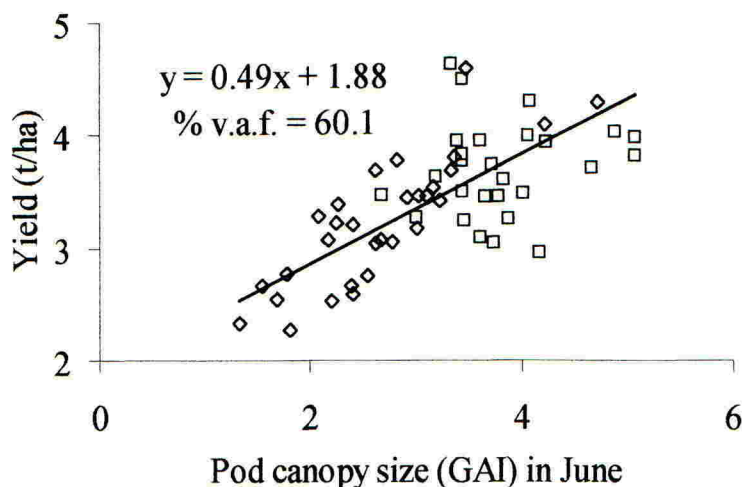


Figure 4: Relationship between pod canopy size (green area index) in June and yield (t ha^{-1} @ 90% DM) of winter oilseed rape (cvs Bristol \diamond , Capitol \square) at Rothamsted in 1997/98.

CONCLUSION

This work confirms that light leaf spot can cause yield losses in excess of 1 t ha^{-1} , even in resistant cultivars, and up to 2 t ha^{-1} in susceptible cultivars. No evidence for yield loss due to winter kill was found in this experiment, although this can be a factor in severe conditions (Baierl *et al.*, 2002). However, evidence was found for LLS infection reducing pod canopy size and thus light interception, reducing numbers of pods and seeds, as well as assimilate availability for seed filling. The effect on canopy size was greater on the susceptible cultivar Bristol than on the resistant cultivar Capitol, and Bristol was thus more responsive to fungicides. With complete control of disease by routine fungicide application, canopy GAI could be almost doubled compared to untreated controls, allowing more than 15% extra incident light to be intercepted. Although the main impact of LLS on yield appeared to be due to reduced canopy size, there were differences in the levels of disease on the pods that could have affected yield due to reduced pod photosynthesis during the critical phase of pod filling. However, due to large inter-plot variations in disease levels, a significant relationship could not be demonstrated. Although fungicide sprays in the autumn delayed onset of LLS infection and reduced maximum leaf area affected, strategies involving spring applications appeared to give better control at the critical phases, increasing canopy size and reducing pod infection. However, plant-growth-regulating effects, providing *c.* 0.5 t ha^{-1} of extra yield, are possible from use of tebuconazole and could not be separated from disease effects in this experiment.

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REFERENCES

- Anon. (1997). UK recommended list for oilseeds 1997. *National Institute of Agricultural Botany*.
- Baierl A; Evans N; Steed J M; Sutherland K G; Fitt B D L (2002). Effects of light leaf spot (*Pyrenopeziza brassicae*) infection on winter survival of leaves and yield of oilseed rape (*Brassica napus*). This volume.
- Fitt B D L; Gladders P; Turner J A; Sutherland K G; Welham S J (1997). Prospects for developing a forecasting scheme to optimise use of fungicides for disease control on winter oilseed rape in the UK. *Aspects of Applied Biology* **48**, 135-142.
- Fitt B D L; Doughty K J; Gladders P; Steed J M; Sutherland K G (1998). Diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. *Annals of Applied Biology* **133**, 155-166.
- Hardwick N V; Fitt B D L; Wale S J; Sweet J B (1991). Oilseed Rape Diseases. Review No. **OS 4**, HGCA, London. pp.131.
- Lunn G D; Spink J H; Stokes D T; Wade A; Clare R W; Scott R K (2001). Canopy management in winter oilseed rape. Project Report No. **OS 49**, HGCA, London.
- Lunn G D; Spink J H; Wade A; Clare R W (2002). Targeting and choice of spring remediation of canopy size in winter oilseed rape (*Brassica napus*). Project Report, HGCA, London. *In Press*

**Anti-oxidative and anti-senescence effects of the strobilurin pyraclostrobin in plants:
A new strategy to cope with environmental stress in cereals**

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ABSTRACT

In addition to its broad spectrum fungicidal activity, the strobilurin pyraclostrobin had positive effects on the crop yield in the absence of pathogen challenge. This physiological effect on the plants was especially apparent under conditions of environmental stress. We have observed that pyraclostrobin prevented both symptom development and yield reduction by physiological leaf spot in barley. Foliar application of pyraclostrobin reduced the production of reactive oxygen intermediates in barley leaf tissues by more than 50% and activated the plant antioxidative system. In addition, pyraclostrobin treatment prevented the release of stress-induced ethylene and premature senescence. Since the physiological leaf spot disease and other environmental stresses are caused by changes in the genetic and metabolic regulation of reactive oxygen intermediates resulting in membrane-leakage, cell death or premature senescence, we postulate that the anti-oxidative and anti-senescence effects of pyraclostrobin are responsible for its ability to improve stress tolerance in plants.

INTRODUCTION

Mitigating plant stress, whether from fungal pathogens or environment factors, is critical to maximising crop yield performance. Today, fungal pathogens can be effectively controlled by broad-spectrum fungicides such as the strobilurin class. Apart from their fungicidal effects, strobilurins such as kresoxim-methyl and pyraclostrobin (*F500*[®]) can cause long-term changes in the metabolism and growth of the treated plants resulting in higher biomass and yield (Köhle *et al.*, 1997; Glaab & Kaiser, 1999). Moreover, increases in biomass paralleled proportional increases in starch and protein. We believe that strobilurin treatment widens the critical bottlenecks for carbon as well as nitrogen assimilation. A partial inhibition of the respiration in leaf tissue caused by kresoxim-methyl decreased the CO₂-compensation point (Köhle *et al.*, 1997) and stimulated nitrate reductase activity (Glaab & Kaiser, 1999). Additionally, strobilurin treatment has been observed to alter the level of several phytohormones and delay plant senescence (Grossmann & Retzlaff, 1997).

A leaf spot disorder of barley with unknown aetiology has become an important issue in various regions of Europe (e.g. up to 40% or 3 t/ha yield loss in Southern Germany). This disorder is referred by several names such as physiological leaf spots (PLS), nonparasitic necrosis, genetic necrosis, and tar spots. PLS symptoms are characterised by necrotic spotting

in the uppermost four leaves and are not caused by a microbial pathogen, but by unknown genetic or abiotic stress factors. However, in some, but not all, regions, *Ramularia collo-cygni* is considered to contribute to the PLS complex (Sachs, 2002).

Less is known about the causal mechanism of nonparasitic necrosis in plants compared to necrosis following a hypersensitive resistance response caused by pathogens (Jabs & Slusarenko, 2000). Nevertheless, Jabs *et al.*, (1996) found some striking similarities between pathogen-dependent hypersensitive response and necrotic lesions induced by excessive light or artificial oxidative stress on *Arabidopsis* plants. Both types of lesions express the same genetic and histochemical markers on the macroscopical and microscopical level, such as callose deposition, production of reactive oxygen intermediates and the increase of stress-related enzymes. Thus, plants respond to abiotic and biotic stress with similar physiological mechanisms. Reactive oxygen intermediates (ROI) seem to play an important role inducing programmed cell death, membrane-leakage, ethylene release, and local necrosis or premature senescence (Jabs & Slusarenko, 2000; Overmyer *et al.*, 2000). Recently, evidence has also been presented for the involvement of oxidative stress in the formation of physiological leaf spot. ROI accumulation in barley leaves was correlated with the severity of PLS as well as with the cultivar-specific susceptibility to PLS in the field (Wu & von Tiedemann, 2002a). The purpose of this study was to achieve a better understanding of the altered plant stress responses after pyraclostrobin treatment under field conditions.

MATERIALS AND METHODS

Table 1. Fungicides used for applications

Code	Active ingredient (g/litre)
PYR+EPX	pyraclostrobin (133) + epoxiconazole (50)
PYR	pyraclostrobin (250)
EPX	epoxiconazole (125)
Strob. 1	Strobilurin 1 (50% w/w)
Strob. 2	Strobilurin 2 (250)
Strob. 3	Strobilurin 3 (250)

For the Physiological leaf spot field trial in Frankendorf, Bavaria, in 2000, the highly sensitive winter barley *cv.* Anthere and the less sensitive *cv.* Gunda were used. Fungicide treatments were applied at GS 39 or GS 51 as follows: PYR+EPX (1.75 litres/ha). All plots were pre-treated with 1.5 litres/ha of Fortress Top (quinoxifen 67 g/litre; fenpropimorph 250 g/litre) at GS 31. Letters above the columns indicate significant differences between treatments ($P < 0.05$, LSD).

For determination of ozone injury, spring barley (*cv.* Scarlett) was grown under glasshouse conditions. Fungicide treatments were applied as foliar sprays (0.1% formulated products) at GS 30 3 days prior to a 2-day ozone fumigation period (150-180 ppb, 7h/d). Ozone injury was determined as described (Wu & von Tiedemann, 2002b; $n = 10$, $P < 0.05$, LSD).

For determination of superoxide production and SOD activity, spring barley (*cv.* Scarlett) was grown under glasshouse conditions. Fungicide treatments were applied as consecutive foliar

sprays (0.1% formulated products) at GS 32 and 39. Examinations were performed at GS 55 and 69 as described (Wu & von Tiedemann, 2002a; $P < 0.05$).

For ethylene determination, young wheat plants (*cv.* Kanzler) were raised in vermiculite substrate under controlled environmental conditions. Fungicide treatments were applied as foliar sprays at GS 12-13 at 3 days or 2 hours before stress treatment as follows (litres/ha): PYR+EPX (1.5); PYR (0.8); Strob. 1 (0.25 kg/ha); Strob. 2 (0.8); Strob. 3 (0.8). The stress regimen consisted of incubating detached shoots at 30°C and 60% humidity for 30 min. Ethylene release was determined as described (Grossmann & Retzlaff, 1997).

RESULTS

Inhibition of physiological leaf spot in barley by pyraclostrobin treatment

Under field conditions, treatment with the fungicide mixture OPERA® (PYR+EPX), containing pyraclostrobin and epoxiconazole, prevented development of PLS symptoms (Fig. 1A). A formulation without a.i. did not reduce leaf spot symptoms (data not shown). The development of PLS necrotic lesions was accompanied by a premature loss of chlorophyll and reduction of photosynthesis in the uppermost leaves (data not shown), resulting in a yield reduction in untreated plots of up to 2.2 t/ha (Fig. 1B). The barley cultivar Anthere, which is highly susceptible to PLS, responded more significantly to the fungicide treatment, strengthening the hypothesis that pyraclostrobin treatment ameliorates oxidative stress leading to nonparasitic leaf spots.

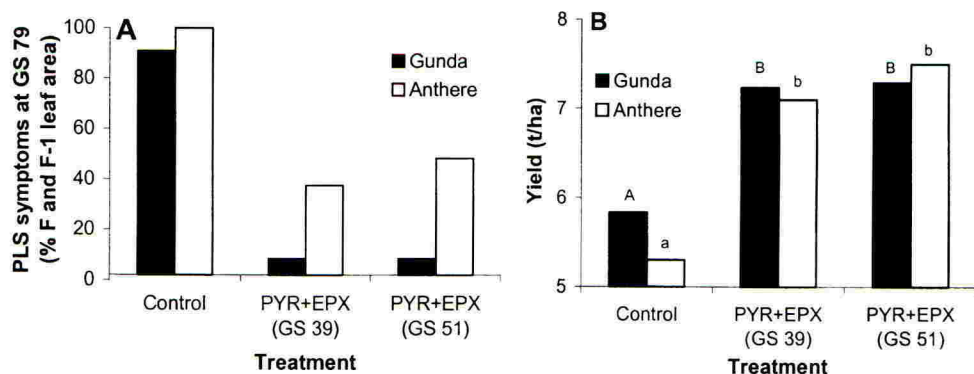


Figure 1. Effects of fungicides on Physiological Leaf spot symptoms in F and F-1 leaves (A) and on yield (B).

Anti-oxidative effects by pyraclostrobin treatment

Fungicide treatment protected barley plants against artificial oxidative stress, such as ozone injury (Fig. 2; $p < 0.05$). Pyraclostrobin was most effective and reduced ozone damage by more than 90%.

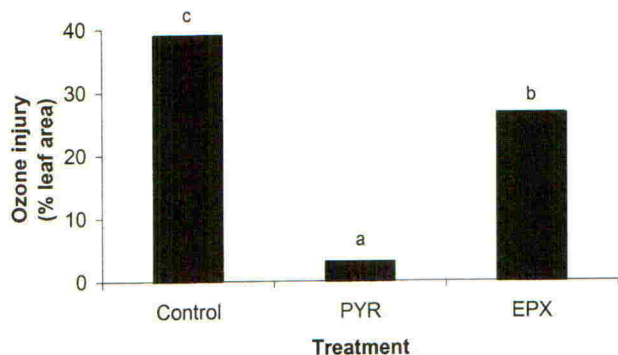


Figure 2. Effects of fungicides on ozone tolerance of barley leaves.

Furthermore, fungicide treatments to barley plants at GS 32 and 39 induced a sustained reduction of superoxide production even when evaluated at the advanced GS 55 ($P < 0.01$, Fig. 3A). Superoxide production was reduced by about 50% by pyraclostrobin, while epoxiconazole was less efficient. The differences between fungicides in reducing ROI production became smaller at GS 69, but pyraclostrobin was still most effective (data not shown). In general, lowered ROI production in barley leaves was directly correlated with the protection of young barley plants from ozone damage by these fungicides.

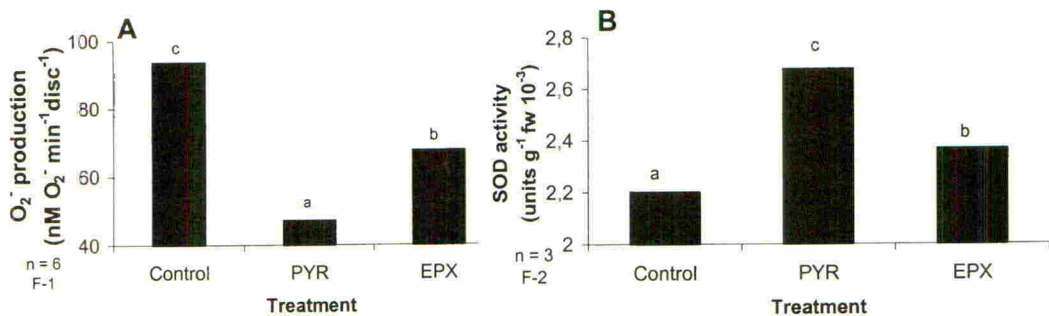


Figure 3. Effects of fungicides on superoxide production (A) and on SOD activity (B) of barley ($P < 0.05$).

Plants exhibiting increased tolerance to oxidative stress typically possess with increased activities of anti-oxidative enzymes, such as superoxide dismutases (SOD), catalases and peroxidases (Smirnov, 1998). SOD activity in leaves is the primary scavenger for superoxide radicals and decreases in ageing leaf tissues, which is consistent with the increase of ROI production during plant senescence. Treatment with pyraclostrobin increased superoxide-scavenging SOD activity in barley leaves at GS 55 and was superior to the effects of epoxiconazole (Fig. 3B), which is in agreement with the strong reduction of superoxide production by pyraclostrobin. Even at more mature growth stages (GS 69) the enhancement of SOD activity by pyraclostrobin over the untreated control and other fungicides was observed (data not shown). Interestingly, this activation of the antioxidative system by pyraclostrobin treatment was found to precede the development of physiological leaf spot symptoms in a winter barley field trial (Köhle *et al.*, in press), indicating that oxidative stress is a cause rather than a consequence of this physiological disorder.

Anti-senescence effects by pyraclostrobin treatment

In addition to ROI production, ethylene release is a rapid plant response to oxidative stress. Ethylene itself is required for sustained ROI production, which drives cell death propagation (Overmyer *et al.*, 2000). Pyraclostrobin treatment resulted in a strong reduction of ethylene production after a short-term drought stress regimen (Fig. 4). Pyraclostrobin applied only 2 hours before stress regimen resulted in more than 80% inhibition of ethylene release indicating a rapid uptake strong intrinsic activity of pyraclostrobin. Although inhibition of ethylene release seems to be a more general physiological side effect of strobilurins (Grossmann & Retzlaff, 1997), other strobilurin-containing fungicides were less effective and showed a delayed onset of the ethylene release inhibition. Even when applied 3 days before the stress regimen, pyraclostrobin treatment reduced ethylene formation for 80 h by more than 50% (Fig. 4B), indicating once more the excellent bioavailability of pyraclostrobin.

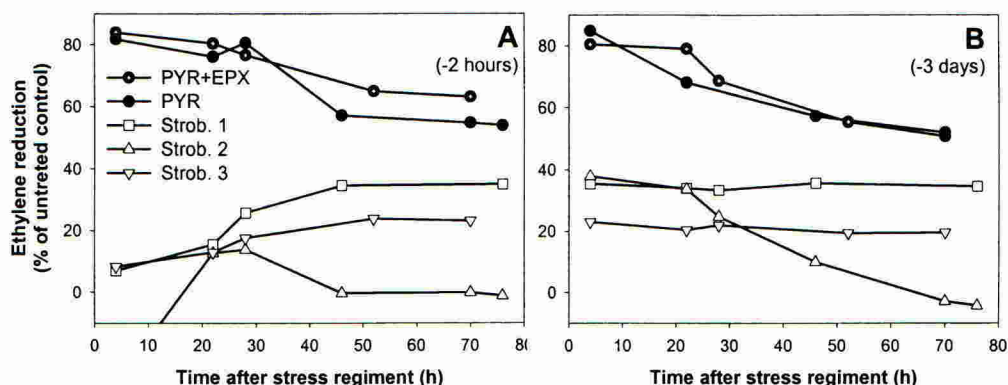


Figure 4. Effects of fungicides on ethylene release from detached and stressed wheat shoots. Fungicide treatments were applied as foliar spray to wheat plants (A) 2 hours or (B) 3 days before stress treatment.

CONCLUSIONS

The physiological leaf spot disease and other environmental stresses are caused by changes in the genetic and metabolic regulation of ROIs (Wu & von Tiedemann, 2002a). ROIs form a feedback amplification cycle in concert with ethylene signaling, resulting in cell death, premature senescence and yield reduction (Overmyer *et al.*, 2000). Therefore, we postulate that both the strong anti-oxidative and anti-senescence effects of pyraclostrobin contribute to its excellent efficacy against PLS and to improved stress tolerance in cereals (Fig. 5). This helps plants to prolong the duration of corn filling and ensures optimal maturation and yield.

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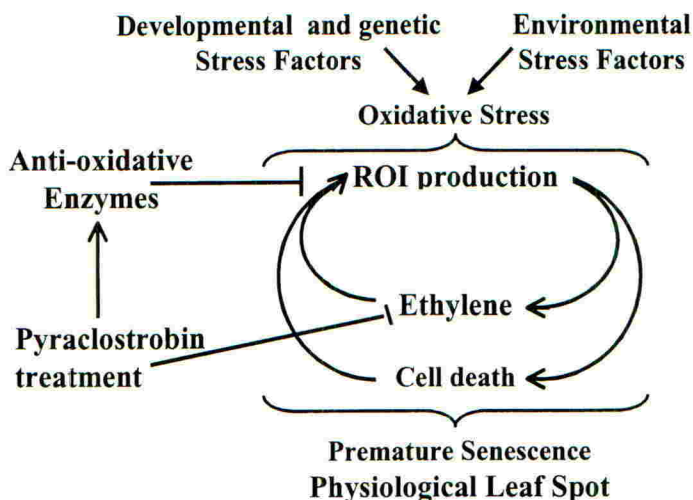


Figure 5. Hypothetical Model for the Action of pyraclostrobin treatment against oxidative plant stress.

REFERENCES

- Glaab J; Kaiser W M (1999). Increased nitrate reductase activity in leaf tissue after application of the fungicide Kresoxim-methyl. *Planta* **207**, 442-448.
- Grossmann K; Retzlaff G (1997). Bioregulatory effects of the fungicidal strobilurin Kresoxim-methyl in wheat (*Triticum aestivum*). *Pesticide Science* **50**, 11-20.
- Jabs T; Dietrich RA; Dangl JL (1996). Initiation of runaway cell death in an Arabidopsis mutant by extracellular superoxide. *Science* **273**, 1853-1856.
- Jabs T; Slusarenko A J (2000). The hypersensitive response. In: *Mechanisms of Resistance to Plant Diseases*, eds AJ Slusarenko *et al.*, pp. 279-323. Kluwer Academic Publishers:
- Koehle H; Grossmann K; Retzlaff G; Akers A (1997): Physiological effects of the new fungicide Juwel[®] on yield in cereals. *Gesunde Pflanzen* **49**, 267-271.
- Koehle H; Grossmann K; Jabs T; Gerhard M; Kaiser W; Glaab J; Conrath U; Seehaus K; Herms S (in press): Physiological effects of the strobilurin fungicide F 500 on plants. In: *Modern Fungicides and Antifungal Compounds III: 13th International Reinhardsbrunn Symposium*.
- Overmyer K; Touminen H; Kettunen R; Betz C; Langebartels C; Sandermann H; Kangasjärvi J (2000). Ozone-sensitive *Arabidopsis rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. *The Plant Cell* **12**, 1849-1862.
- Sachs E (2002). Monitoring on the spread of *Ramularia* leaf spot disease on winter barley in Germany in 2000. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **54**, 31-35.
- Smirnov M (1998). Plant resistance to environmental stress. *Current Opinion in Biotechnology* **9**, 214-219.
- Wu Y X; von Tiedemann A (2002a). Evidence for oxidative stress involved in physiological leaf spot formation in winter and spring barley. *Phytopathology* **92**, 145-155.
- Wu Y X; von Tiedemann A (2002b). Impact of fungicides on active oxygen species and antioxidant enzymes in spring barley (*Hordeum vulgare* L.) exposed to ozone. *Environmental Pollution* **116**, 37-47.