POSTER SESSION 9D

MANAGEMENT OF PESTS AND DISEASES IN ARABLE CROPS

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Poster Papers

9D-1 to 9D-19

Field experience with sitespecific application of fungicides to winter wheat

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ABSTRACT

Sitespecific applications of fungicides and herbicides have been tested in the field in Denmark and abroad. The testing was based on a system linking a DGPS/tractor terminal and a spray controller. Application maps were constructed in advance. The methodology has shown promising results. In field trials, fungicide application maps have been based on detailed observations of disease incidence and canopy densities. Yield increases were found in 1996. Farmers using the system were satisfied and some reduced overall application rates. Some simple decision rules can be applied by farm managers at the present time. Decision rules and data acquisition need to be simplified and automated to achieve full exploration of the potential.

INTRODUCTION

In intensive agriculture, the control of pests and diseases has changed from routine treatments to control strategies with rational and field specific components. Decisions on treatment need and dosage are becoming more complex due to the increasing number of factors of quantified importance. The increasing complexity makes it advisable to include decision aids involving detailed weather-based models and sitespecific applications.

The recent development of technologies for sitespecific applications has made it possible to adjust applications according to the specific demands on the land. This technology involves DGPS (Differential Global Positioning System) combined with a tractor terminal and implement controller. The controller must be capable of varying application rates while moving according to values recorded on an application map. With a conventional sprayer, the application rate can be varied by a change in the volume applied.

The potential of this methodology has been shown for herbicides (Heisel *et al.*, 1997) and fungicides (Secher, 1997). It is known that disease epidemics can develop more severely in some areas of a field, thus resulting in a variation in treatment needs (Bjerre *et al.*, 1998). The appropriate dosage necessary to control a disease can vary according to the disease incidence and the physiological response of the plant according to light interception and nutritional status. In addition, the efficacy of a treatment is related to the concentration of active ingredient on the leaf surface and therefore related to the leaf area index at the time of application (Secher, 1998). Since disease severity, crop physiological response and fungicide uptake can vary spatially, fungicide performance might be improved if application is on a sitespecific basis.

In this paper, a technical solution for sitespecific application of pesticides is presented, together with results from field trials, and implemented at a practical level with farm managers.

MATERIALS AND METHODS

Technology for sitespecific application

The technical components have been provided by HARDI INTERNATIONAL A/S, Denmark. The system is based on the HARDI PILOT spray controller linked to a tractor terminal with DGPS. The HARDI PILOT is a controller automatically adjusting the volume applied by changing the spray pressure. To counteract the risk of getting an unsatisfactory application because the spraying pressure is a too high or too low, the method is limited to application of dosages in the interval plus or minus 25 percent on each side of a mean value. This interval might be expanded up to plus or minus 50 percent with air assisted spraying (HARDI TWIN SYSTEM) since the air stream will facilitate deposition and reduce drift.

The tractor terminals have been the Massey Ferguson Fieldstar/Dronningborg Agrivision terminal or the CLAAS Agrocom ACT. The application map is constructed on a computer before spraying, using a programme compatible with the tractor terminal. When the map is constructed, it can be transferred to the tractor terminal using a PCMCIA data card. The sequence and configuration of the full system are illustrated in Figure 1.

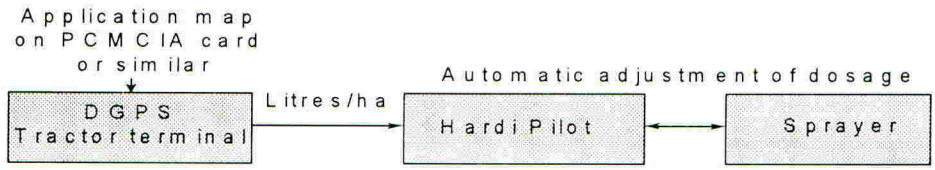

Figure 1. The components in the system for sitespecific application of pesticides.

Field trials

Field trials have been done since 1996. Trial designs are reported by Secher (1997) and Bjerre *et al.* (1998). Two series of field trials have been done. One series compared yields obtained using standard treatment and sitespecific applications and the other gathered detailed information on disease variation and other factors varying at the intra-field level (Bjerre *et al.*, 1998).

Implementation with farmers

From 1997, the system has been implemented on a number of farms in Denmark (3), UK (1), Germany (1) and France (1). The implementation on farms has the objective of testing the system's applicability at farm level and testing perception of the technology by farm managers. The farmers had different amounts of experience with precision farming. As a result of this, the data available for constructing application maps did vary between farms. Basically, the

construction of application maps did follow a general sequence (as presented in Table 1), based on the available information and a field walk.

Table 1. General steps in the construction of application maps.

Step	Title/Phase	Description
1	Data acquisition	Yield maps, soil maps, field walk.
2	Treatment need	Deciding the average application rate: could the rate applied with a conventional treatment be reduced by 5-15 % because of better targeted sitespecific applications.
3	Canopy density adjustment	The average dosage is adjusted guided by the previous years' yield maps. The yield used as an estimate of this year's canopy density. The density of the crop has an impact on both fungicide and herbicide efficiency.
4	Disease conduciveness	Finally, the map is corrected in the areas where the farmer has experience of either high or low treatment need (for example areas which normally have high disease incidence).

RESULTS

Technology for sitespecific application

The performance of the systems was satisfactory. The combined reaction time of both DGPS/tractor terminal and spray controller was measured as 8 seconds for passing a pre-mapped change in application rate of plus or minus 15 litres/ha. Four seconds of the delay were caused by the DGPS/terminal not reading the change in application rate and thus not transmitting the change in application rate. The controller was close to the target volume after 2 seconds, but needed additional 2 seconds to fine tune the target value. Without corrections, 8 seconds are equivalent to approximately 16 metres, when driving at 7 km/h. If the driving speed was used as a mean to compensate for low or high levels of spray pressure, it was possible to vary volume rates by up to 50 percent. When mounted on self propelled sprayers with hydraulic speed change or on tractors with clutch free gear shifts, this is an option.

Field trials

In 1996 a field trial comparing a standard treatment and the sitespecific application resulted in a higher yield with the sitespecific treatment (Table 2). For the standard treatment, dose was

Table 2. Results from a trial comparing sitespecific application of fungicides with conventional application in winter wheat, Denmark 1996. The trial was treated twice, mainly against powdery mildew (Secher, 1997).

	Fungicide units			
Treatment	Minimum	Maximum	Average	Yield in dt/ha
Conventional			0.84	70.9
Sitespecific application	0.67	1.02	0.84	73.9
LSD 0.95				2.9

calculated according to the average disease incidence of plots (Secher *et al.*, 1995). The sitespecific treatment dose was calculated according to the individual disease incidence and further adjusted according to the crop density (more dense crops received higher doses). A similar trial was done in 1997, but no treatment effects were found, probably due to a low incidence of powdery mildew (*Erysiphe graminis*) in the experimental field.

In 1997 detailed investigations of the amount of disease within 3 fields of winter wheat were done. The amount of powdery mildew was variable within the field (Figure 2), and for septoria disease, substantial intra-field variation also occured. The greatest amount of mildew disease was found in areas close to shelterbelts, forest edges or other vegetation (Bjerre *et al.*, 1998). Crop density, expressed as Ratio Vegetation Index, also had a substantial intra-field variation, but did not have a clear effect on the amount of mildew disease.

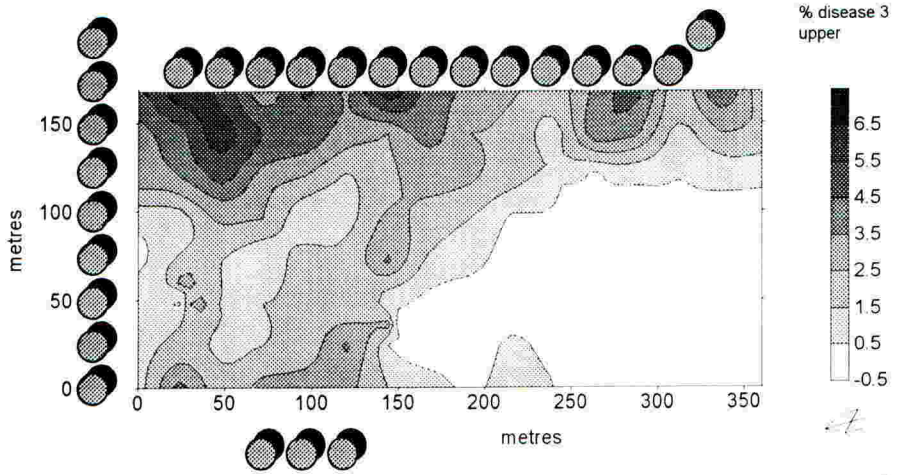

Figure 2. Powdery mildew in part of a field of winter wheat on 11 July 1997 (GS 77); per cent disease on the 3 upper leaves. Filled grey and black circles outside the map denote shelterbelts or other vegetation with similar effects (Bjerre *et al.*, 1998).

Implementation with farmers

Farmers and farm managers were generally satisfied with the system. A farmer not practising precision farming on the whole farm decided to stop after the first season. Two farmers reduced the average application rates by 10 percent and 13 percent respectively due to an expectation of higher overall efficacy achieved with the pesticides applied sitespecifically. The other farmers have not yet decided on reductions in the overall application rate, but a good understanding of principles and potentials were achieved by all participants.

An example is shown in Figure 3. On this farm no yield maps or crop density maps were available at the time of application. Adjustments were therefore solely based on the knowledge of the managers and a field walk. A dense crop was observed in the clay containing parts of the land resulting in a higher application rate. A low application rate was applied on the west side of the field where the crop was poor and damaged. A higher application rate was applied in the more disease conducive micro-climates in the vicinity of trees and hedges.

The yield maps were used as an estimate of crop densities where no other information was available on crop densities. In some fields infra-red pictures or geodetic pictures were taken from aeroplane to help the construct the application maps.

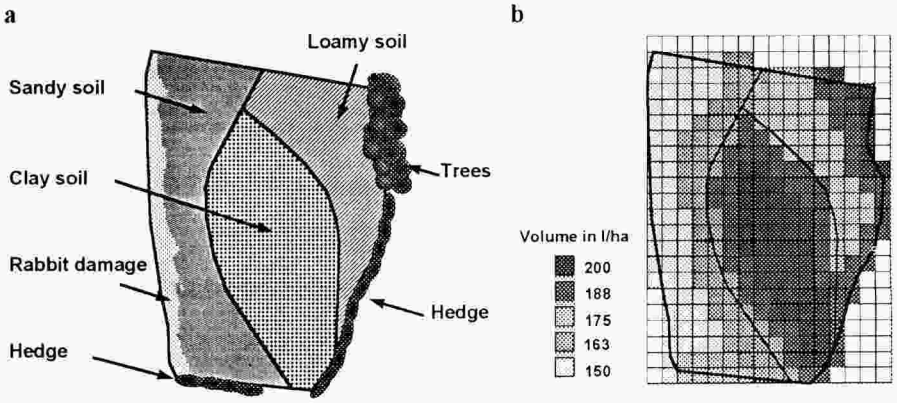

Figure 3. "Harry lamb" field at "Rectory farm", Beckingham, UK. Field size = 10.53 ha; (a) description of soil types, surrounding vegetation and an area with damage by rabbits; (b) fungicide application map for treatment on 17 June 1997.

DISCUSSION

Sitespecific application of pesticides has in field trials and in field tests shown encouraging results with potential reductions in pesticide use or yield increase. In the trials done up to now the treatments have been based on a very detailed and for practice unacceptable registration. In field tests application maps are based on a rather simple registration, the farmers' prior knowledge and maps of yield or crop density (Table 1). Farmers who have collected yield maps for several years, and are eager to utilize this new technology, will probably get started by using these simple principles. As yield maps show a considerable variation between seasons (Lark, 1996), only a rather low correlation can probably be expected between a yield map from a single or a few seasons and current crop densities. A better basis for producing application maps will be achieved by using current maps of crop density.

In the future, sitespecific application will be based on automatized registrations and model calculated application maps. Sensors under development for measuring plant density or weed coverage will find a natural use. Either mounted directly on farm implements or as services offered by advisers or special firms focused on this.

Today a number of questions remain which demand further research and development. Most important is the development of simple registration methods and models for calculating disease variation within the field, based on constant factors that do not need to be registered every year. It is also of importance to develop efficient tools for the interpretation of yield maps, especially in relation to estimation of crop densities. In combination with weather records, yield maps from previous years can probably give a good estimate on the present and the future crop density. Finally, the making of models for calculation of dosage and the development of real

decision support systems will be an important step in the development of application maps based on a technical and objective basis.

Expensive and complex equipment and very large data sets that have to be processed within short intervals make simultaneous dose adjustment according to for example crop density during a run across the field difficult. Therefore it is likely that sitespecific crop protection will be based on predetermined application maps in the near future.

CONCLUSIONS

The field experiments and tests confirm that sitespecific application of pesticides can lead to a more purposeful and efficient utilization of the applied substances. This can be utilized to reduce the total consumption and to benefit both environment and yield. It is likely that sitespecific application can produce a completely new concept for application of pesticides – application according to crop density instead of the current practice – application according to the ground below the crop. An important condition for the future development is the acquisition of the knowledge necessary to produce good application maps, including development of sensor-based methods for data acquisition, simplified registration methods and a thorough documentation of the profitability of the methods.

ACKNOWLEDGEMENTS

The authors would like to thank The Danish Ministry for the Environment for financial support for some of the work reported in this article.

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Reduced dosages of strobilurins for disease management in winter wheat

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ABSTRACT

The strobilurins azoxystrobin, kresoxim-methyl and CGA 279202 (proposed name trifloxystrobin) have been tested in field trials in winter wheat at recommended full and reduced rates either alone or in co-formulations with EBI-fungicides. Both azoxystrobin and kresoxim-methyl controlled septoria as well or better at half rate compared with full rate of the Danish standard product Tilt top (125 g propiconazole + 375 g fenpropimorph). Similarly kresoxim-methyl controlled mildew well at half dose rate. In all the trials half rates of strobilurins gave better yield responses than full rates of the standard fungicide. Trials comparing 33% of full dose rates of different strobilurins showed that different products had advantages and disadvantages. The co-formulation kresoxim-methyl + epoxiconazole gave good control of all major diseases of wheat and produced good yield responses, indicating its potential for use at reduced rates. In 37 trials comparing full, 50% and 25% rates of either azoxystrobin or kresoxim-methyl + fenpropimorph, the full rate continually failed to give the best economic benefit, when deducting costs of fungicide and application from the harvested yield. The differences between 50% and 25% rates were, however, often marginal, indicating that the best economic return can be achieved by using 25% to 50% rate, depending on the spectrum and severity of diseases.

INTRODUCTION

For more than 10 years, Denmark has used reduced and split dosages of fungicides in cereals. Both farming practise and trial results with EBI fungicides have generally shown good results when using reduced and adjusted dose rates. Experiments have shown that reduced rates can provide acceptable control of cereal diseases without an increased risk of yield losses (Jørgensen & Nielsen, 1992). The success of reduced dose strategies relies on spraying when disease incidence is low and adjusting spray intervals relative to the dosages used (Jørgensen, 1994).

Strobilurin analogues are a novel group of fungicides that have been under development since the early 1980's. Azoxystrobin and kresoxim-methyl were presented in 1992 (Ammermann *et al.*, 1992; Godwin *et al.*, 1992) and became commercially available in 1996. CGA 279202 with the proposed common name trifloxystrobin was presented in 1998 (Margot *et al.*, 1998). Compared to fungicides already in use, strobilurins have shown potential for increased efficacy and yield responses for several diseases of cereals (Godwin *et al.*, 1992; Hanhart &

Frahm, 1996). In 1998 the first strobilurin (azoxystrobin) was registered in Denmark. Results using reduced rates of strobilurins in Denmark are based on trial work done from 1993-98. Strobilurins have a better and longer preventive effect than the EBI fungicides, whereas the curative effect is either less or similar to that of products like propiconazole and tebuconazole (Jørgensen *et al.*, In Press; Godwin *et al.*, In Press). To achieve maximum benefit from strobilurins, new low dose strategies have been tested to provide good, economic recommendations for farmers. This report describes some of the experiments done in winter wheat to evaluate the effects of using reduced dosages.

MATERIALS AND METHODS

Field trials were done at The Danish Institute of Agricultural Sciences (DIAS) or The Danish Agricultural Advisory Centre (DAAC). The design of the trials was a randomized complete block with four replicates and a plot size of 25-32 m^2 at DIAS. The trial design at DAAC was a systematic complete block design with 5 replicates. The fungicides were applied with knapsack sprayers at low pressure (2-3 bar) using flat fan nozzles in a volume of 200-300 litres/ha. Disease assessments reported in this paper were all done at DIAS, as per cent leaf area covered by the individual diseases. Attacks of septoria were found in almost all trials with *Septoria tritici* dominant, but mixed infections with *Stagonospora nodorum* were also seen. *Erysiphe graminis* and *Puccinia striiformis* were found in fewer trials. Before statistical analysis, disease data was logit-transformed. The plots were harvested with a plot combine harvester and the grain yield was corrected to 15% moisture content. When calculating net yields (profit) the prices used were: Tilt top 350 dkr per litre, Amistar 490 dkr per litre, Mentor 485 dkr per litre and 60 dkr per application; grain price, 800 dkr per tonne. The following products were tested:

Products	**normal rate**	**active ingredients per litre**	**Code**
Tilt top	1.0 l/ha	125 g propiconazole+375 g fenpropimorph	Prop+fen
Amistar	1.0 l/ha	250 g azoxystrobin per litre	Azoxy
Amistar Pro	2.0 l/ha	100 g azoxystrobin + 280 g fenpropimorph	Azoxy+fen
Mentor	0.7 l/ha	150 g kresoxim-methyl + 300 g fenpropimorph	Kr-Me+fen
Diamant	1.0 l/ha	125 g kresoxim-methyl + 125 g epoxiconazole	Kr-Me+epox
Stratego	1.0 l/ha	188 g CGA 279202 +125 g propiconazole	Trif+prop
BAS 490 F	0.25 l/ha	500 g kresoxim-methyl	Kr-Me

RESULTS

Field trials using 2-3 dosages of the new products in comparison with a standard product showed that all the strobilurins have potential to be used at reduced dose (Table 1). Both kresoxim-methyl and azoxystrobin used alone showed variability in effectiveness, which should be considered when using recommended reduced rates. Azoxystrobin and kresoxim-methyl gave insufficient control of mildew and yellow rust, respectively. No trials have been done with the strobilurin CGA 279202 used alone. All co-formulations with strobilurins and EBI-fungicides had a broader and greater effect on disease control. Generally, a half rate of all strobilurin-products gave better or similar control to a full dose of Tilt top, which has dominated the market in Denmark for more than 10 years. Half dose rates of all strobilurins in all the trials increased the yield compared to the standard.

Table 1. Percentage leaf area attacked by diseases in winter wheat and yield response in field trials using 100%, 75% and 50% rate of strobilurins. Treatments were applied at GS 31 and 45-59. Treatments followed by different letters were significantly different.

Treatments Per cent dose per ha	% area attacked			No. of green leaves GS 75-85	Yield, yield increases (t/ha)
	Septoria GS 71-75	Mildew GS 71-75	Yellow rust GS 65-73		
Untreated	6.5 a	13.9 a	25.0 a	1.3	6.73
2 x 100% Prop+fen	2.9 b	1.9 b	0.0 d	2.3	0.78
2 x 100% Kr-Me	1.7 c	0.6 c	1.0 c	2.5	1.34
2 x 50% Kr-Me	2.7 b	1.4 bc	2.0 bc	2.2	1.25
LSD 95	-	-	-	*0.5*	*0.45*
No. of trials	*5*	*6*	*1*	*3*	*6*
Untreated	21.8 a	15.0 a	36.7 a	0.9	7.23
2 x 100% Prop+fen	5.5 b	2.6 b	0.2 c	1.3	1.01
2 x 100% Kr-Me+fen	3.3 c	0.1 d	0.4 c	1.6	1.57
2 x 75% Kr-Me+fen	4.7 bc	0.3 d	1.5 bc	1.6	1.42
2 x 50% Kr-Me+fen	6.6 b	0.9 c	3.5 b	1.4	1.15
LSD 95	-	-	-	*0.2*	*0.35*
No. of trials	*7*	*5*	*1*	*9*	*10*
Untreated	25.3 a	17.5 a	-	0.6	7.00
2 x 100% Prop+fen	8.3 b	9.1 b	-	1.0	0.84
2 x 100% Kr-Me+epox	0.9 d	0.3 c	-	1.5	1.95
2 x 75% Kr-Me+epox	2.0 cd	1.0 c	-	1.4	1.72
2 x 50% Kr-Me+epox	3.2 c	2.1 c	-	1.2	1.59
LSD 95	-	-		*0.3*	*0.31*
No. of trials	*4*	*2*		*4*	*4*
Untreated	7.1 a	7.4 a	25.7 a	1.1	7.20
2 x 100% Prop+fen	2.5 b	1.6 c	0 b	2.3	1.00
2 x 100% Azoxy	1.2 c	2.8 bc	0 b	2.5	1.62
2 x 75% Azoxy	1.4 bc	3.3 b	0 b	2.3	1.58
2 x 50% Azoxy	2.1 bc	4.2 b	0.1 b	2.2	1.31
LSD 95	-	-	-	*0.2*	*0.48*
No. of trials	*6*	*7*	*3*	*6*	*8*
Untreated	20.0	18.9 a	-	1.0	5.35
2 x 100% Prop+fen	6.1	5.8 b	-	1.6	1.14
2 x 100% Azoxy+fen	2.8	3.5 b	-	1.8	1.98
2 x 75% Azoxy+fen	3.2	4.5 b	-	1.7	1.85
2 x 50% Azoxy+fen	4.8	7.6 b	-	1.8	1.73
LSD 95	*1.3*	-	-	*ns*	*0.46*
No. of trials	*4*	*4*	-	*2*	*4*
Untreated	19.6 a	19.6 a	60.0	0.7	6.37
2 x 100% Prop+fen	7.1 b	5.5 b	10.0	1.2	1.14
2 x 100% Trif+prop	3.3 c	2.0 cd	3.0	1.4	1.77
2 x 75% Trif+prop	4.7 c	3.1 bcd	4.3	1.3	1.62
1 x 50% Trif+prop	6.3 bc	4.4 bc	3.8	1.4	1.47
LSD 95	-	-	-	*0.2*	*0.24*
No. of trials	*4*	*4*	*1*	*4*	*4*

In trials comparing 33% rate of different strobilurins, the effectivness of the different products is clearly shown (Table 2). Two treatments of 33% rate of kresoxim-methyl+epoxiconazole gave excellent control of septoria (>90%) followed by CGA 279202+propiconazole and azoxystrobin+fenpropimorph. Products with kresoxim-methyl gave better control of *Erysiphe graminis*, even at 33% rate, compared to other products. Little information is available about the control of *Puccinia striiformis* using reduced doses of strobilurins. However, the co-formulations including either propiconazole or epoxiconazole were expected to give effective control. Azoxystrobin and kresoxim-methyl both have good preventive effects against yellow rust but azoxystrobin gave greater control of this disease (similar to that of propiconazole).

The economic benefit from using reduced dose strategies has been measured in trials done by the advisory centre (Table 3 and 4). Although strobilurins always gave a greater yield response than the standard product (Table 4), the higher cost of the products reduced the economic benefit considerably. Full dose rate failed to give the best net yield response in either of the trials series (Table 3). The differences between 50% and 25% rates were, however, marginal, indicating that the best economic return can be achieved by using 25-50% of the normal dose rate depending on the dominance of diseases.

Table 2. Percentage leaf area attacked by diseases in winter wheat and yield response in 1996-98 using 33% of the full dose rate. Treatments were applied at GS 31-32 and 45-59.

Treatments Litre per ha	% area attacked						No. of green leaves	Yield, yield increase (t/ha)	
	Mildew		Septoria			Yellow Rust			
	1996	1998	1996	1997	1998	1998	1997	1996	1997
Untreated	23.9	18.5	25.4	19.3	34.2	30.0	0.6	4.78	7.83
2 x 0.33 l Prop+fen	8.4	10.5	11.3	8.3	15.4	2.8	0.9	0.97	0.49
2 x 0.23 l Kr-Me+fen	4.5	2.2	8.6	6.9	12.4	4.3	1.1	1.29	0.78
2 x 0.66 l Azoxy+fen	6.4	9.0	5.8	4.4	12.2	2.2	1.3	1.48	1.08
2 x 0.33 l Trif.+prop	7.3	4.5	6.1	-	4.8	1.2	-	1.39	-
2 x 0.33 l Kr-Me+epox	-	0.9	-	1.6	2.3	0.6	1.3	-	1.30
No. of trials	4	1	4	3	3	2	4	4	4
Growth stage	73-75	73	71-73	71-75	69-73	69-71	85		
LSD95						2.0	0.2	0.29	0.15

Table 3. Average yield increases and net yield from 2 treatments at GS 31-32 and 49-59 in 23 trials with kresoxim-methyl+fenpropimorph 1995-97 and 14 trials with azoxystrobin in 1996 and 1997.

Treatments Litre per ha	Yield increase (t/ha)	net yield response* (t/ha)	Per cent of trials giving profit
2 x 0.70 l Kr-Me+fen	1.05	0.05	48
2 x 0.35 l Kr-Me+fen	0.84	0.26	70
2 x 0.18 l Kr-Me+fen	0.70	0.34	83
2 x 1.0 l Azoxy	1.10	-0.28	29
2 x 0.50 l Azoxy	0.99	0.23	79
2 x 0.25 l Azoxy	0.78	0.32	86

*Yield response after deduction of costs

Table 4. Yield increase and yield minus cost, when comparing 50% and 25% dose rate of propiconazole + fenpropimorph and kresoxim-methyl + fenpropimorph in 14 trials and half dose of propiconazole + fenpropimorph and azoxystrobin in 11 trials in 1995-96.

Treatment	Yield increase (t/ha)	Net yield response (t/ha)*
2 x 50 % dose Prop+fen	0.40	-0.18
2 x 50% dose Kr-Me+fen	0.86	0.28
2 x 25% dose Prop+fen	0.28	0.09
2 x 25% dose Kr-Me+fen	0.73	0.36
2 x 50% dose Prop+fen	0.44	-0.15
2 x 50% dose Azoxy	0.87	0.11

*Yield response after deduction of costs

DISCUSSION

Farmers are generally concerned about keeping their variable costs as low as possible, which means that they are now focussing more on net yield response than gross yield. Data from many trials in Denmark using strobilurins indicate that there are good possibilities of continuing the reduced dose strategy, which has been developed over the last 10 years using EBI-fungicides. Modification and adjustments to thresholds used previously will be done in order to maximise the potential of strobilurins.

Results from field experiments using reduced dosages of strobilurin products alone have generally given better control of mildew and septoria diseases in winter wheat and greater yields compared with traditional EBI-fungicides used at reduced rates (Table 2). This gives the farmers an opportunity to improve their net return from fungicide treatments, despite the higher prices of the strobilurins. The use of co-formulations of strobilurins and triazoles has further reduced diseases and increased yields.

Azoxystrobin provided good control of septoria, especially at the end of the season. Early season assessments, however, clearly indicated a better effect from EBI products. With the severity of septoria normally occurring under Danish conditions, dosages between 25% and 50% have generally given acceptable control. To get the full benefit of azoxystrobin's preventive effect and also a greater yield response, the recommended dose rate and timing for septoria treatment is being revised. Trials from 1996 and 1997 indicated that a threshold for application of 4 days of rain might give a better result than the 8 days threshold used previously (Jørgensen *et al.*, In Press), something which is still under investigation. For satisfactory mildew control, azoxystrobin should be mixed with a fungicide effective against mildew, such as fenpropimorph, fenpropidin or quinoxyfen.

The effectiveness of kresoxim-methyl and other co-formulations with strobilurins against mildew indicates that the previous strategy of applying reduced rates in crops with early slight attacks of mildew should continue. A similar strategy is also used for EBI's. Strobilurins, however, provide better opportunities of improving both disease control and yield.

Co-formulations of strobilurins with triazoles gave excellent control of all important diseases and good yield responses. The potential for control using these co-formulations should be carefully examined, as trials comparing 33% rate of these products indicated that in most cases they provided a satisfactory control as long as the timing was correct. Using more than half dose rate in Denmark would in most years be unnecessary and could not be justified due to the general political pressure to reduce pesticide input. This fact is emphasized under Danish conditions as even for azoxystrobin and kresoxim-methyl+fenpropimorph two treatments at 25-50% rate gave best net yields.

As for the EBI-fungicides, the risk of developing resistance to strobilurins is considered to be moderate (Godwin *et al.*, In Press). The risk of increasing resistance development by using reduced dosages should be assessed as it has been for EBI- fungicides for more than 15 years. In Denmark, despite use of split and reduced dose strategies for more than 10 years, there has been a similar shift in sensitivity to that in other countries using intensive control of fungal disease applying higher dosages of EBI-fungicides (Nielsen & Munk, 1997). It is recommended that strobilurins should not be applied using repeated application of sub-optimal dosages (Godwin *et al.*, In Press). In future it will be important to monitor changes in sensitivity to strobilurins, and to encourage the use of effective dosages.

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The management of *Stagonospora nodorum* on winter wheat in south west England

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ABSTRACT

Two experiments were carried out on winter wheat in Cornwall in 1997. In Experiment 1, the activity of fungicides applied as single sprays at GS 39 against *Stagonospora nodorum* was examined on cv. Brigadier. Good eradicant activity against *S. nodorum* on leaf 2 was demonstrated by triazole fungicides, particularly metconazole, tebuconazole and epoxiconazole. Flusilazole was more effective at full dose than at reduced doses. Strobilurin fungicides gave variable disease control. Azoxystrobin demonstrated moderate eradicant activity but kresoxim-methyl + fenpropimorph was less effective, similar to the protectant fungicide chlorothalonil. The anilinopyrimidine fungicide cyprodinil showed good activity against *S. nodorum* but its lack of activity against *S. tritici* compromised disease control. Yield was severely reduced by *S. nodorum* and untreated plots yielded 2.17 t/ha. Full dose kresoxim-methyl + epoxiconazole and epoxiconazole alone increased yield to 4.78 and 5.21 t/ha. Experiment 2 examined the interaction of host resistance and fungicide dose. By GS 75, most cultivars required a minimum of three-quarter dose to give adequate control of a disease complex of *S. nodorum* and *S. tritici* on the flag leaf. Glume blotch was more severe on cv. Admiral, and was little affected by fungicides. The combination of severe *S. nodorum* on upper leaves and ears caused considerable yield loss. Admiral yielded only 0.54 t/ha without fungicide treatment and 1.38 t/ha with a full dose of tebuconazole. The yield of Brigadier was also severely reduced and was lower than that of more resistant cultivars ($P<0.05$). The most resistant cultivar, Spark, yielded 3.07 t/ha without fungicide and 5.35 t/ha with a full dose of tebuconazole.

INTRODUCTION

Stagonospora nodorum (Syn: *Septoria nodorum*) infects the leaves and ears of wheat to cause leaf spot and glume blotch respectively. It is primarily a trash-borne disease, but can be seed-borne. It is potentially the most damaging foliar disease of winter wheat, but since the 1980s it has generally been confined to a low incidence in the UK (Polley & Thomas, 1991). However, *S. nodorum* regularly affects crops of winter wheat grown in south-western England, where under favourable weather conditions (warm and wet), the relatively short incubation period of *S. nodorum* of 6-14 days (Djurle *et al.*, 1996) results in rapid colonisation of the upper leaves and ears. The disease can cause substantial yield losses if it is not adequately controlled.

MATERIALS AND METHODS

Two experiments were established at a site in Cornwall in October 1996. Experiment 1 examined nine fungicides on plots (20 m x 2.15 m) of the susceptible cv. Brigadier, each at full

label recommended dose, three-quarter dose, half dose and quarter dose. Untreated controls were also included. The fungicides and their full label recommended doses of product were: metconazole (ACF 474, 1.5 l/ha), azoxystrobin (Amistar, 1.0 l/ha), chlorothalonil (Bravo 500, 2.0 l/ha), fenpropimorph + kresoxim-methyl (Ensign, 0.7 l/ha), tebuconazole (Folicur, 1.0 l/ha), flusilazole (Genie, 0.4 l/ha), epoxiconazole + kresoxim-methyl (Landmark, 1.0 l/ha), epoxiconazole (Opus, 1.0 l/ha), and cyprodinil (Unix, 1.0 kg/ha). In Experiment 2, six cultivars (Admiral, Brigadier, Hunter, Hussar, Mercia and Spark) each received a single application of tebuconazole (as Folicur) at full label recommended dose (1.0 l/ha), three-quarter dose, half, quarter and zero dose (i.e. untreated). In each experiment, fungicides were applied when the flag leaf was emerged (GS 39) using an Oxford Precision Sprayer fitted with TeeJet XR11003 nozzles operating at 200 kPa pressure. Each of the treatments was randomised and replicated three times. All plots were sampled by randomly collecting 10 main shoots per plot on 17 June (GS 69) and on 10 July (GS 75). Disease was assessed as the percentage leaf area affected. Glume blotch was assessed on 10 randomly selected ears per plot on 22 July (GS 85) as percentage ear area affected. Plots were harvested using a Sampo plot combine and yields calculated at 85% dry matter.

RESULTS AND DISCUSSION

Weather and disease development

Dry weather in April did not favour the development of *S. nodorum* or *S. tritici*, and by 25 April, when the penultimate leaf was emerging, the upper three leaves showed no disease symptoms. Wet weather between 25 and 28 April enabled *Stagonospora* and *Septoria* to infect eventual leaf 2, and further rain between 3 May and 20 May enabled both pathogens to infect the flag leaf. During both periods, rainfall was sufficiently heavy to splash spores to upper leaves (Figure 1). Fungicides were applied on 14 May when the flag leaf was just fully emerged on most plants. Although the upper four leaves were largely free from symptoms of *Septoria* or *Stagonospora* at that time, effective disease control on leaf 2 required considerable eradicant activity by the fungicides (up to 19 days) plus protection during May and June. The flag leaf emerged during the period 4-14 May, and disease control on that leaf also required some eradicant activity (up to 10 days) plus forward protection until the end of June. Ears were not exposed when fungicides were applied and any control of glume blotch reflected the ability of fungicides to reduce inoculum on the upper leaves, limiting transfer of conidia to ears.

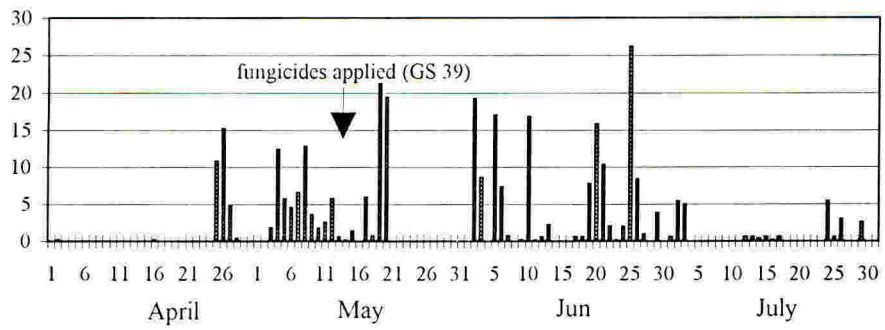

Figure 1. Daily rainfall measured at the site from April to July 1997.

Experiment 1

The effects of fungicides on *S. nodorum* at GS 69 are shown in Figure 2.

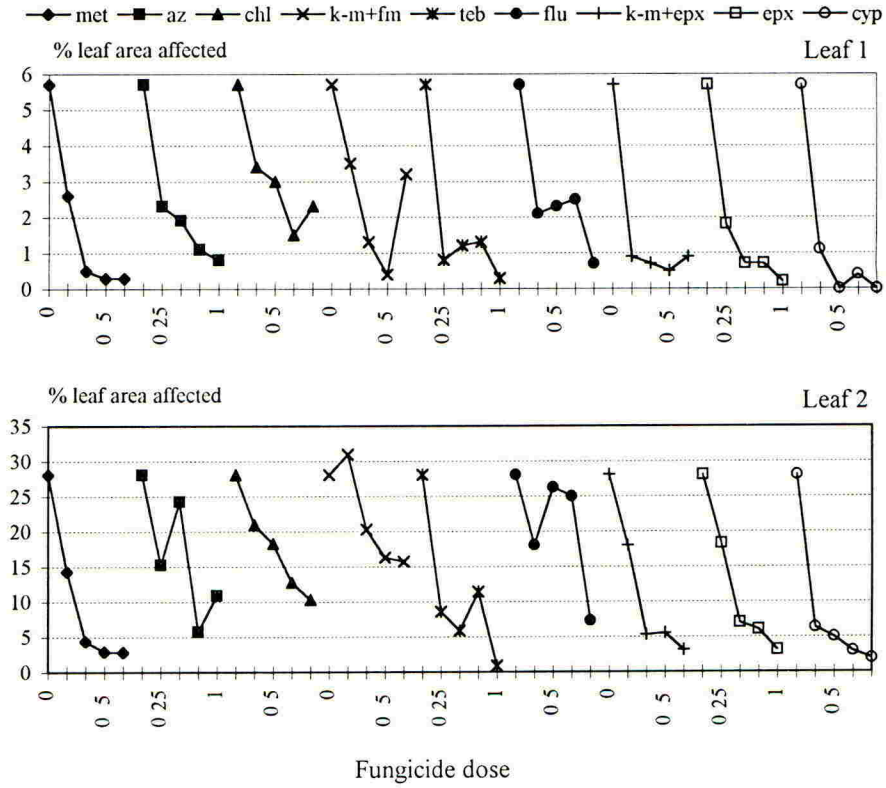

Figure 2. Effect of fungicides and dose on *S. nodorum* on upper leaves of cv. Brigadier at GS 69.

(az - azoxystrobin; chl - chlorothalonil; cyp - cyprodinil; epx - epoxiconazole; flu - flusilazole; k-m+fm - kresoxim-methyl + fenpropimorph; k-m+epx - kresoxim-methyl + epoxiconazole; met - metconazole; teb - tebuconazole)

Half dose cyprodinil gave good control of *S. nodorum* on the flag leaf at GS 69. Metconazole, tebuconazole and epoxiconazole also gave good control. On leaf 2, kresoxim-methyl + fenpropimorph and chlorothalonil were least effective. Metconazole, tebuconazole, kresoxim-methyl + epoxiconazole, epoxiconazole and cyprodinil all gave good control at half dose but a full dose of flusilazole was required for effective eradicant activity (Figure 2).

At GS 75, epoxiconazole and kresoxim-methyl + epoxiconazole showed good persistence at full and three-quarter dose against disease on leaf 1. Chlorothalonil and kresoxim-methyl + fenpropimorph failed to reduce the levels of disease substantially. The apparently poor performance of cyprodinil is probably a reflection of its failure to control *S. tritici*. A full dose of flusilazole was necessary to achieve any disease control (Figure 3).

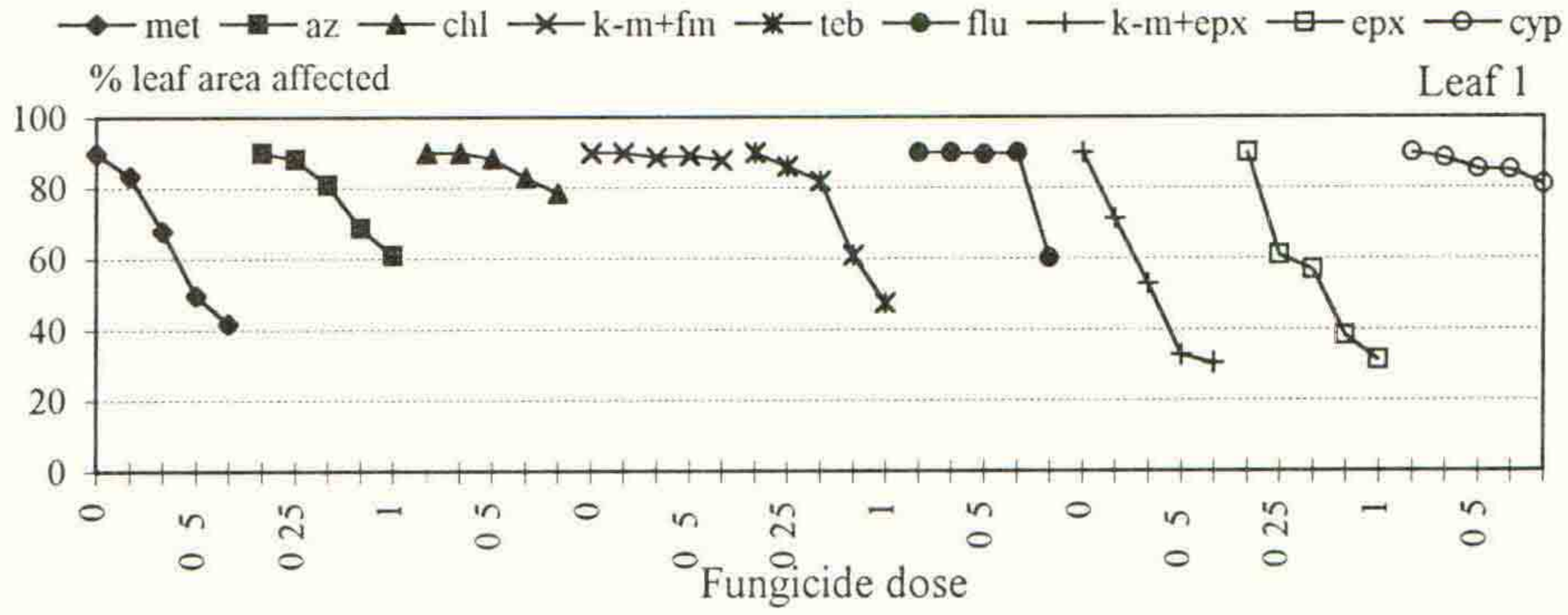

Figure 3. Effect of fungicides and dose on *S. nodorum/S. tritici* disease complex on the flag leaf of cv. Brigadier at GS 75.

S. nodorum caused severe glume blotch. None of the fungicide treatments applied at GS 39 affected the severity of glume blotch assessed at GS 85. This can be explained by the considerable rainfall experienced during June which would have been sufficient to splash conidia of *S. nodorum* from infected lower leaves to unprotected ears. It has been reported that upward splash of conidia to a height of 2m is possible (Arseniuk *et al.*, 1998).

The rapid destruction of green leaf area, mainly by *S. nodorum*, and the high levels of glume blotch severely limited yield. Untreated plots gave an average yield of just 2.17 t/ha (Figure 4).

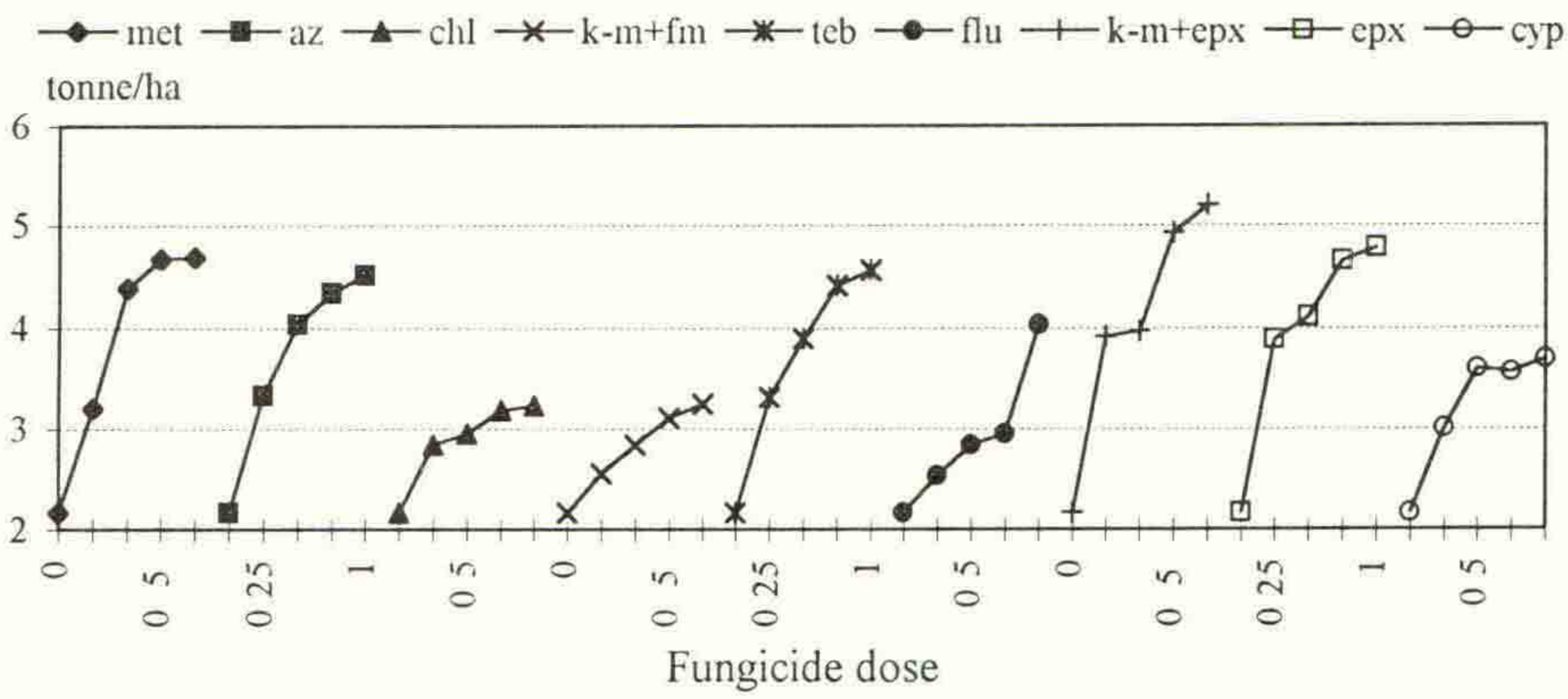

Figure 4. Effect of fungicides and dose on grain yield of cv. Brigadier.

Experiment 2

Quarter dose tebuconazole gave good control of *S. nodorum* on leaf 1 at GS 69 (Figure 5). On leaf 2, more eradicant activity was required and three-quarter or full dose was necessary to give good control of *S. nodorum* on Brigadier, but on Admiral, the quarter dose was still effective.

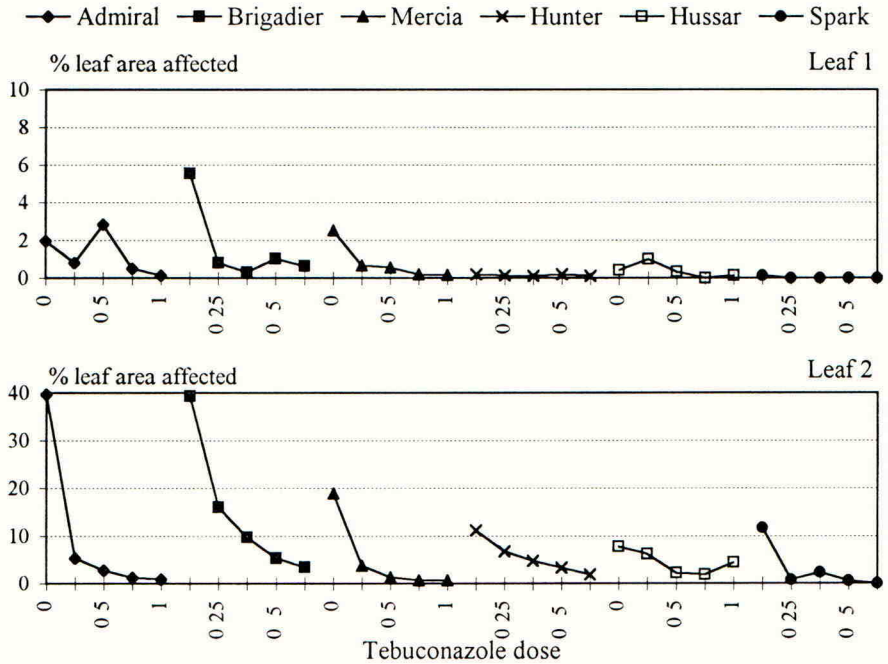

Figure 5. Effect of tebuconazole dose on *S. nodorum* on the upper leaves of six cultivars at GS 69.

By GS 75, the flag leaf carried high levels of *S. nodorum* and *S. tritici* (Figure 6). Admiral and Brigadier were severely affected by the disease complex. The disease recorded on Hussar was probably due mainly to *S. tritici*. Spark was the most resistant cultivar. Three-quarter dose was necessary to give maximum disease control on the flag leaf for all cultivars.

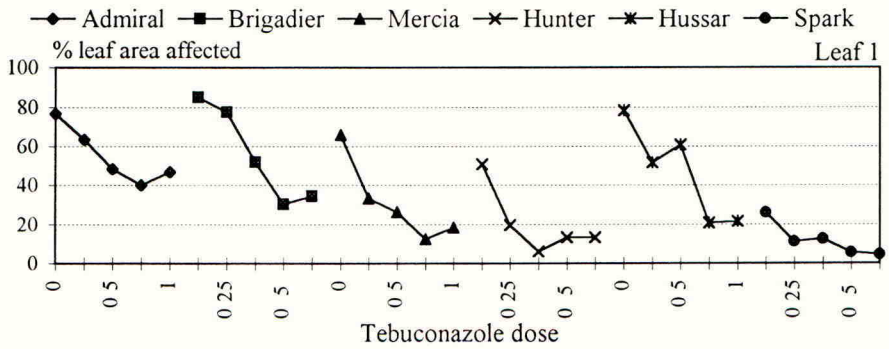

Figure 6. Effect of tebuconazole dose on *S. nodorum/S. tritici* disease complex on the flag leaf of six cultivars at GS 75.

As in Experiment 1, fungicide sprays applied at GS 39 did not affect the severity of glume blotch, but Admiral was more severely affected than other cultivars ($P<0.05$). Admiral gave the lowest yield without fungicide treatment (0.54 t/ha). The yield of untreated Brigadier was also low. The mean yields of Mercia, Hunter and Hussar were similar and greater than the mean yields of Brigadier and Admiral ($P<0.05$). The value of genetic resistance was demonstrated by Spark which gave a greater yield when untreated than untreated Brigadier and Mercia or treated Admiral ($P<0.05$) (Figure 7).

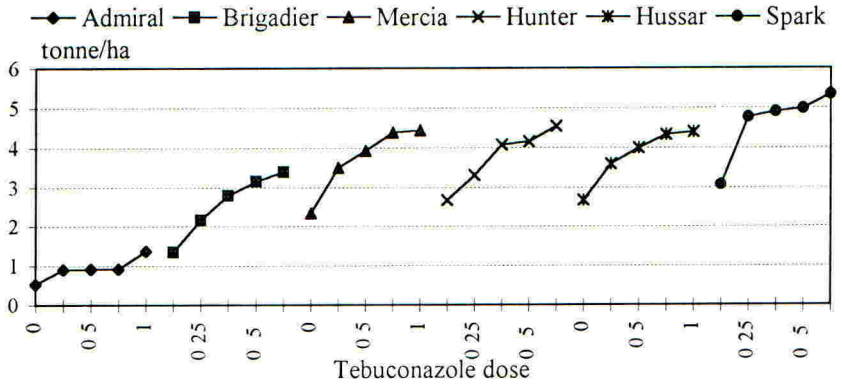

Figure 7. Effect of tebuconazole dose on yield of six cultivars.

Admiral required a full dose of tebuconazole to increase its yield ($P<0.05$), but a quarter dose increased the yield of the other cultivars compared with the untreated ($P<0.05$). Under the conditions of severe disease pressure experienced at this site, single fungicide sprays were clearly unable to give satisfactory disease control, but the work has identified fungicides with good activity against *S. nodorum* (e.g. epoxiconazole, cyprodinil, metconazole, tebuconazole) and has also identified the benefits of genetic resistance, particularly in cv Spark.

ACKNOWLEDGEMENTS

We thank the Home-Grown Cereals Authority for funding the work, Alex Stephens for providing the trial site, and Chris Dyer for analysing the data.

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Fungal diseases of white lupin (*Lupinus albus*) and their control

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ABSTRACT

A field experiment is described in which the relative importance of different fungal pathogens of white lupin (*Lupinus albus*) and their potential for control by fungicides were assessed. The involvement of fungal pathogens (e.g. *Fusarium avenaceum*) in overwinter losses of lupins was examined. Application of inoculum of *F.avenaceum* caused significant disease and slightly increased the number of plants lost in the winter. Seed treatment with iprodione + carbendazim increased plant survival in the winter, decreased the number of plants infected by *F.avenaceum* and increased seed yield. Prochloraz applied as an autumn spray also decreased plant infection by *F.avenaceum* and increased grain yield. Application of tebuconazole during the summer effectively controlled both rust (*Uromyces lupinicolus*) and brown spot (augmented by artificially applied inoculum of *Pleiochaeta setosa*) and significantly increased seed yield. Autumn fungicides also decreased the severity of foliar diseases, assessed in June and July.

INTRODUCTION

The white lupin (*Lupinus albus*) has considerable potential as a new high-quality protein crop in the UK. Genotypes being developed for autumn-sowing have a florally determinate habit, which ensures that the crop ripens, and are cold tolerant. Several fungi have been identified as threats to lupins in the UK (Bateman *et al.*, 1991). Severe frost is the principal cause of overwinter losses but evidence suggests that plant pathogenic fungi may also be involved. Fungi, such as *Fusarium avenaceum*, have been implicated in overwinter losses because of their frequent isolation from injured plants (Bateman *et al.*, 1991), their pathogenicity in glasshouse tests (Bateman, 1997) and increased plant survival with autumn-applied fungicides. Natural occurrence of leaf diseases in white lupin in the summer months is erratic. Rust (*Uromyces lupinicolus*), brown spot (*Pleiochaeta setosa*) and powdery mildew (*Oidium erysiphoides*) all occur at Rothamsted and are known to cause losses in seed yield when untreated.

MATERIALS AND METHODS

A field experiment at Rothamsted in 1996-97 was one of a series designed to investigate the involvement of fungal pathogens in overwinter losses of white lupin and the incidence and control of leaf diseases in the spring and summer. The experiment site was on clay-loam with flints of pH 6.5-7.2 and the lupin crop (cv. Lucyane) followed set-aside and spring oats in the previous two years. There were 48 plots (9 m x 5.4 m) in three randomized blocks. A 3-m strip at the end of each plot was used for destructive sampling and the remainder was used for yield, plant counts and *in situ* disease assessments. Treatments are shown in Table 1.

Table 1. Treatments applied to lupins in a field experiment in 1996-97.

Factor	Treatment
Sowing date	4 September 1996 (early)
	2 October 1996 (late)
Application of *Fusarium avenaceum* inoculum	None
	Inoculated
Seed treatment fungicide	None
	Iprodione + carbendazim (105 g carbendazim + 53 g iprodione 100 kg^{-1}; Germi Pro UFB)
	Fludioxonil (400 ml a.i. 100 kg^{-1}; Beret Gold)
Autumn fungicide spray	None
	Prochloraz (450 g a.i. ha^{-1}; Sportak 45), 14 Nov. 1996
Summer fungicide spray	None
	Tebuconazole (250 g a.i. ha^{-1}; Folicur), 13 May 1997, 9 June 1997

Inocula of the fungal pathogens *Fusarium avenaceum* and *Pleiochaeta setosa* were prepared by growing the fungi on oat grains sterilised by autoclaving in 5-l flasks for 4-6 wk; inocula were then spread out to dry and stored at 4°C until required.

Seed beds were prepared by subsoiling, ploughing and furrow-pressing. The *F.avenaceum* inoculum was broadcast on the seed bed (1 kg per plot); plots were then rotary harrowed and drilled the following day. The seed was inoculated, by mixing, with MicroBio HiStick *Rhizobium* Inoculant (MicroBio Ltd, Hemel Hempstead, UK) at 4g kg^{-1} and drilled at 40 seeds m^{-2}. Herbicides were applied to the seed bed (pendimethalin as Stomp 400 SC) and in November (carbetamide as Carbetamex and simazine as MSS Simazine 50 FL). Deltamethrin (Decis) was applied in November to control thrips. *Pleiochaeta setosa* inoculum was broadcast over all plots on 17 March 1997 (350 g per plot).

Plants were counted each month in the same three 1-m lengths of row per plot. Ten plants were taken at intervals from random positions in the sampling areas and assessed for frost damage and fungal necrosis. Damage to each plant was scored as: 0 = no damage, 1 = 1-25% of tissue damaged, 2 = 26-50% damaged, 3 = 51-75% damaged and 4 = 76-100% damaged. Foliar diseases were assessed *in situ*. Ten plants per plot were selected at random and scored as: 0 = no disease present, 1 = trace of disease, 2 = disease only present in lower leaves and 3 = disease spreading to upper leaves. Fungi present on plants were isolated and identified after surface-sterilising plant pieces in sodium hypochlorite (2% available chlorine), rinsing twice in sterile water, placing on 1/5 concentration potato dextrose agar (PDA) and incubating at 20°C for 1-2 wk.

RESULTS

Overwinter losses

More plants survived through the winter and spring and seed yield was greater in early-sown than in late-sown plots (Table 2). Most plant death occurred between January and March and was particularly noticeable in the late-sown plots where plant numbers decreased by more than 50% during these months. Application of *Fusarium avenaceum* inoculum resulted in a slight decrease in the plant population between November and January; no effect was observed in the spring. Seed treatment with iprodione + carbendazim increased plant survival in winter and early spring and all fungicide treatments increased yield relative to the untreated control.

Table 2. Effects of sowing date, added inoculum of *Fusarium avenaceum* and seed treatments or autumn fungicide spray on plant population and seed yield.

	Plants m^{-2}								Seed yield (t ha^{-1})
	5 Nov.	28 Nov.	14 Jan.	7 Mar.	7 Apr.	7 May	9 Jun.	16 Jul.	
Sowing time									
Early	33.2	32.4	30.6	20.9	18.4	17.7	17.0	16.8	1.11
Late	33.9	33.7	33.2	16.1	10.3	10.1	9.3	8.9	0.13
SED (30 df)	1.85	1.81	1.81	1.48	1.57	1.57	1.56	1.24	0.11
P	0.7	0.5	0.2	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
Inoculum									
None	35.4	34.7	33.8	19.3	15.0	14.5	13.8	13.4	0.63
+ *F.avenaceum*	31.7	31.3	30.0	17.8	13.6	13.3	12.5	12.3	0.60
SED (30 df)	1.85	1.81	1.81	1.48	1.57	1.57	1.56	1.24	0.11
P	0.06	0.07	0.05	0.3	0.4	0.4	0.4	0.4	0.8
Seed treatment/autumn fungicide									
None	30.4	29.8	28.6	17.2	13.6	13.6	13.0	12.5	0.22
Iprodione + carbendazim	38.4	38.2	37.1	23.1	16.3	16.0	15.0	14.6	1.30
Fludioxonil	33.2	32.7	31.5	16.2	13.1	12.6	12.3	12.4	1.21
Prochloraz	32.3	31.5	31.5	17.7	14.3	13.4	12.3	11.8	1.72
SED (30 df)	2.63	2.56	2.56	2.09	2.22	2.22	2.21	1.75	0.22
P	0.03	0.02	0.02	0.01	0.5	0.5	0.6	0.4	0.002

In February, necrosis caused by frost was more severe in late-sown plots (score for hypocotyl, 1.76, score for root, 2.0) than in early-sown plots (score for hypocotyl, 0.54, score for root, 0.68). Late-sown plants were more susceptible to infection by *Fusarium avenaceum*; the fungus was isolated from 44% of late-sown plants compared with 19% of early-sown plants (P =

<0.001). *F.avenaceum* was isolated from more plants in plots that had inoculum applied than in those that had not (Table 3). In plots receiving inoculum of *F.avenaceum*, the percentage recovery of the fungus was less (by more than 20%) in plots in which a fungicide seed treatment or autumn fungicide spray had been used. The percentage of plants from which *F.avenaceum* was recovered was significantly smaller in those plots in which either iprodione + carbendazim as a seed treatment or prochloraz as an autumn spray had been used (Table 3). *Cylindrocarpon destructans*, a minor pathogen, was isolated commonly. This fungus was recovered from plants in the greatest numbers where *F.avenaceum* inoculum had not been applied to plots (Table 3).

Table 3. Effects of added inoculum of *Fusarium avenaceum* and seed treatment or autumn fungicide spray on the frequency of isolation of fungi from lower hypocotyl or upper root region of plants of white lupin sampled on 3 February.

	Logit (% plants) (back-transformed means)	
	Fusarium avenaceum	*Cylindrocarpon destructans*
Inoculum		
None added	-1.076 (9.9)	-0.371 (31.8)
+ Inoculum	0.270 (62.7)	-0.783 (16.8)
SED (30 df)	0.1128	0.1174
P	<0.001	<0.001
Seed treatment		
None	-0.212 (39.0)	-0.511 (26.0)
Iprodione + carbendazim	-0.560 (24.1)	-0.711 (18.9)
Fludioxonil	-0.298 (35.0)	-0.649 (21.0)
Prochloraz	-0.539 (24.9)	-0.436 (29.0)
SED (30 df)	0.1595	0.1661
P	0.1	0.3

Leaf diseases

The inoculum of *Pleiochaeta setosa* was effective; typical brown spots were observed on leaves, stems and pods and symptoms were severe when uncontrolled. A natural infection of rust (*Uromyces lupinicolus*) developed and spread across the experimental plots. Both diseases were well controlled by two applications of tebuconazole (Table 4) and treatment resulted in a marked increase in seed yield. The seed treatments and autumn fungicide spray also reduced the severity of brown spot and rust and increased seed yields.

Table 4. Effects of summer and autumn fungicide treatments on severity of rust and brown spot (0-3 score) and seed yield.

	Severity of brown spot	Severity of rust		Seed yield (t ha^{-1})
	13 June	6 June	16 July	
Summer fungicide				
None	1.23	1.97	1.63	0.28
Tebuconazole	0.48	0.95	0.19	1.94
SED (18 df)	0.1344	0.1251	0.0898	0.174
P	<0.001	<0.001	<0.001	<0.001
Seed treatment/autumn fungicide				
None	1.25	2.08	1.75	0.22
Iprodione + carbendazim	0.68	1.17	0.67	1.30
Fludioxonil	0.69	1.17	0.63	1.21
Prochloraz	0.78	1.33	0.58	1.72
SED (18 df)	0.1551	0.1445	0.1037	0.220
P	0.005	<0.001	<0.001	0.002

DISCUSSION

The most important factor affecting lupin plant survival during the winter was sowing date. Late sowing resulted in poor plant structure, making the plants more susceptible to frost damage and to infection by fungal pathogens (e.g. *Fusarium avenaceum*). Differences between the two sowing dates became apparent from March 1997 when heavy losses were sustained by the smaller and weaker late-sown plants. Shield *et al.* (1996) showed that lupin crops sown in early September at Rothamsted established well and were sufficiently cold-hardy to survive early winter frosts, whereas crops sown in late October either lost many plants or were destroyed completely. The importance of fungal pathogens in causing overwinter losses is not clear. The involvement of pathogenic fungi has been implicated because prochloraz, applied as a spray in the autumn, has consistently decreased plant losses over a number of years (Bateman *et al.*, 1997). In the 1997 experiment, fungal pathogens appeared to affect the crop early in the winter. The application of inoculum of *F.avenaceum* reduced plant survival between November and January and the seed treatment with iprodione + carbendazim was effective in decreasing plant losses during this period, presumably by controlling the fungal pathogens. The use of seed treatment fungicides in experiments over several years has not given consistent effects. Iprodione + carbendazim were effective in decreasing early plant loss between November and March, decreasing the number of plants infected with *F.avenaceum* and increasing seed yield. Fludioxonil, a fungicide with specific activity against fusarium diseases, was not effective in

reducing plant loss but significantly increased seed yield. In a similar experiment in 1995-96 however, none of the seed treatment fungicides tested (iprodione + carbendazim, fenpiclonil and fludioxonil; data not shown) had any effect on plant survival or seed yield. Bateman *et al.* (1997) also reported that seed treatment fungicides had little effect but noted that their value on sites where lupins have been grown frequently or where infested seed is used remained to be determined. Spray application of prochloraz in the autumn had no positive effect on plant survival in 1996-97, although it had proved effective in previous years (Bateman *et al.*, 1997). The fungicide did, however, decrease the number of plants infected by *F.avenaceum* when sampled in February 1997 and increased seed yield.

The most important foliar diseases in the UK at the present time are rust and brown spot. Field experiments in 1996 and 1997 have shown the devastating effect of failing to control rust; total crop loss has been the result. Both rust and brown spot were well controlled by applications of tebuconazole during the summer. The fungicide seed treatments and autumn fungicide spray also appeared to have an effect in reducing the severity of foliar disease in the summer. Natural occurrence of leaf disease is erratic and inoculum of the lupin-specific *P.setosa* (brown spot pathogen) was successful in causing disease. In an experiment in 1995-96 (data not shown) attempts to inoculate the crop with *P.setosa* in the dry conditions of that season were unsuccessful. Periods of rain in 1997 allowed the fungus to sporulate and disease to spread within the crop. Natural occurrence of brown spot, from infected seed or crop residues, is relatively common; an infection of white lupins at Rothamsted in spring 1998 (data not shown) caused considerable damage to the crop when untreated.

ACKNOWLEDGEMENTS

Funding for this research by the Ministry of Agriculture, Fisheries and Food is gratefully acknowledged.

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Fungicide evaluation and risk assessment of wheat stem-base diseases using PCR

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ABSTRACT

Four wheat cultivars differing in susceptibility to stem-base diseases were untreated or treated with each of four fungicides with different activity spectra against stem-base pathogens. Diseases were assessed visually before and after fungicide applications. PCR was used to detect pathogens, to resolve disease identification problems in samples at early growth stages, before treatments were applied, and to confirm fungicidal effects. Fungicides caused no significant disease-related yield increases; this was predicted by detection of small amounts of disease and of fungal DNA at early growth stages.

INTRODUCTION

Many wheat crops are treated routinely with fungicides to control stem-base diseases, especially eyespot (*Tapesia* spp.). Efficient use of these fungicides depends on an accurate assessment of disease risk. For eyespot, this is usually done by assessing the extent of leaf sheath penetration at Zadoks growth stage (GS) 30-32. This procedure, established for the first eyespot fungicides, mostly benzimidazoles, is not necessarily useful when using newer fungicides that have good protectant activity and might be applied earlier or when the symptoms of eyespot are obscured by those of brown foot rot (*Fusarium* spp. or *Microdochium nivale*) or sharp eyespot (*Rhizoctonia cerealis*).

This paper describes part of a project aimed at putting into practice PCR-based diagnostics to identify and quantify fungal pathogens in wheat shoot bases at early growth stages and to determine thresholds for effective use of modern fungicides.

MATERIALS AND METHODS

Three similar experiments of four randomized blocks of 20 plots (10 m x 3 m) were done in 1996-7 at Rothamsted (Hertfordshire), Harper Adams Agricultural College (Shropshire) and Morley Research Centre (Norfolk). In each, untreated seed of wheat cvs Brigadier, Lynx, Mercia or Soissons, which have different susceptibility ratings to stem-base diseases, was drilled in early-mid October. Each cultivar was untreated or treated at GS 30-32 with azoxystrobin (250 g a.i. ha^{-1} as Amistar), cyprodinil (750 g a.i. ha^{-1} as Unix), flusilazole (200 g a.i. ha^{-1} as Sanction) or prochloraz (350 g a.i. ha^{-1} as Sportak).

Table 1. Incidence of disease symptoms on shoot bases assessed before and soon after fungicides were applied, Rothamsted experiment.

	Logit (% plants) (back-transformed values in parentheses)		
Cultivar	GS 22	GS 30(-31)	GS 32-33
Eyespot lesions (penetrating)			
Lynx	-1.95 (1.5)	-2.00 (1.3)	-2.04 (1.2)
Brigadier	-1.39 (5.4)	-0.71 (19.0)	-1.15 (8.6)
Mercia	-1.36 (5.7)	-1.25 (7.1)	-1.37 (5.6)
Soissons	-1.32 (6.2)	-0.94 (12.9)	-1.03 (10.8)
SED (73 d.f.)	0.133	0.139	0.155
P	<0.001	<0.001	<0.001
Brown lesions other than penetrating eyespot or distinct sharp eyespot			
Lynx	-0.92 (13.3)	-0.60 (22.6)	-0.68 (19.9)
Brigadier	-1.02 (11.0)	-0.74 (18.0)	-0.75 (17.8)
Mercia	-1.13 (9.0)	-0.65 (21.0)	-0.94 (12.7)
Soissons	-1.10 (9.5)	-0.45 (28.3)	-0.80 (16.4)
SED (73 d.f.)	0.099	0.098	0.100
P	0.2	0.03	0.07
Distinct sharp eyespot			
Lynx	-1.77 (2.3)	-1.53 (4.0)	-1.30 (6.4)
Brigadier	-1.56 (3.7)	-1.28 (6.7)	-1.00 (11.4)
Mercia	-1.62 (3.3)	-1.66 (3.0)	-1.32 (6.2)
Soissons	-1.67 (2.9)	-1.43 (4.9)	-0.91 (13.4)
SED (73 d.f.)	0.134	0.134	0.128
P	0.5	0.04	0.003

These fungicides were chosen because of their different activity spectra against stem-base diseases. Standard husbandry practices were otherwise used on each of the farms; these included application of epoxiconazole at GS 39 and additional fungicides (mixtures including one or more of fenpropimorph, fenpropidin, carbendazim, flusilazole and flutriafol) after flag leaf emergence to control leaf diseases.

Thirty plants, three from each of 10 sampling positions, were taken from each plot at GS 22-25 (February), GS 30-32 (April, immediately before fungicide treatments were applied), GS 32-33 (2-3 wk after treatment), GS 39-59 and GS 75-77. Shoot bases of whole plants or stem bases of main shoots (last two samples) were assessed visually for incidence and, on stems (last two samples), for severity of eyespot, sharp eyespot, brown foot rot and indeterminate symptoms. The basal 3-5 cm lengths were cut off, chopped, frozen, freeze-dried and milled. DNA was extracted in 20 ml CTAB buffer (hexadecyltrimethylammonium bromide, 8 g/l; sarkosyl, 10 g/l; sorbitol, 25 g/l; NaCl, 87.7 g/l; EDTA, 8 g/l; polyvinylpolypyrrolidine, 10 g/l) at 65°C for 2 h. Potassium acetate (6.7 ml 5M) and chloroform (5 ml) were added to the

tubes, mixed and placed at -20°C before being centrifuged at 3000 *g* for 15 min. The aqueous phase (0.6 ml) was transferred to 1.2 ml ice-cold ethanol to precipitate the DNA. After at least 1 h at 4°C the samples were centrifuged at 3000 *g* for 10 min. The supernatant was carefully discarded and the pellet washed twice in ice-cold 70% ethanol before being air-dried and resuspended in TE buffer. A volume of each extract containing 200 ng DNA was PCR-amplified using specific primers to identify *T. herpotrichoides*, *T. acuformis*, *M. nivale* var. *nivale*, *M. n.* var. *majus*, *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *R. cerealis* (Doohan *et al.*, 1998; Nicholson & Parry, 1996; Nicholson *et al.*, 1996, 1997; Parry & Nicholson, 1996; Turner *et al.*, 1998). DNA of these fungi in extracts containing total DNA at standard concentrations was quantified by competitive PCR (Nicholson *et al.*, 1996, 1997).

RESULTS AND DISCUSSION

Detailed results from only one of the experiments, that at Rothamsted, are presented. Disease incidences and severities were different at the other sites, but the main pathogens identified by PCR, the main effects of treatments and the overall conclusions are the same. Results from the fourth sample, at GS 51-59, are not presented as they do not add further to the points being made here. Cultivar x fungicide interactions were usually not significant and are presented only for grain yields.

Table 2. Effects of cultivars and fungicides on eyespot incidence and severity at GS 75(-77[a]), Rothamsted experiment.

	Logit (% main stems) (backtransformed values in parentheses)		
	Eyespot (all severities)	Moderate-severe eyespot	Severe eyespot
Cultivar			
Lynx	-0.66 (20.7)	-1.68 (2.8)	-2.14 (0.9)
Brigadier	-0.22 (38.6)	-0.83 (15.4)	-1.93 (1.6)
Mercia	-0.50 (26.5)	-1.15 (8.6)	-1.12 (0.9)
Soissons	-0.25 (37.3)	-0.78 (16.8)	-1.84 (2.0)
SED (57 df)	0.136	0.142	0.080
P	0.01	<0.001	<0.001
Fungicide			
None	-0.08 (45.4)	-0.78 (17.0)	-1.98 (1.4)
Prochloraz	-0.45 (28.3)	-1.13 (9.0)	-2.05 (1.1)
Cyprodinil	-0.90 (13.7)	-1.59 (3.5)	-2.12 (0.9)
Azoxystrobin	-0.10 (44.7)	-0.93 (13.0)	-1.97 (1.4)
Flusilazole	-0.50 (26.5)	-1.14 (8.8)	-1.92 (1.6)
SED (57 df)	0.152	0.159	0.090
P	<0.001	<0.001	0.2

[a]Mainly cv. Soissons

Table 3. Effects of cultivars and fungicides on incidence and severity of brown foot rot and sharp eyespot at GS 75(-77[a]), Rothamsted experiment.

	Logit (% main stems) (backtransformed values in parentheses)			
	Sharp eyespot	Moderate -severe sharp eyespot	Brown foot rot[b]	Moderate-severe brown foot rot[b]
Cultivar				
Lynx	-0.69 (19.6)	-1.84 (2.0)	-1.74 (2.5)	-2.03 (1.2)
Brigadier	-0.63 (21.8)	-1.36 (5.7)	-1.48 (4.4)	-1.89 (1.7)
Mercia	-0.70 (19.3)	-1.40 (5.2)	-0.60 (22.6)	-1.32 (6.2)
Soissons	-1.39 (5.4)	-1.79 (2.2)	-0.70 (19.2)	-1.45 (4.8)
SED (57 df)	0.104	0.120	0.123	0.093
P	<0.001	<0.001	<0.001	<0.001
Fungicide				
None	-0.76 (17.4)	-1.52 (4.1)	-0.94 (12.8)	-1.47 (4.5)
Prochloraz	-0.75 (17.6)	-1.56 (3.8)	-0.85 (14.9)	-1.53 (4.0)
Cyprodinil	-0.67 (20.2)	-1.42 (5.1)	-1.53 (4.0)	-2.04 (1.2)
Az'strobin	-1.50 (4.2)	-2.10 (1.0)	-1.27 (6.8)	-1.76 (2.4)
Flus'zole	-0.56 (24.0)	-1.39 (5.3)	-1.06 (10.2)	-1.56 (3.8)
SED (57 df)	0.116	0.135	0.138	0.104
P	<0.001	<0.001	<0.001	<0.001

[a]Mainly cv. Soissons. [b]Probably includes some indistinct sharp eyespot

A small incidence of penetrating eyespot was found at GS 22 (Table 1). Incidence at GS 30-31 suggested a greater incidence on cv. Brigadier than on Lynx and Mercia that might necessitate fungicide treatment, especially if the indeterminate brown lesions included some eyespot (cf. Polley & Turner, 1995). Although it was considered likely that these brown lesions might also include eyespot or sharp eyespot, their incidence related less closely to NIAB ratings for eyespot susceptibility than did the penetrating eyespot incidence. This uncertainty was a particular problem because the incidence of brown lesions was relatively large (and even larger and more confusing in samples from a similar experiment at Rothamsted in 1998, not described here). Shoot-base disease incidences at GS 32-33, soon after fungicide treatments, suggest that some disease control had occurred, although this was not significant ($P \leq 0.05$), except for a small effect of flusilazole on sharp eyespot (not shown). Disease symptoms were more easily identified at GS 75, when eyespot was decreased by all fungicides except azoxystrobin (Table 2), sharp eyespot by azoxystrobin and brown foot rot by cyprodinil and azoxystrobin (Table 3).

PCR detected all fungi except *F. graminearum*, which was not identified in any sample. The most frequent of the pathogens were: *T. acuformis* (results for this pathogen only are shown, as an example, in Table 4) was detected in trace amounts, except in cv. Lynx, at GS 22 and in all cvs (trace in Mercia) at GS 30; *T. herpotrichoides* was not detected at GS 22 but was

Table 4. Incidence[a] of DNA of *Tapesia acuformis* in shoot and stem bases at three sampling times.

Cultivar	GS 22	GS 30(-31)	GS 75(-77)[b]				
			None	P	C	A	F
Lynx	-	+	+	+	(+)	+	(+)
Brigadier	(+)	+	+	+	+	+	+
Mercia	(+)	(+)	+	M	(+)	+	+
Soissons	(+)	+	+	+	+	+	+

[a]-, absent; +, present; (+), present as a trace; M, missing value. [b]Fungicides applied at GS 30-32: P, prochloraz; C, cyprodinil; A, azoxystrobin; F, flusilazole.

Table 5. Effects of cultivars and fungicides on grain yield (t ha^{-1} at 85% DM), Rothamsted experiment.

Fungicide	Cultivar				
	All	Lynx	Brig.	Mercia	Soissons
None	8.60	9.83	8.53	8.07	7.97
Prochloraz	8.53	9.20	9.11	7.94	7.85
Cyprodinil	8.81	9.89	8.65	8.37	8.32
Azoxystrobin	9.31	10.29	10.58	7.86	8.53
Flusilazole	8.76	9.58	9.35	7.95	8.17
SED (57 df)	0.237		0.474		
P	0.02		0.10		
All	-	9.76	9.24	8.04	8.17
SED (57 df)	-		0.212		
P	-		<0.001		

present (trace amounts in cv. Lynx) at GS 30; *M. n.* var. *nivale* (trace amounts at GS 22) and *R. cerealis* were present in pre-treatment samples in all cultivars. Amounts of DNA of all fungi in these early samples were insufficient for quantification; quantification is achievable in the ranges e.g. 0.02-50 ng fungal DNA/mg dry weight for *T. herpotrichoides* (using 4 fg competitor DNA per reaction) and 0.005-50 ng fungal DNA/mg dry weight for *T. acuformis* (using 16 fg competitor DNA per reaction), using DNA from 0.2 mg dry weight plant material per 50 µl reaction mixture (Nicholson *et al.*, 1997). This confirmed a low incidence of infection. *M. n.* var. *nivale* was responsible for most of the poorly diagnosed symptoms,

especially in mixed infections. At GS 75, *T. acuformis* was present after all fungicide treatments (Table 4), but usually as a trace after cyprodinil and on cv. Lynx. *T. herpotrichoides* was present (except on Lynx), but usually in trace amounts in fungicide-treated plants. *M. n.* var. *nivale* was present at GS 75 but usually as a trace in Soissons or after azoxystrobin treatment. *R. cerealis* was also detected at GS 75 but usually as a trace after cyprodinil and not after azoxystrobin. These results generally concur with cultivar susceptibilites and effects of fungicides on visible disease. They also confirm the identities of the principal pathogens although inconsistencies between incidences of brown foot rot and *M. n.* var. *nivale* (e.g. on cv. Soissons at GS 77) suggest that this disease may have had other causes.

Grain yields (Table 5) were increased only by azoxystrobin for cvs Lynx and Brigadier, and not as a result of stem-base disease control by any fungicide. Results from three similar experiments completed in 1997 showed that small amounts of identifiable stem-base disease and correspondingly small amounts of pathogen DNA before stem extension led to amounts of disease during grain-filling that did not justify the application of fungicides, even on the most susceptible cultivars. Subsequent experiments in the series are expected to resolve further the diagnosis problems that occur in early samples by the use of PCR and to lead to the identification of sampling times and fungal DNA concentrations at which fungicide sprays are justified economically.

ACKNOWLEDGEMENT

This research is funded by the Home-Grown Cereals Authority.

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Evaluation of fungicide seed treatments against Fusarium diseases of wheat using PCR diagnostic tests

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ABSTRACT

PCR diagnostic tests were used to identify and quantify pathogen inoculum on seed as part of seed treatment efficacy trials. Seed lots were assessed for pathogens present by standard plate counts and quantitative PCR before being treated with Sibutol (bitertanol 375 g/l; fuberidazole 23 g/l), Beret Gold (fludioxonil 24.3 g/l) or left untreated. Seed was drilled at two sites on two drilling dates. Stand counts and disease assessments (visual and PCR based) taken at emergence and establishment showed differences between seed treatments.

INTRODUCTION

Fusarium seedling blight of wheat in the UK is caused predominantly by *Microdochium nivale* and *Fusarium culmorum*. In 1992 and 1993, 99% of UK seed samples were found to be infected with *M.nivale* (Cockerell and Rennie, 1996). Assessment of samples is routinely undertaken by measuring the percentage of seed infected by plating surface sterilised seeds onto potato dextrose agar (PDA). This simple test only detects the fastest growing pathogens present within each seed and takes no account of the inoculum concentration within individual seeds. Recent advances in PCR-based diagnosis of plant pathogens offer an hitherto unavailable opportunity to diagnose and quantify pathogens associated with *Fusarium* diseases of wheat (Doohan *et al.*, 1998; Nicholson *et al.*, 1998). Using these techniques the two subspecies of *M.nivale* (var. *majus* and var. *nivale*) can be readily distinguished (Nicholson *et al.*, 1996). These techniques are being used to identify and quantify pathogen inoculum on seed and seedlings as part of seed treatment efficacy trials.

MATERIALS AND METHOD

Plots of winter wheat cv. Hussar (1.5 x 12 m) were inoculated with spores of either *M.nivale* var. *majus* and var. *nivale* or *F.culmorum* and *F.graminearum* at a rate of 10^5 spores/ml, 33 ml/m^2 at mid anthesis. Plots were subsequently mist irrigated for two weeks at a rate of 30 seconds per half hour for 12 hours per day (08:00 to 20:00). At harvest, seed was cleaned and dried to 16% moisture content. Seed samples from each plot (8 g) were crushed and DNA extracted in 30 ml CTAB buffer overnight. Potassium acetate 5M (10 ml) and chloroform (5 ml) were added, tubes

mixed, placed at -20°C until frozen, thawed, mixed again and then centrifuged at 3000 g for 15 min. The aqueous phase (1 ml) was transferred to 0.8 ml isopropanol, mixed and incubated at 18°C for 30 min to precipitate DNA. Samples were centrifuged at 4000 g for 15 min, the supernatant discarded and the pellet washed twice with 70% ethanol. Pellets were air dried and resuspended in 0.2 ml TE buffer overnight. Total DNA was quantified based on absorbance at 260 and 280 nm and samples were diluted to 100 ng DNA /µl in TE buffer. The presence of *F.culmorum*, *F.graminearum*, *M.nivale* var. *nivale* and *M.nivale* var. *majus* was detected within seed samples by PCR using conditions and primers described by Doohan *et al.* (1998) and Nicholson *et al.* (1998). Aliquots of template DNA (5 µl) were amplified in a final reaction volume of 25 µl using a PTC-100 Thermal Cycler (MJ Research, Massachusetts, USA). DNA was amplified using a 'touchdown' PCR programme according to Parry & Nicholson (1996). Four seed lots were subsequently selected to be used in a seed treatment trial. These were (1) commercially produced untreated seed (cv. Hussar), (2) seed from plots inoculated with a mixture of *M.nivale* var. *nivale* and *majus* at anthesis, (3) seed from plots inoculated with a mixture of *F.culmorum* and *F.graminearum* at anthesis and (4) seed from plots only mist irrigated at anthesis.

Pathogen DNA was quantified within these seed samples by competitive PCR, according to Doohan *et al.* (1998) and Nicholson *et al.* (1998). Percentage infection of seeds by *M.nivale* and *Fusarium* species was determined using standard plating techniques. Two hundred seeds were surface sterilised and plated onto Potato Dextrose Agar (PDA) amended with 130 mg/l streptomycin sulphate (5 seeds /plate). A selection of *Fusarium* isolates were identified on a preliminary basis by colony and spore morphology.

Seed was treated according to manufacturers' instructions with Beret Gold (active ingredient (AI) fludioxonil 5 g/100 kg seed) and Sibutol (AI bitertanol 56 g/100 kg seed and fuberidazole 3.5 g/100 kg seed); untreated seed was used as a control. Seed was drilled at two sites in the UK (Harper Adams, Shropshire (1.5 x 6 m plots) and Whittlesford, Cambridge (1.5 x 4.5 m plots)) at two drilling dates (mid-October and late-January) at a drilling rate of 275 seeds/m². Stand counts and disease assessments were taken at emergence and establishment. Disease assessments were based on 30 plants visually assessed for disease and scored 0 - 3 for healthy, slight, moderate or severe symptoms. Disease indices (total disease score divided by total number of plants) were divided by three to give a value between 0 - 1 and arcsine transformed before analysis. Stand counts and disease indices were analysed using ANOVA with site, seed lot and seed treatment as factors.

After visual assessment the 30 plants were freeze-dried and milled to a powder and incubated in CTAB for 2 h at 65°C to extract DNA. At emergence, all the shoot was sampled; at establishment, the lower 5 cm of shoot was sampled. Subsequent extraction steps, quantification of DNA and PCR amplification were as for seed material. Due to low detection rates at emergence of the early drilled plots subsequent sampling involved extracting DNA only from diseased plants within each plot. The volume of CTAB buffer used in the extraction was reduced to 5 ml /g freeze dried plant material. Volumes of potassium acetate and chloroform were reduced accordingly.

RESULTS AND DISCUSSION

The percentage infection and PCR quantification of pathogens within the four seed lots are shown in Table 1. *M.nivale* and *Fusarium* spp. were isolated from all four seed lots using the plating technique. The commercially produced seed (Seed Lot 1) had the least amount of infection. The seed from the uninoculated/misted plots (Seed Lot 4) had a high amount of *M.nivale*, equal to the seed from plots inoculated with both *M.nivale* subspecies (Seed Lot 2). This indicates that either the method of inoculation was inefficient for *M.nivale* or the *M.nivale* inoculum had little pathogenicity. The latter is unlikely since five isolates of each subspecies were used. It also indicates that *M.nivale* was the prominent natural inoculum at the site in 1997. The seed from the *Fusarium* inoculated plot had the highest amount of *Fusarium* infection. The predominant species present on this seed was identified as *F.culmorum*.

Table 1. Percentage infection from plate counts and pathogen DNA quantified by competitive PCR for each seed lot.

Seed Lot	% Infection		Pathogen DNA (ng/mg total DNA)			
	M.nivale	*Fusarium*	*M.n.nivale*	*M.n.majus*	*F.culmorum*	*F.graminearum*
1	25	4	0.42	ND	ND	ND
2	51	10	0.51	0.32	ND	ND
3	17.5	69	0.26	0.13	0.74	NQ
4	54	14.5	0.46	0.15	ND	ND

ND, not detectable; NQ, detectable but not quantifiable.

The quantification of pathogen DNA by competitive PCR is in general agreement with the percentage infection data. Quantitative PCR was not as sensitive as plate counts in this experiment. This was because plate counts can quantify a single colony forming unit within 200 seeds which was lower than the level of detection for the PCR procedure used. However, quantification of pathogen DNA gives a more accurate measurement of the fungal biomass as compared to presence or absence on individual seeds and can readily distinguish between species and subspecies. When pathogen DNA could not be quantified then it was recorded as either not detectable or not quantifiable (when the pathogen was detected but was present at too low an amount to be quantified). *M.nivale* var. *nivale* was detected in all four seed lots compared to *M.nivale* var. *majus* which was not detected in the commercially produced seed. *F.graminearum* was only detected in Seed Lot 3 and it was present at too low an amount to be quantified. *F.graminearum* is usually associated with warmer climates, which could well explain the low amount present. The two *Fusarium* species were co-inoculated and *F.culmorum*, which is more associated with the UK climate probably outcompeted *F.graminearum*. *F.culmorum* was not detected by PCR in Seed Lots 1, 2 and 4 although it was present at low amounts according to the plate counts. It was likely that this pathogen was present as very low biomass in these seed lots, again possibly due to competition from the dominant pathogen within these seed lots, *M.nivale*. PCR from single seed extractions did detect *F.culmorum* within these seed batches (results not

given).

Data from the early drilling showed no significant difference between sites. There was no interaction between seed lot and seed treatment except for the stand count data at establishment. This was due to the high stand count for the untreated, commercially produced seed (Seed Lot 1). This result was probably due to the greater vigour of this seed lot compared to the others (as indicated by the higher thousand grain weight and percentage germination in the presence or absence of seed treatment - results not given). Combined transformed disease index and stand count means of each seed treatment from both sites are shown in Figures 1 and 2 respectively. For the early drilled plots, both seed treatments significantly increased stand counts and decreased disease indices. There was no significant difference between stand counts of each seed treatment at emergence, however fludioxonil gave a higher stand count than bitertanol and fuberidazole at establishment. Fludioxonil controlled disease better than bitertanol and fuberidazole at emergence and establishment. The difference in control was greater at establishment, indicating that fludioxonil may give more persistent control.

The late drilling was scheduled to be drilled in late November but due to adverse weather conditions could not be drilled until late January. When the plots were drilled the seed bed was in poor condition; consequently stand counts were low at emergence and establishment at both sites. Complete data was only obtained for the Harper Adams site (Table 2). Both seed treatments increased stand counts and reduced disease compared to the untreated but there was no significant difference between seed treatments.

Table 2. Combined mean transformed disease index and stand count for each seed treatment in the late drilled plots (Harper Adams site only).

	Transformed disease index		Stand count (plants/m row)	
	Emergence	Establishment	Emergence	Establishment
Untreated	23.0 (0.47)	27.9 (0.67)	12.9	7.1
bit & fub	3.1 (0.03)	22.7 (0.45)	23.7	12.1
fludioxonil	0.0 (0.00)	19.5 (0.35)	23.9	11.4
LSD ($P < 0.05$)	3.5	5.1	4.0	2.4

Values shown in parenthesis are mean disease indices. bit = bitertanol; fub = fuberidazole.

PCR analysis of diseased seedlings is currently available only from the Harper Adams site. This data showed that only *M.nivale* (both subspecies) could be detected on diseased seedlings from the early drilled plots. On the late drilled plots both *M.nivale* (both subspecies) and *F.culmorum* were detected on diseased seedlings irrespective of treatment. PCR results from samples of all 30 plants taken at emergence of the early drilled plots detected *M.nivale* in eight untreated plots at an amount too low to be quantified. To increase the sensitivity of detection only DNA from diseased plants was extracted. This resulted in a higher detection rate but did not allow quantification of pathogens present due to the biased sampling strategy. Methods are currently

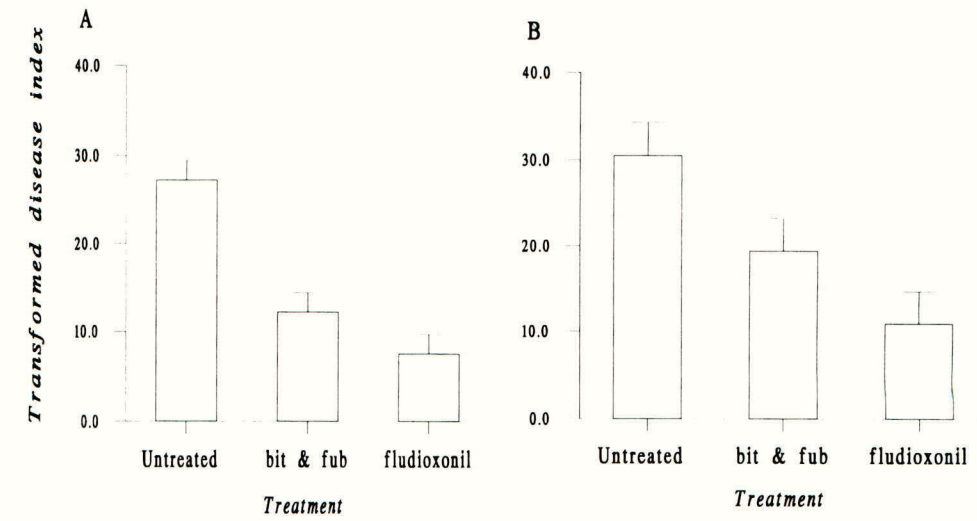

Figure 1. Mean transformed disease index at (A) emergence and (B) establishment for the early drilled plots. Error bars represent LSD at 5% significance level (bit & fub = bitertanol and fuberidazole).

Figure 2. Mean stand count at (A) emergence and (B) establishment for the early drilled plots. Error bars represent LSD at 5% significance level (bit & fub = bitertanol and fuberidazole).

being assessed to improve the sensitivity of the assay.

The use of these molecular techniques within fungicide efficacy trials will lead to a greater understanding of the effects of fungicides on individual species within disease complexes. Results from these trials indicate that both seed treatments provide good control of *M. nivale* and *F. culmorum*, with fludioxonil showing greater and more persistent control of these pathogens than bitertanol and fuberidazole.

ACKNOWLEDGEMENT

S G Edwards and N C Glynn acknowledge funding from Novartis Crop Protection AG and field trial assistance from members of staff at Whittlesford, particularly Carolyn Thomas and Kuldip Mudhar.

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ITITECH: a survey to improve the evaluation of relationships between cultural practices and cereal disease incidence

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ABSTRACT

The aim of this work was to study the relationships between cultural practices used in growing French cereal crops and the incidence of the important components of the foot and root disease complex (eyespot, sharp eyespot, take-all and *Fusarium* spp.) using a survey of 894 cereal fields from 1987 to 1994. Different methods, especially multivariate analyses, were used to analyse these data. The results show the effects of crop rotation, sowing date, fertilizer use and fungicide use. The effects of some factors are interesting because of their indirect role on disease incidence associated with intensive cropping. The "ITITECH" method could be used for other arable crops. It should be an essential complement to field experiments, especially in the study of complex sustainable farming systems.

INTRODUCTION

To optimize the management of cereal crop protection regimes to minimize disease risk for a given set of environmental and economic contraints, the complex relationships between cultural practices and pest and/or disease incidence need to be understood. This study concerns the foot and root disease complex: eyespot (*Tapesia yallundae* and *Tapesia acuformis*), sharp eyespot (*Rhizoctonia cerealis*), take-all (*Gaeumannomyces graminis* var. *tritici*) and foot rot (*Fusarium roseum, Microdochium nivale*). These foot and root pathogens survive as saprophytes on crop debris which serves as inoculum for future crops. Agronomic factors and their influence on saprophytic development can greatly influence disease incidence. Previous work shows the influence of cultural practices such as fertilizer use, soil management and crop rotation on disease development. Side effects of fungicides on evolution of pathogen populations have also been described but no synthesis at the cropping system level has been attempted. The current work aims to give an unbiased description of the effects of the cultural practices used in growing French cereal crops, to describe the incidence of the major components of the foot and root disease complex and then to study the relationships between these two sets of data to identify the cultural practices which act favourably or unfavourably on disease incidence. The hypothesis was that the sum of minor effects of several factors on disease development can be as great as a large effect of an important factor.

METHODS AND MATERIALS

The method chosen was a survey called "ITITECH". Data were collected from 894 cereal fields from 1987 to 1994 in different regions of France, from Institutes (INRA, ITCF),

agricultural colleges and mostly from farmer cooperatives (UNCAA). About 50 plants per crop were sent to INRA-Le Rheu during grain filling (growth stage 83-85 on BBCH growth scale) to be examined. Incidence and severity of diseases and sensitivity of pathogens to fungicides (especially MBC fungicides) were determined, according to the methods of Cavelier & Le Page (1985) and Lucas *et al.* (1989).

Two categories of variables were analysed:

- explanatory variables were the 38 qualitative variables describing cultural practices such as soil cultivation, fertilizer use, sowing date and the fungicide and other treatments during the crop growing season;
- explained quantitative variables were incidence and severity indices of diseases. The two species of *Tapesia* and MBC sensitive and MBC resistant strains were distinguished.

Statistical methods used included elementary statistics, then multidimensional analyses using the programme SPADN (Lebart *et al.*, 1984) which allowed the representation of all the variables in a low dimensional space. Linear or non-linear relationships were established between variables.

RESULTS

Description of variables

Explanatory variables: cultural practices

The factorial analysis done on all the cultural practice variables allowed these variables to be placed in five classes (Table 1) using an increasing hierarchical classification. Classes 1 and 5 were characterized by low inputs, 2 and 3 were characterized by a high level of inputs and 4 was without fungicide treatments.

Table 1. Classification of cultural practices.

Classes (number of plots observed)	Significant variables	% of plots / class
1 (97)	no use of fungicides	100
	no K or P fertilizers	93
	no herbicides (dicots)	80
	superficial soil cultivation	47
2 (150)	one fungicide treatment	75
	use of herbicides (dicots)	96
	use of herbicides (monocots)	87
	use of growth regulators	62
	no K or P fertilizers	90
	normal sowing date	100

continuation of Table 1

3 (351)	use of fungicides	78
	use of K and P fertilizers	94
	use of herbicides (dicots)	99
	use of herbicides (monocots)	89
	deep soil cultivation	81
	use of growth regulators	67
	normal sowing date	100
4 (123)	no use of fungicides	98
	use of K and P fertilizers	92
	use of herbicides (dicots)	100
	use of herbicides (monocots)	90
	deep soil cultivation	90
	normal sowing date	100
5 (194)	no use of fungicides	78
	use of K and P fertilizers	72
	no use of growth regulators	90
	superficial soil cultivation	47
	no herbicides (monocots)	73
	late sowing date	100

Explained variables: plant diseases

A principal component analysis grouped these variables into five classes (Table 2).

Table 2. Classification of disease incidence.

Classes (number of plots observed)	Significant variables	% of stems (or plants for *G. graminis*) / class
1 (200)	severe lesions	40
"severe eyespot class"	slight and medium lesions	26
	T. acuformis strains	40
	T. yallundae strains	28
2 (36)	severe infections	33
"severe take-all class"	slight and medium infections	52
3 (73)	severe lesions	19
"severe *Fusarium* class"	medium lesions	27
4 (166)	severe lesions	13
"severe sharp eyespot class "	medium lesions	13
5 (412)	no eyespot	91
"low disease class"	no take-all	63
	no rhizoctonia	94
	severe eyespot lesions	2.5

Characterization of variables

Disease incidence in relation to cultural practices for eyespot

As a first step towards the analysis of the influence of cultural practices on disease incidence, a screening test where each variable is described by all the others (DESCO procedure of SPADN) was applied. The null hypothesis of no relationship was rejected at $P = 0.005$ and the test values were ≥ 3 (Table 3).

Table 3. Relationships between eyespot and cultural practices.

Parameters describing eyespot	Significant parameters describing cultural practices	Mean values*
stems without lesions	cultivar Vizir	98
	previous crop soya	97
	previous crop sorghum	96
	previous crop sunflower	89
	no use of growth regulators	85.5
stems with medium or severe lesions	cultivar Thésée	8
	previous crop beet	27
	previous crop pea	30
	use of herbicides (monocots)	12
	lime improvement	10
	use of growth regulators	9
T. acuformis strains resistant to MBC	previous crop beet	40
	pre-previous crop wheat	33
	use of organic matter	28
	lime improvement	37
	use of growth regulators	28.5
T. yallundae strains resistant to MBC	cultivar Thésée	33
	previous crop wheat	34
T. yallundae strains sensitive to MBC	late sowing date	25
	no herbicides (monocots)	17
	no herbicides (dicots)	28
	no fungicides	15
	use of fungicides	4

* = % of plots characterized by cultural practice parameters related to eyespot parameters.

These relationships were only descriptive, but one conclusion could be drawn: the corresponding "cultural practice" variables for the group "severe eyespot incidence", (treatment with monocotyledon herbicides, use of growth regulators and use of lime - improvement) seem more like indicators of the cereal growing system which favours disease than direct causes of disease themselves.

Characterization of the influence of cultural practices on incidence of eyespot

All the statistical analyses used were based on the two patterns of data. Eyespot was the disease that was most closely related to cultural practices; 50% of the variation in this disease was explained by cultural practice components. According to the classes identified by the first analyses, a discriminant analysis estimated the contributions of each characteristic to the structure of the factorial pattern retained (four axes). According to its high weight, eyespot was well characterized by the first axis. The negative coordinates were related to incidence of severe eyespot and the positive coordinates to no incidence of this disease (Table 4).

Table 4. Contributions to the first axis in the discriminant factorial analysis.

Significant variables	Contribution	$cosin^2$	Coordinates
Eyespot	59.3	0.93	-
Lime improvment	5	0.89	-
Cv. Camp Rémy	1.3	0.89	-
Growth regulators	6.1	0.97	-
Pea	1.6	0.55	-
Sunflower	1.8	0.70	+
P2O5 (excess)	5.8	0.96	+
Cv. Vizir	5.7	0.78	+
Sorghum	2.2	0.86	+
Soya	1.5	0.89	+

The results of this analysis suggesting that crop cultivar and previous crop were the dominant factors in the system were confirmed by a chi-squared test (test values were respectively 10.4 and 10.1, the number of degrees of freedom was 16).

DISCUSSION AND CONCLUSION

In the case of eyespot, the results suggest an important effect of crop rotation: positive relationships existed with cereals as the previous or pre-previous crop and with peas as the previous crop. Soya, sorghum, sunflower as the previous crop were related to plots without eyespot. Beet as the previous crop was related to severe disease caused by *T. acuformis* strains resistant to MBC fungicides. This result suggests an indirect effect of this previous crop related to intensive cropping in Northern France. In the same way a relationship existed with MBC resistant strains which could be considered as an indirect indicator of a short crop rotation. Sowing date is known to influence eyespot considerably; early sowing greatly increases disease incidence. In this study, this factor was less important than other factors such as crop rotation. An excess of phosphate fertilizer decreased disease incidence, perhaps directly through plant responses or indirectly through the soil microflora. In the same way, the absence of lime improvement was related to absence of eyespot.

The variable "crop cultivar" was preponderant in the case of eyespot. 36 cultivars were used; only Camp Rémy, Recital, Renan, Soissons, Thésée and Vizir had different effects. Camp Rémy and Thésée were susceptible to eyespot unlike Vizir, however, Vizir was grown in Southern France, and in this region the epidemiology of eyespot is different from that in other regions due to different climatic conditions. In this study, fungicides were used not only against eyespot but also against foliar cereal diseases. It is the reason why fungicide treatment had an indirect effect or an indicator role. Clear relationships were not observed between fungicide applications and absence of eyespot. But relationships were observed; no fungicide treatments and the presence of eyespot caused by MBC-sensitive *T. yallundae* strains, and inversely the application of all categories of fungicides and a rare occurence of MBC-sensitive *T. yallundae* strains. This result suggests that the effects of non-specific fungicides are no more important than those of other components of the cropping system but exert a selection pressure on the pathogen population. There was a clear positive relationship between growth regulator use and eyespot incidence. A direct effect on the physiology of the plant could be envisaged. But these kind of clear relationships confirm the hypothesis that intensive cultural practices affect eyespot incidence.

"ITITECH" shows that foot and root diseases of cereals are greatly influenced by cultural practices. The importance of this dependence is sufficient to identify agricultural determinants able to be used for supervised methods of preventive and input-thrifty control.

The variability in some factors (e.g. nitrogen fertilizer use or ploughing methods) was not sufficient. Variations in disease development between the different regions were tested: no seasonal or geographic effect was statistically significant. "ITITECH" could allow explanatory calculations to be done. It should be an essential complement to field experiments. This method could be used for other arable crops (soil borne pathogens of oilseed crops) and vegetables. The results should be confirmed and relative importance factors should be determined by other experimental methods, leading to the development of models to estimate disease risk as a function of cropping system (Colbach *et al.*, 1997).

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A survey of *Tapesia yallundae* and *Tapesia acuformis* in UK winter wheat crops using a polymerase chain reaction diagnostic assay

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ABSTRACT

Polymerase chain reaction (PCR) assays have been developed by Novartis to detect and quantify the presence of W-type (*Tapesia yallundae*) and R-type (*T. acuformis*) syn. *Pseudocercosporella herpotrichoides*, the causal agents of eyespot in cereals. Results from the analysis of over 200 plant samples taken from commercial wheat crops in England and Scotland during the spring of 1998 are presented. The influence of agronomic factors (cultivar, soil texture, soil type, crop rotation, drilling date) on the incidence and severity of the two pathotypes are discussed.

INTRODUCTION

Eyespot disease of wheat, barley and rye in temperate regions is caused by *Pseudocercosporella herpotrichoides* (Fitt *et al.*, 1988). This pathogen has been classified into two major pathotypes W-type (*Tapesia yallundae*) and R-type (*T. acuformis*) (Dyer *et al.*, 1996). The disease is difficult to diagnose by visual symptoms, particularly at early crop growth stages with symptoms of eyespot, sharp eyespot and *Fusarium* spp. and *Microdochium nivale* foot rots often being indistinguishable (Goulds & Polley 1990). The inability to correctly identify disease symptoms can result in inappropriate or badly timed control measures which can lead to inadequate disease control and subsequent losses in yield and quality (Nicholson *et al.*, 1996). It has been previously reported that Novartis has developed polymerase chain reaction (PCR) assays for each of the two pathotypes (Beck *et al.*, 1996). These assays allow both the incidence of W- and R-type eyespot to be determined and some quantification of disease severity. This paper reports the results of a survey of eyespot in UK winter wheat crops using this PCR assay.

MATERIALS AND METHODS

Samples of plants were taken from commercial crops of winter wheat in England and Scotland. Each sample consisted of 30 whole plants taken at random from the crop. These samples were then either delivered by hand or sent by first class post for analysis at the Novartis Plant Pathology Laboratory. With each sample, information was provided by the sampler on location, cultivar, drilling date, soil texture, previous cropping and crop growth stage at sampling.

Extracts were prepared by bulk maceration of 4 cm of stem tissue from the 30 whole plants, using a Homex 6 homogeniser, in an equal volume of proprietary extraction buffer per weight of wheat tissue. Extracts were aliquoted into eppendorf tubes, boiled and microcentrifuged. Diagnostic PCRs were run with 1 µl of 1:10 diluted wheat, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 100 µM of each dTTP, dATP, dCTP and dGTP, 50 pmol of each primer and 2.5 units of AmpliTaq polymerase (Perkin-Elmer) in a final volume of 50 µl. Reactions were run for 35 cycles, each consisting of 15 s at 94°C and 1 min at 75°C. The PCR products were analysed on an ethidium bromide-stained (EtBr) gel by using 10 µl of the PCR products. Results were expressed according to fragment intensity on the EtBr gel on a 0-5 scale. These values correlate to an increasing concentration of genomic DNA for the specific pathotype (Beck *et al.*, 1996).

RESULTS

The results of the survey were analysed to investigate the effects of different factors on the incidence and severity of W- and R-type eyespot. In each of Tables 1-5, results show incidence of the pathotypes as a percentage of total samples tested in any one category. The tables show the proportion of crops infected with either W- or R-type, and the proportion of samples giving positive results for both W- and R-type, indicating that a mixed population was present in the sample. Mean data for severity (amount of pathotype DNA) are shown to include only those samples which were positive for that pathotype since the inclusion of data for all samples (both positive and negative for the pathotype) would tend to reflect the trends in incidence.

Soil Texture

Samples were categorised according to the ADAS Soil Texture (85) System into heavy, medium and light soils. The majority of samples were from medium and heavy soils with only a small number of samples received from soils with light texture (Table 1). The results showed a high incidence of R-type in all three soil categories with the proportion of samples infected with W-type being greater on heavy soils. The eyespot severity was similar for all soils for both R-type and W-type; crops grown on heavy soils were more severely affected.

Cultivar

Crops of over 20 different cultivars were tested; this inevitably meant that for some cultivars a very small number of samples was available and therefore these have been grouped together and excluded from the analysis. For the majority of cultivars both W- and R-type infected 30 - 45% of samples. However, for Soissons, 54% of samples had both pathotypes, whereas Riband had only 14% of samples infected by both (Table 2). Due to the generally high incidence of infection by R-type (range 62-77% apart from cv. Hereward at 91%) this was mostly a reflection of the incidence of W-type on these two cultivars - Soissons with 77% of samples infected and Riband with only 20%. The remaining cultivars had 49-70% of samples infected with W-type. The severity score for infection by R-type was in the range of 2.1-2.7, apart from cv. Reaper which was more severely infected (3.1). The severity score for W-type was 1.4 - 2.4, apart from on cv. Riband (1.0).

Table 1. The effect of soil texture on incidence and severity of W and R-type eyespot.

	% samples infected			Mean severity of infection, 0-5 scale #	
Soil texture (no. samples tested)	R- and W-type	R-type	W -type	R-type	W-type
Light* (17)	18	59	18	2.8	1.3
Medium* (112)	26	79	38	2.0	1.6
Heavy* (84)	45	74	62	2.7	2.0

*according to ADAS Soil Texture (85) System
mean value for infected samples

Table 2. The effect of cultivar on incidence and severity of W and R-type.

	% samples infected			Mean severity of infection, 0-5 scale #	
Cultivar (no. samples tested)	R- and W-type	R-type	W -type	R-type	W-type
Brigadier (29)	38	69	52	2.7	1.9
Charger (10)	40	70	50	2.1	1.4
Consort (37)	32	65	49	2.1	1.9
Hereward (11)	45	91	55	2.6	1.5
Reaper (10)	30	70	70	3.1	2.3
Rialto (12)	33	67	58	2.4	2.1
Riband (35)	14	77	20	2.3	1.0
Soissons (13)	54	62	77	2.3	2.4
Others (52)	27	73	33	2.7	1.7

mean value for infected samples

Drilling Date

The proportion of samples infected with both W- and R-type appeared to have been unaffected by the drilling date of the crop from which they were sampled (Table 3). There is a suggestion that the latest drilled crops (after 1 November) were more severely affected but this is perhaps a reflection of the very small number of samples tested. For the individual pathotypes, R-type occurred more frequently in the early drilled crops whereas W-type was more frequently observed in samples from crops drilled after 1 October. The severity of infection was only moderately affected by drilling date with no clear pattern apparent for either pathotype.

Table 3. The effect of drilling date on incidence and severity of W and R-type eyespot.

	% samples infected			Mean severity of infection, 0-5 scale #	
Drilling date (no. samples tested)	R- and W-type	R-type	W -type	R-type	W-type
Before 15 Sept. (43)	26	86	33	2.3	1.6
16 - 30 Sept. (96)	31	75	41	2.4	2.2
1 - 15 Oct. (45)	36	60	56	2.9	1.7
15 - 31 Oct. (22)	32	55	50	2.6	1.5
After 1 Nov. (4)	50	75	75	2.3	1.0

mean value for infected samples

Previous cropping

A large proportion of samples were taken from crops that had wheat as the previous crop (Table 4), which might be expected to increase the incidence and severity of eyespot in the following crop. For W-type, wheat as a preceding crop seemed to increase the incidence of eyespot but not for R-type. However barley as a previous crop appeared to decrease the incidence of both W-type alone and the mixture of both pathotypes, and decreased the severity of attack of W-type. The severity of R-type eyespot was not affected by preceding crop.

Location

Results for geographical location are presented in Table 5. Due to the small numbers of samples from either individual counties or groups of adjacent counties, interpretation of the data was rather difficult. However, where these data were grouped to cover larger areas of the UK, trends became more apparent. Scotland had the highest incidence of R-type but the lowest incidence and severity of W-type and therefore the lowest proportion of samples infected by both pathotypes. In England there seemed to be little regional variation between the pathotypes, although the data suggest that the incidence and severity of R-type was lowest in the south of England.

Table 4. The effect of previous cropping on incidence and severity of W and R-type eyespot.

Previous crop (no. samples tested)	% samples infected			Mean severity of infection, 0-5 scale #	
	R- and W-type	R-type	W -type	R-type	W-type
Barley (10)	0	60	20	2.2	1.0
Oilseed rape (28)	29	71	39	2.4	1.8
Peas/beans (16)	25	81	25	2.2	1.8
Wheat (140)	35	72	49	2.5	1.9
Other (26)	23	62	38	2.4	1.4

mean value for infected samples

Table 5. The effect location on incidence and severity of W and R-type eyespot.

Location (no. samples tested)	% samples infected			Mean severity of infection, 0-5 scale #	
	R and W-type	R-type	W -type	R-type	W-type
Aberdeenshire & Angus (9)	11	100	11	3.3	1.0
Perthshire (7)	0	86	0	1.7	0.0
E. Lothian & Fife (4)	50	100	50	2.8	1.0
Scotland (20)	**15**	**95**	**15**	**2.7**	**1.0**
Durham & Northumberland (8)	38	88	38	3.7	1.0
Yorkshire (35)	37	77	54	2.0	1.8
Nottinghamshire (13)	38	85	38	3.4	3.6
Cheshire & Shropshire (8)	38	50	38	3.0	2.3
Northern England (64)	**38**	**77**	**47**	**2.6**	**2.1**
Lincolnshire (24)	42	71	58	3.1	1.6
Norfolk (14)	14	79	21	2.2	1.0
Suffolk (10)	10	80	10	2.4	1.0
Cambridgeshire (17)	47	65	65	2.6	2.3
Essex (19)	63	74	74	2.6	2.7
Eastern England (84)	**39**	**73**	**51**	**2.6**	**2.1**
Northants & Oxon (12)	17	50	50	1.8	1.3
Herts, Beds, Bucks (12)	25	58	33	2.4	2.5
Hants, Kent & Sussex (13)	15	46	38	2.0	1.6
Dorset, Somerset & Wilts (9)	56	100	56	2.2	2.2
Southern England (46)	**26**	**61**	**43**	**2.1**	**1.9**

mean value for infected samples

DISCUSSION

The information presented in this paper confirms that eyespot is a disease which frequently occurs in UK winter wheat crops. Many of the survey results confirm previous results; for example that eyespot is more severe on medium to heavy soil types. However the PCR assay allowed the rapid and accurate differentiation of the W and R pathotypes, which provides new information on the influence of drilling date and cultivar on the incidence of these pathotypes.

Research using other PCR assays for eyespot has so far concluded that the lack of correlation between early and late season amounts of DNA make the use of PCR systems for establishing disease thresholds to guide fungicide treatment unlikely (Burnett *et al.*, 1997). The use of PCR to survey wheat crops to improve understanding of the factors affecting the risk of severe eyespot epidemics may prove to be a more effective way to use the technology, given the narrow range of timing available for fungicide application to give effective disease control and the influence of climatic conditions during the spring on the epidemic development. On a practical level for the farmer and advisor, the effects of agronomic factors on the risks that severe eyespot epidemics will occur will prove to be useful information in decision making.

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A model for the prediction of yield loss in wheat due to take-all disease caused by *Gaeumannomyces graminis* var. *tritici*

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ABSTRACT

Gaeumannomyces graminis var. *tritici*, the cause of take-all, is found in agricultural soils throughout the world, yet its effect on crop loss in susceptible species varies widely. Documentation of factors responsible for these differences is infrequent and sometimes contradictory. A study of this disease which commenced in 1994, has examined factors influencing disease development in wheat from which a mathematical model has been created to predict the losses in yield due to this pathogen.

Through quantification of inoculum pressure, the soil and environmental factors influencing inoculum expression and the time at which a crop is sown relative to local conditions, a measure of the importance of take-all occurring in a current wheat crop has been determined. The accuracy of this model has been confirmed over a three year period in European field trials by comparing yields in take-all infested wheat treated and untreated with the fungicide, MON65500, for the control of this pathogen.

INTRODUCTION

One of the most important factors in the development of take-all is the quantity of inoculum present at the start of the season. It has long been known that crop rotations have an important impact on the development of take-all through their effect on inoculum levels (Garrett, 1940). Quantification of inoculum levels and their use for predicting subsequent development of the disease have been previously attempted (Hornby, 1981; Herdina *et al.*, 1997). The subsequent level of disease expression is linked to many other factors, including pH, soil type, fertilisation, biological antagonism, time and density of sowing, moisture and temperature (Cook, 1981; Colbach *et al.*, 1997). It is the multiplication effect of these factors on inoculum concentration and their subsequent interaction with the host that determines the eventual yield loss due to take-all. The value in a take-all prediction model will lie in the accuracy with which it can predict not just the level of disease expression but its effect on yield loss. This paper describes a model for the prediction of take-all yield loss and its recovery through the use of a take-all seed treatment fungicide, MON65500.

METHODS

Inoculum quantification

Inoculum levels were quantified in relation to crop rotations, and the known susceptibility of different species to take-all, similar to the system of Colbach *et al.* (1994). A level of 1-4 was derived from knowledge of the two previous crops in the rotation. Previous crops of wheat, barley or triticale were classed as high contributors to inoculum; rye, maize, grass and set-aside were classed as medium contributors to inoculum and oilseed rape, linseed, sugar beet, potatoes, peas, beans and oats were classed as low contributors to inoculum. The inoculum level at drilling of the wheat crop was then calculated according to table 1.

Table 1. Crop matrix used for determining inoculum levels in wheat crops in European model.

Inoculum level	Crop	Crop year -1	Crop year -2
1	wheat	low	low
1	wheat	low	medium
2	wheat	medium	low
2	wheat	low	high
3	wheat	medium	medium
3	wheat	medium	high
3	wheat	high	low
4	wheat	high	medium
4	wheat	high	high

Crop rotational data region by region were derived from Farmstat for the UK, Klefmann for Germany and S.C.E.E.S. (Service Central des Etudes et Enquêtes statistiques) and I.T.C.F. (Institut Technique des Céréales et des Fourrages) in France to calculate the inoculum distribution by areas.

Climate data

Meteorological data for up to the last 20 years were collected from local meteorological stations covering all areas of France, UK and Germany. For France and the UK, average monthly soil temperatures were calculated from daily temperatures taken at 12 noon at a soil depth of 10 cm. For Germany average monthly temperatures were calculated from mean daily air temperatures (3 recordings per 24 h period). Monthly soil moisture data were calculated from rainfall sums for the same regions. Knowledge of the effects of temperature and moisture on infection of wheat by *Gaeumannomyces graminis* var. *tritici* (Ggt) and on its development within the crop, were taken from published sources. These were used to define a series of temperature and moisture cut-offs for Ggt growth relative to time of year, producing a matrix of 81 different combinations. From these a 20-year climatic impact on take-all was calculated by country (Figure 1).

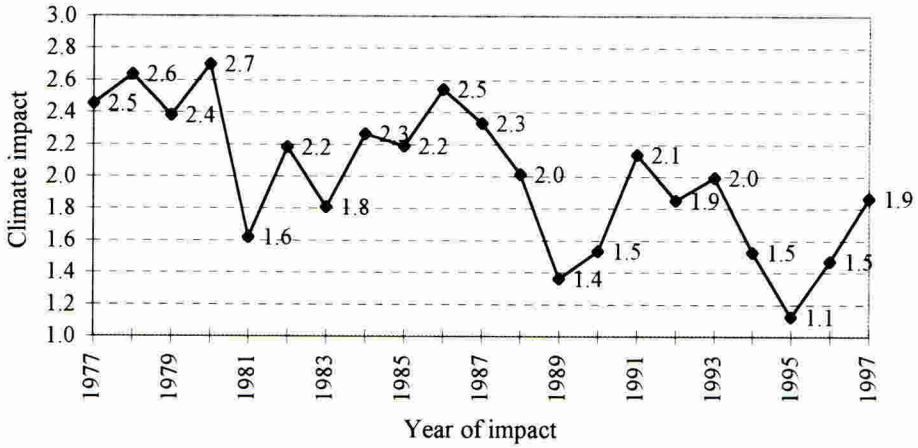

Figure 1. Climatic impact on take-all over the last 20 years in the UK (means from all wheat-growing counties; <1.5 = low impact; 1.5-2.5 = medium impact; >2.5 = high impact).

Soil analyses

Data of soil type distribution were collected by Farmstat for the UK and by PSL (Produce Studies Limited) for France and Germany. Soil types, defined as light, medium and heavy soils according to the classifications of Anon. (1985), were linked to wheat areas and used to adjust climatic impacts on disease, region by region (county, département or länder). Laboratory analyses of soil samples taken from take-all experiments conducted in wheat fields in these three countries were also made.

Field trials

Small-plot and large-strip field trials were done in France, Germany and the UK. Randomised block designs had a minimum of four replicates with plot areas from 3.3 m^2 to 20 m^2, all being double plots, the left-hand plot used for sampling and the right-hand plot for harvest. Large-strip trials were drilled and harvested with farm equipment using plots with areas of 0.3 - 0.6 ha. Assessments of take-all were taken as root infections by Ggt (GS 13-75) from at least 20 plants per plot, 100 plants per treatment, calculating incidence of take-all (number of plants with take-all / number of plants assessed x 100), intensity of take-all using a take-all index (TAI = (0a + 10b + 30c + 60d+100e) / t .where a, b, c, d, e represent the number of plants in each of 5 categories: 0 = healthy roots; 1= 1-10% roots infected; 2 = 11-30% roots infected; 3 = 31-60% roots infected; 4 = 61-100% roots infected; and t is the total number of plants examined (a+b+c+d+e)), and percent whitehead incidence (GS 69-75). Yields were recorded in tonnes per ha at 15% moisture (GS 92). Treatments comprised a control, being a basic seed treatment having little or no effect on take-all, and the basic treatment with the addition of the take-all fungicide, MON65500 (125g a.i./l FS; Monsanto) at 25 g a.i./100 kg seed. The basic treatment was: 5 g of fludioxonil /100 kg seed or 90 g of bitertanol + 5.5 g of fuberidazole/100 kg seed in the UK, 60 g of guazatine /100 kg seed in Ireland, 5 g of fludioxonil + 1 g of tebuconazole/100 kg seed or 5 g of fludioxonil + 20 g of

difenoconazole/100 kg seed in Germany, and 5 g of fludioxonil + 50 g anthraquinone / 100 kg seed in France.

RESULTS AND DISCUSSION

Analysis of data obtained from nearly 200 field trials showed close links between take-all development and a number of factors. The inoculum concentration based on rotation was a major factor in the level of disease expression, this being closely linked with date of sowing (Figure 2). Soil characteristics were also important, take-all expression was closely linked to clay, sand, organic matter and pH. Sensitivities around these were made for their incorporation into the model. Yield potentials for different soil types were calculated from first wheat yields, yield loss through take-all was calculated from trials data and potential yield recovery through use of MON65500 was predicted (Table 2). Through the studies of regional data, the yield loss through take-all could be calculated by area or country. For example in the UK it was calculated that 560000 hectares were at high risk from take-all.

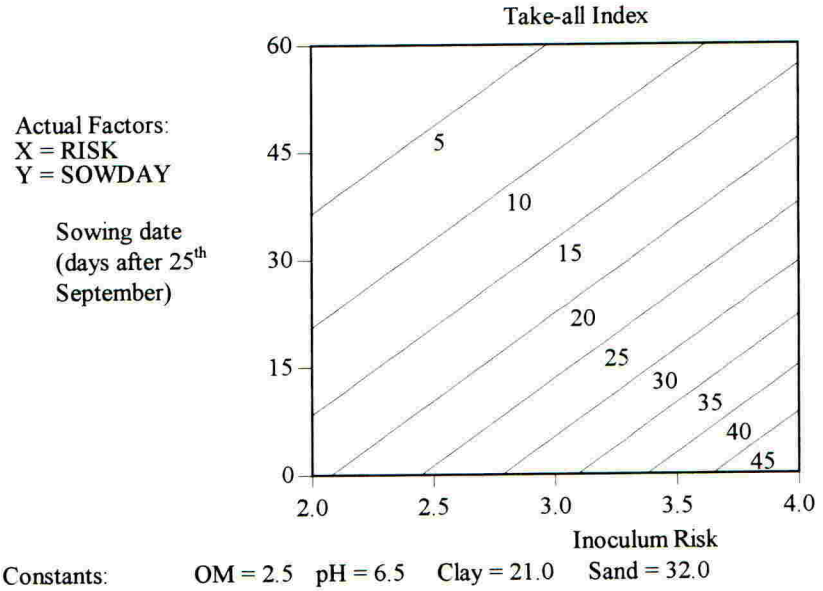

Figure 2. The effect of sowing date and inoculum risk on the level of take-all in wheat (data from 3 yr field programme in France, Germany & UK).

Validation of the accuracy of the model was made by examining the data from field trials conducted in different take-all inoculum situations and under different weather conditions. Each field trial was classified by the two preceding crops to give the inoculum threshold and by the weather conditions prevailing during the life of the crop to give the climatic impact. The yield increases through use of MON65500 compared to the control, closely matched those predicted by the model (Figure 3). In two of the three years take-all was low, thus

these data are if anything conservative estimates of the potential yield recovery expected over a 20-year period.

Table 2. The expected yield recovery (t/ha) from take-all through use of MON65500 under different rotational (inoculum) and climatic conditions, the 20-year adjustment factors and the mean expected yield recovery per country per inoculum class.

	Climatic impact			Expected yield recovery		
Inoculum level	low	medium	high	UK	France	Germany
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.25	0.09	0.05	0.08
3	0.20	0.35	0.80	0.48	0.37	0.45
4	0.40	0.80	1.60	1.00	0.76	0.94
20-year climatic influence						
UK	25%	38%	37%			
Fr	50%	30%	20%			
G	32%	35%	33%			

Fr, France; G, Germany.

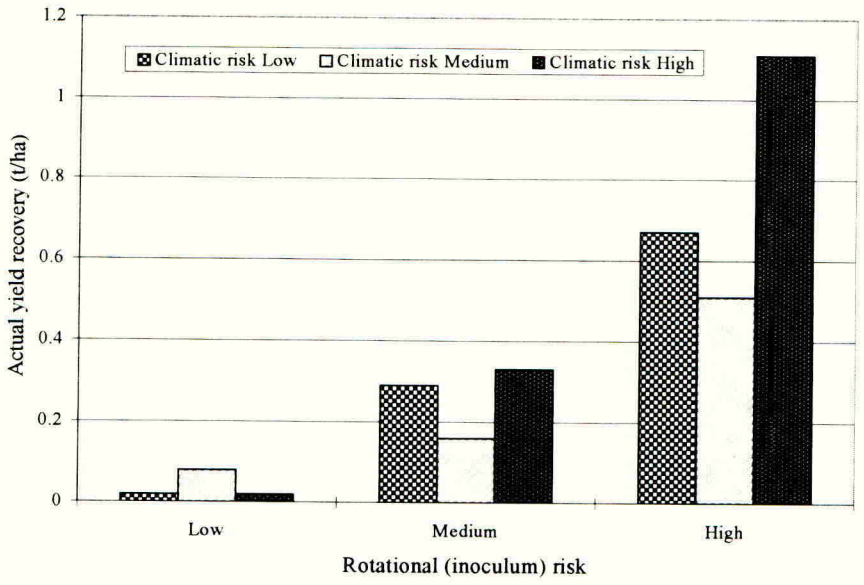

Figure 3. Yield recoveries from 191 field trials classified by preceding two crops and by climatic risk - 1994-1997.

CONCLUSION

The importance of several factors on the quantity of infective Ggt inoculum and its expression as take-all in winter wheat have been quantified. The accuracy of a model predicting yield recovery from use of a take-all fungicide has been validated from approximately 200 field trials conducted over a 3-year period. This model, although still in the process of finalisation, may offer a wheat producer the opportunity to evaluate risk from this disease and provide a measure of yield loss associated with risk level. It could provide a tool to optimise wheat rotations, to time drilling and to carefully target and apply inputs for optimum return.

AKNOWLEDGEMENTS

We thank Gregory Ong and Gerard DeKerchove for statistical analyses and all colleagues who have contributed to the field trials programme used to obtain data for this study.

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The use of a polymerase chain reaction diagnostic test to detect and estimate the severity of stem base diseases in winter wheat

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ABSTRACT

Specific polymerase chain reaction (PCR) tests have been developed by the John Innes Centre to detect and quantify nine fungal species commonly causing stem base diseases of cereals. These tests allow precise and reliable evaluation of the dynamics of the different species in the stem base disease complex.

In both 1997 and 1998 AgrEvo carried out a survey of winter wheat in the UK to establish the occurrence and severity of both *Tapesia acuformis* (R-type eyespot) and *Tapesia yallundae* (W-type eyespot), *Rhizoctonia cerealis* and several species of *Fusarium* and *Microdochium*. This survey ("Spotcheck") has confirmed that *T. acuformis* is the predominant pathogen in the stem base complex and that *T. yallundae* very rarely occurs. It has also shown that crop rotation, drilling date and geography influence the abundance of the different pathogens.

INTRODUCTION

The 'stem-base complex' comprises *Fusarium culmorum, F. graminearum, F. avenaceum* and *F. poae* along with *Microdochium nivale* var. *majus* and *nivale*, all of which cause foot rot. In addition the complex may contain *Tapesia yallundae* and *T. acuformis* (true eyespot) and *Rhizoctonia cerealis* (sharp eyespot). The true eyespot pathogen was formerly classified as wheat (W-) and rye (R-) adapted pathotypes of *Pseudocercosporella herpotrichoides*, but these have recently been re-classified as *T. yallundae* and *T. acuformis* respectively in recognition of their being distinct species.

Visual diagnosis of stem base disease is problematic, and is particularly difficult to assess during early growth stages, since the symptoms of all foot rot components can be indistinguishable at this time. Visual symptom assessment also fails to account for the relative contribution of the different species outlined above to tissue browning. This is of key importance where different species are known to have differential sensitivity to fungicides (exemplified by *T. yallundae* and *T. acuformis*). Clearly, the inability to diagnose disease correctly may result in the adoption of inappropriate or poorly timed control measures leading to inadequate disease control and subsequent yield and/or quality losses.

Until recently, no methods have been available for identification and quantification of individual fungal species present within plant tissue. Molecular techniques being developed will assist in the study of disease complexes, as these utilise fungal species-specific DNA markers. The sensitivity of such disease detection methods can be enhanced by polymerase chain reaction

(PCR) technology, which allows small quantities of fungal DNA in early infection stages to be 'amplified' for subsequent analysis. John Innes Centre (JIC) has developed specific PCR assays, for the detection of nine fungal species involved in the 'stem-base' complex, from plant tissue extracts. The diagnostic capabilities of these tests have been further refined to allow quantitative analysis of each species, which gives a unique insight into the dynamics of the foot rot complex pathogens in response to environmental and chemical influences.

MATERIALS AND METHODS

Seedling samples were collected for testing according to set guidelines in collaboration with key distributors. Assays were conducted by JIC in 1997, and by Adgen Limited in 1988 under JIC consultancy and using the same methodology. Main tillers were collected from 30 wheat plants in an evenly spaced diagonal field transect. Roots were removed before despatch, to facilitate handling. A form giving information on the location, sampling date, previous cropping, wheat cultivar, soil type and drilling date accompanied each sample. On arrival at the testing facility, assays were immediately carried out on plant tissue extracts. Samples were cleaned, and sections of stem were selected and chopped before being freeze-dried. DNA was extracted and PCR tests carried out alongside an internal control for each pathogen (Nicholson *et al.*, 1998). The amount of target (pathogen) and internal control product amplified from each sample were compared, and the severity of each disease estimated using a 0-5 scale. Results from the tests were communicated to the initiator within ca. 7 days.

Results for 1997 and 1998 are tabulated below. Disease severity is expressed as a Disease Index: $\frac{(N\times 0) + (N \times 1) + (N \times 2) + (N \times 3) + (N \times 4) + (N \times 5)}{\Sigma N \times \text{number of classes}} \times 100.$

RESULTS AND DISCUSSION

Disease incidence

The incidence of each foot rot complex pathogen in all 1997 and 1998 samples is summarised in Table 1, which also represents the first field survey of UK *Tapesia* populations analysed by PCR diagnostics. It is known that *Tapesia* populations are a dynamic mixture of two species, the relative proportions of which can shift both within the growing season and from year to year. In addition, there are geographic differences in the species composition of eyespot populations in different countries. In the UK, it has been widely accepted that there has been a shift in the relative proportion of the species over the last decade to almost exclusive *T. acuformis* predominance. This is confirmed by results summarised in Table 1. Overall, in both years *T. acuformis* occurred in around 70% of samples, while *T. yallundae* was present in <1%. It is not known what stimulated this shift, although differential sensitivity of *Tapesia* spp. to some triazole fungicides is suspected. The role of the sexual stage of the disease in providing airborne ascospores for long range dispersal may also be of importance in stimulating population shifts, especially in view of recent changes in straw management practices, which allow the sexual stage to flourish by provision of large areas of standing stubble. The sexual stage is initiated from specific mating types present in normal lesions, which cross sexually to form fruiting bodies, or apothecia, from which airborne ascospores are actively discharged. Apothecia form

on standing stubble (not green tissue), and require at least 2 months to develop with a mean daily temperature of 3-8°C and high rainfall (Dyer *et al.*, 1994). They are typically formed after harvest from mid-October to July, with a peak from late January to March. Both asexual and sexual spores of each species have the same basic infection process leading to lesion formation, although lesions are usually seen further up the stem when initiated by ascospores. Inter-species variations in the basic infection process are known to occur in the vegetative cycle (Daniels *et al.*, 1991), but mechanisms have only been confirmed for the sexual cycle of T. *yallundae* (Daniels *et al.*, 1996). *T. yallundae* is able to grow within the host cell walls, this growth mechanism being facilitated by the secretion of extracellular enzymes, and tissue browning during leaf sheath penetration is very evident. *T. acuformis* has a random invasive growth habit, resulting in rapid leaf sheath penetration. Tissue browning is significantly delayed, so that *T. acuformis* is often well-established in the absence of visible symptoms. This can clearly be problematic when visible symptoms are used (a) to select for tests based on re-isolation and (b) for conventional disease scoring. Clearly, PCR based analysis of randomly sampled stem bases is not subject to this bias.

In both species rapidly growing 'runner hyphae' are formed, which initiate the key infection structures responsible for colonisation of successive leaf sheaths. Runner hyphae are highly susceptible to prochloraz. The physico-chemical properties of this chemical aid post-spray redistribution to the leaf sheath surfaces, which are the preferred location for runner hyphae.

Table 1. Incidence and severity of stem base complex pathogens.

	% of tested samples with one or more pathogens (Disease Index)	
Pathogen	1997	1998
Tapesia acuformis	77.0 (30.1)	58.0 (17.1)
Tapesia yallundae	0.2 (<1)	0.7 (<1)
Rhizoctonia cerealis	30.7 (11.6)	17.3 (3.4)
Microdochium nivale var *nivale*	36.4 (14.7)	37.1 (9.3)
Microdochium nivale var *majus*	4.7 (1.3)	59.7 (24.0)
Fusarium culmorum	0	31.8 (5.4)
Fusarium avenaceum	0	24.7 (4.5)
*Fusarium graminareum**	-	18.7 (3.4)
*Fusarium poae**	-	8.5 (1.4)
Number of samples tested	401	283

*tested in 1998 only

In combination, data from the two years indicate the extreme variation between seasons in the overall incidence of foot rot pathogens.

Disease severity was related to disease incidence, but in 1998 fewer samples were detected with a high incidence of *T. acuformis*. *M. nivale* var. *majus* occurred in many samples in 1998 and was more abundant than other foot rot pathogens. Where *Fusarium spp.* were regularly detected in 1998, they tended to be present at a low incidence and their contribution to yield losses was probably negligible.

Regional distribution

Table 2. Occurrence of main pathogens of stem base complex by region.

	% of samples with pathogens			
UK region	*T. acuformis*	*R. cerealis*	*M. nivale*	*M. majus*
East Anglia (160*)	61	19	48	30
Midlands (78*)	71	18	42	27
N. E. England (148*)	77	24	41	24
N. W. England (41*)	80	41	22	22
Scotland & Borders (90*)	77	19	21	42
Southern England (150*)	64	32	35	23

* = sample size.

In all regions *T. acuformis* was the dominant pathogen with 60-80% of samples infected. In northern England and Scotland 77% or more of samples were infected, while infections were slightly lower in East Anglia and Southern England where drier weather may influence infection. *M. nivale* var. *nivale* was the next most common disease in three regions, although in Scotland *M. nivale* var. *majus* was more prevalent.

Drilling date

Table 3. Effect of drilling date on incidence and severity of the main stem base pathogens.

	% of samples with pathogens (Disease Index)			
Drilling Date	*T. acuformis*	*R. cerealis*	*M. nivale*	*M. majus*
Aug to early September (37*)	76 (28.4)	21 (7.7)	35 (10.4)	27 (10.8)
Mid to end September (252*)	69 (25.0)	26 (9.0)	46 (15.9)	33 (13.7)
Early to mid October (280*)	65 (25.1)	27 (8.4)	34 (11.4)	26 (9.1)
End October & November (58*)	52 (17.0)	19 (5.7)	22 (7.8)	22 (9.8)

* = sample size

The abundance and severity of *T. acuformis* declined with later crop drilling, but over 50% of crops drilled in late October or November were still infected with eyespot. All the diseases occurred less often in the latest drilled crops, except *R. cerealis* which was affected least by drilling date. *M. nivale* var. *majus* and *M. nivale* var. *nivale* occurred most frequently, and a little more severely, on mid to end of September drilled crops.

Crop rotation

Over 60% of samples were infected with *T. acuformis*, and most samples were taken from crops following cereals (Table 4). Where samples were taken following a broad-leaved crop, a large proportion (62%) were infected with *T. acuformis* indicating that break crops had little effect on the reduction of eyespot inoculum.

CONCLUSIONS

The "Spotcheck" survey showed that in 1997 and 1998, *Tapesia acuformis* ('R-type' true eyespot) was ubiquitous and predominant in UK field populations. *T. yallundae* ('W-type' eyespot) was detected at very low levels, making little contribution to cereal eyespot disease. The incidence of *T. acuformis* was greater in Northern England and Scotland, and was only moderately influenced by drilling date. Break crops did not appear to influence *T. acuformis* incidence, and there was little effect of different cultivars on disease abundance or severity. *Rhizoctonia cerealis* was also an important component of foot rot populations, but the incidence and severity was much less in 1998 than 1997. As with true eyespot, previous cropping had little influence on disease incidence or severity of sharp eyspot. *Microdochium* and *Fusarium* species were present across the UK, particularly in 1998, indicating that these species warrant further consideration as important components of the stem base complex. Thus, the analytical approach exemplified by the "Spotcheck" survey provides a detailed overview of the dynamics of stem base pathogen populations, and can be used to provide early indication of the presence and quantity of fungal species. This would allow timely applications of an appropriate fungicide to be made, optimising product useage and efficacy. This is of particular importance for pathogens such as *T. acuformis* which can colonise vascular tissues of stem base leaf sheaths in the absence of substantial tissue browning. When timed to coincide with the initial stages of infection, the imidazole fungicide prochloraz continues to provide useful control of *Tapesia* species, it can also reduce infections of *R. cerealis* and *Microdochium/Fusarium spp.* if they are developing at the time of application.

ACKNOWLEDGMENTS

We would like to thank everyone who assisted in the "Spotcheck" survey, in particular those who collected samples, provided agronomic information, conducted tests and collated the data.

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Table 4. Effect of previous crop on incidence and severity of main stem base pathogens.

Crop type	% of samples with pathogens (Disease Index)			
	T. acuformis	*R. cerealis*	*M. nivale*	*M. majus*
Broad-leaved crops (174*)	62 (19.3)	26 (9.8)	41 (14.4)	33 (13.2)
Cereals (442*)	73 (27.8)	25 (7.7)	36 (12.0)	26 (9.9)
Grass/Maize/ Onions (15*)	60 (14.4)	13 (6.7)	13 (3.3)	13 (11.1)
Set-aside (23*)	61 (16.7)	26 (7.2)	30 (12.3)	35 (10.1)

* = sample size

The extent of the host range of *T. acuformis* has not been fully established, and there is increasing evidence that this species can infect ryegrasses, which would indicate an increased chance of *T. acuformis* occurring in crops drilled after grass leys. Broadening of host range might be expected to result from an increased prevalence of the sexual stage, thus enhancing the capacity of the fungus for adaptation through increased genetic variability. A survey of 45 UK set-aside sites in 1992-1994 found *Tapesia* apothecia on 50% of the sites (Dyer & Lucas, 1995).

Crop cultivar

Table 5. Effect of crop cultivar on incidence and severity of *T. acuformis*.

Cultivar	Incidence (% of samples)	Severity (Disease index)	NIAB Eyespot Rating
Brigadier (126*)	76	30	5
Buster (20*)	75	29	6
Consort (69*)	64	21	5
Hereward (42*)	69	22	5
Hussar (33*)	70	29	5
Soissons (26*)	73	22	4
Reaper (38*)	74	27	5
Rialto (37*)	68	30	6
Riband (177*)	67	21	5

* = sample size

Over 30 different cultivars of wheat were sent for testing, but the nine cultivars listed above represent 83% of all the samples. It might be expected that the most susceptible cultivar on the NIAB list (Soissons) would have had the worst incidence and severity of *T. acuformis*. However, this was not the case, since Soissons had a small disease index (Table 5) and had only a moderately high incidence of the pathogen. Two cultivars classified as most resistant to eyespot by NIAB (Rialto and Buster) scored highly on severity of disease. NIAB ratings are based on visual symptoms of eyespot infection, but as symptoms are not always visible, it is only a guide. Results demonstrated that all the cultivars are susceptible to *T. acuformis* infection.

The effect of site, season and cultivar on disease management strategies for winter oilseed rape grown in England and Scotland

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ABSTRACT

The contribution of cultivar resistance and fungicides to disease control and yield in winter oilseed rape was investigated at sites in the east of England and in the north of Scotland. A range of fungicides applied at different times and doses were evaluated on the cultivars Bristol, susceptible to light leaf spot and resistant to stem canker and Rocket, resistant to light leaf spot and susceptible to stem canker. Disease pressure varied between years and locations and this paper compares the disease incidence and yield responses to different fungicide treatments at the locations in Suffolk in 1995 and Aberdeen in 1996. In the Aberdeen experiment light leaf spot caused extensive plant death over the winter and decreased the yield of cv. Bristol by an estimated 3.0 t/ha.; a full two-spray programme failed to increase the yield of this cultivar to that of cv. Rocket. In contrast, cv. Bristol out -yielded cv. Rocket by 0.6 t/ha in the Suffolk experiment, where the incidence of stem canker was high, but light leaf spot was less damaging. Differences in disease control efficacy and the importance of correct timing to maximise the response to treatment were also identified. These data demonstrate the need for integrated disease management strategies for oilseed rape depending on geographical location and seasonal disease risk.

INTRODUCTION

It is of concern that, despite improvements in oilseed rape cultivar resistance and more widespread use of fungicides, the severity of light leaf spot (*Pyrenopeziza brassicae*) and stem canker (*Leptosphaeria maculans*) in England and Wales in the 1994 and 1995 harvest years was comparable to that recorded in the late 1970's. There is no doubt that good disease control can be achieved with fungicides (Sansford *et al.*, 1996) and a range of new fungicides have been introduced for use on oilseed rape. However, a fundamental understanding of their properties and of the biology of the pathogens is required to optimise their performance.

Disease-yield loss relationships have been derived for light leaf spot and stem canker (Sansford *et al.*, 1996), stem rot (*Sclerotinia sclerotiorum*) and dark pod spot (*Alternaria brassicae*) (Fitt *et al.*, 1997). Estimates of national yield losses for these diseases suggest that stem canker and light leaf spot are the most important diseases; together they have caused yield losses of up to £80 million/annum in recent years. (Losses from stem rot were up to £1.5 million). These losses occurred in commercial crops, many of which had been sprayed with fungicides. Fungicide use has increased in recent years and expenditure on fungicides is about £9 million/annum (Gladders, 1998). However, there is little evidence that increased fungicide use has led to improved disease control. To optimise fungicide use, detailed knowledge of product efficacy in relation to dose and timing is required.

Four experiments on optimising fungicide use against light leaf spot and stem canker were done in each of the harvest years 1995, 1996 and 1997. This paper describes results from two of these experiments, in England in 1995 and Scotland in 1996.

MATERIALS AND METHODS

Two cultivars, which showed different resistance characteristics to light leaf spot and stem canker, were selected from the NIAB Recommended and Descriptive Lists of Oilseed Crops (Anon., 1995); cultivar (cv.) Bristol, susceptible to light leaf spot (resistance rating 2) but with moderate resistance to canker (5) and cv. Rocket, susceptible to canker (4) but resistant to light leaf spot (7).

Table 1. Fungicide treatment applied, products used and dose applied.

Fungicide product, common name and concentration			Application rate (l or kg/ha), full (1) or half (½) dose and timing	
	Product	Active ingredient (concentration g a.i./l or kg)	Autumn (A)	Stem extension (S)
1.	Untreated			
2.	Punch C	flusilazole (250) + carbendazim (125)	0.8 (1)*	0.8 (1)
3.	Punch C		0.8 (1)	
4.	Punch C		0.4 (½)	0.4 (½)
5.	Bavistin DF #	carbendazim (500)	1.0 (1)	
6.	Bavistin DF		0.5 (½)	0.5 (½)
7.	Folicur	tebuconazole (250)	1.0 (1)	
8.	Folicur		0.5 (½)	0.5 (½)
9.	Plover	difenconazole (250)	0.5 (1)	
10.	Plover		0.25 (½)	0.25 (½)

* full or half dose applied in autumn and at stem extension. # Carbate Flowable was used at the Aberdeen site.

The fungicides were selected to provide data on the efficacy of different active ingredients against the two diseases. Treatments were applied either once at full dose in the autumn (mid-November) or as split half-doses in the autumn and spring (early stem extension) (Table 1). Two full dose sprays of flusilazole plus carbendazim were used as a standard against which to compare the effectiveness of half-dose treatments. Carbendazim treatments were re-evaluated following concerns about resistance to MBC fungicides in light leaf spot in

Scotland. Ten plants per plot were assessed for presence of diseases on a whole plant basis in December, at early stem extension and at pod ripening. The percentages of leaf, stem or pod areas affected by each disease on each individual plant were recorded. Prior to stem extension, the samples were incubated in polyethylene bags at room temperature for 24 h to enhance the development of symptoms of light leaf spot before laboratory assessment. Stem cankers were recorded on a 0 – 4 scale, where 0 = no disease, 1 = less than half stem girdled by a lesion, 2 = more than half stem girdled by a lesion, 3 = whole stem girdled by a lesion and 4 = plant dead. A canker index on a 0 to 100 scale was calculated as:

$$\frac{b + 2c + 3d + 4e}{a + b + c + d + e} \times \frac{100}{4}$$

Where a, b, c, d, and e were the numbers of plants in each category. The experiments were arranged in a two-way factorial design with additional controls in a split-plot layout of four replicate blocks; cultivars being completely randomised in main plots and fungicides completely randomised on sub-plots. Sprays were applied to plots of at least 50 m² by knapsack sprayer. Plots were swathed before yields were taken by plot combine harvester and seed weights were adjusted to 91% dry matter.

RESULTS

Yield

In Suffolk in 1995, the untreated yield of cv. Bristol (4.98 t/ha) was greater ($P < 0.05$) than that of cv. Rocket by 0.63 t/ha (Table 2). Yields were increased by difenconazole, flusilazole plus carbendazim or tebuconazole ($P < 0.05$). The full dose treatment in the autumn tended to give a greater yield than the two half-dose treatments.

There were effects of fungicide ($P < 0.001$) and cultivar ($P < 0.05$) on yield at the Aberdeen site in 1996. Average yields of Rocket were 3.65 t/ha compared with 1.58 t/ha for cv. Bristol. The greatest yield on each cultivar was with two full doses of flusilazole + carbendazim and the lowest yields were where carbendazim alone had been applied to cv. Bristol (Table 3).

Disease

In Suffolk, light leaf spot was common only in the experiment in the 1994/95 season. The leaf spotting phase of phoma (*Phoma lingam*) was widespread at the time of the autumn spray application on 24 November 1994 at the 8-leaf crop growth stage. In December (20 days after treatment) there was little light leaf spot but the incidence of phoma leaf spot was great (90% plants affected) with 1.4 and 1.1 % leaf area affected for untreated cv. Bristol and cv. Rocket, respectively. The amount of phoma leaf spotting was decreased by all treatments except carbendazim alone (Table 2). At the beginning of stem extension, on 4 April, the severity of light leaf spot had increased on cv. Bristol only (4.6% leaf area affected on untreated plots). The severity of phoma leaf spot had declined to 0.2 % leaf area affected on untreated plots of both cultivars due to leaf senescence. The disease severity on stems on 20 July was decreased by all fungicide treatments (Table 2) but more effectively by the azole fungicides than by carbendazim alone. Cankers were evident on the stems, with no differences between cultivars in untreated plots. A canker index showed little effect of treatment on cv. Rocket, but there were reductions ($P < 0.05$) in disease severity on cv. Bristol. A full dose of flusilazole plus

carbendazim or difenconazole in the autumn reduced the stem canker index ($P<0.05$). When the first sprays were applied to the Aberdeen experiment on 20 November 1995, light leaf spot was already well established (but only detectable after incubation) on cv. Bristol (70% plants, 7.2% leaf area affected) and also apparent on cv. Rocket (17% plants, 1.2% leaf area affected).

Table 2. Yield (t/ha at 91% dm) and severity of stem canker and light leaf spot on 20 July 1995 in the Suffolk experiment.

Treatment number and timing			Yield (t/ha at 91% dm)			Stem canker index			% stem area affected by light leaf spot (severity)		
	A	S	Bristol	Rocket	Mean	Bristol	Rocket	Mean	Bristol	Rocket	Mean
1.			4.98	4.35	4.67	61.1	54.4	57.8	6.9	0.0	-
2.	1	1	5.19	4.68	4.94	11.1	31.1	21.1	1.9	0.0	-
3.	1	-	5.22	4.71	4.97	12.8	31.1	22.0	1.8	0.0	-
4.	½	½	5.18	5.01	5.10	29.4	42.8	36.1	4.8	0.0	-
5.	1	-	4.81	4.71	4.76	46.1	43.9	45.0	6.9	0.0	-
6.	½	½	4.97	4.50	4.74	50.0	53.3	51.7	6.9	0.0	-
7.	1	-	5.19	4.58	4.89	36.7	40.0	38.4	3.3	0.0	-
8.	½	½	5.10	4.66	4.88	46.7	52.2	49.5	3.4	0.0	-
9.	1	-	5.32	5.22	5.27	7.8	31.7	19.8	3.2	0.0	-
10.	½	½	4.99	4.99	4.99	13.9	38.9	26.4	3.8	0.0	-
Cultivar mean			5.10	4.74	4.92	31.6	41.9	36.8	4.3	0.0	-
SED (44 df)										(22 df)	
Cultivar				0.031			6.60			1.18	
Fungicide				0.145			11.01			0.90	
Cultivar x fungicide				NS			NS				
CV%				5.1							

By the 15 December (26 days after treatment), the % leaf area affected by light leaf spot had increased to 16.9% on untreated plots of cv. Bristol and to 3.5% on those of Rocket. Disease severity on cv. Bristol was reduced by all treatments except for carbendazim alone ($P < 0.05$) but disease severity was already small on cv. Rocket. There were differences in light leaf spot control between full and half doses apparent for flusilazole + carbendazim. Phoma leaf spot was not recorded on either cultivar.

By stem extension (27 March), there were differences in control plots between cultivars in phoma leaf spot severity and incidence (cv. Bristol none, and cv. Rocket 0.1% leaf area; 3.3% plants). Light leaf spot had greatly decreased to affect less than 1% leaf area (cv. Bristol 0.8 %; 26.7 % plants and cv. Rocket 0.4 %; 6.7 % plants).

By 20 May (GS 3,5), light leaf spot severity had increased and 7.0% of the leaf area of cv. Rocket and 2.4% of cv. Bristol were affected in untreated plots (Table 3). This difference was attributed to severe loss of plants in untreated Bristol plots and produced the anomaly that treated plots had more light leaf spot than the untreated plots. There were large differences in the severity of light leaf spot ($P = 0.001$). Despite loss of plants, cv. Bristol showed a greater

disease incidence for light leaf spot and treatments had relatively little effort on disease incidence. Flusilazole + carbendazim and tebuconazole were the most effective products.

Table 3. Yield (t/ha at 91% dm) and severity of light leaf spot on 15 December 1995 and 20 May 1996 in Aberdeen.

Treatment number and timing			Yield (t/ha at 91% dm)			Stem canker index			% stem area affected by light leaf spot (severity)		
	A	S	Bristol	Rocket	Mean	Bristol	Rocket	Mean	Bristol	Rocket	Mean
1.			1.04	3.50	2.27	16.9	3.40	10.15	2.43	7.00	4.72
2.	1	1	2.44	4.06	3.25	0.10	0.00	0.05	5.47	1.83	3.65
3.	1	-	1.68	3.78	2.73	0.83	0.00	0.42	5.33	1.17	3.25
4.	½	½	1.36	3.73	2.55	4.07	0.00	2.04	3.87	2.13	3.00
5.	1	-	1.08	3.48	2.28	23.47	1.63	12.55	2.77	6.77	4.77
6.	½	½	1.24	3.47	2.36	10.10	2.43	6.27	3.30	4.17	3.74
7.	1	-	2.19	3.75	2.97	0.60	0.00	0.3	6.77	1.03	3.90
8.	½	½	1.74	3.84	2.79	1.60	0.00	0.8	6.63	2.00	4.32
9.	1	-	1.70	3.50	2.60	3.63	0.00	1.82	4.00	2.33	3.17
10.	½	½	1.30	3.39	2.35	8.90	0.17	4.54	5.23	2.90	4.07
Cultivar mean			1.58	3.65	2.62	7.02	0.76	3.89	4.58	3.13	3.86
SED (44 df)											
Cultivar				0.323			1.401			0.862	
Fungicide				0.179			1.698			1.148	
Cultivar x fungicide				0.403			2.692			1.778	
CV%				11.8							

Treatments had a little effect on the severity of light leaf spot on the pods (1.8% pod area affected in untreated cv. Bristol plots; 2.1% in cv. Rocket). There were treatment differences in light leaf spot severity on stems (reduced from 1.7% stem area in untreated plots to 0.4 or 0.9% following flusilazole plus carbendazim treatment in the autumn or as a split treatment respectively in cv. Rocket) but not in light leaf spot incidence. However, results were complicated by loss of plants in the untreated control Bristol plots, which had less disease than treated plots. These effects did not apply to cv. Rocket and results on this cultivar probably reflect disease control potential rather better than those on cv. Bristol.

CONCLUSION

Annual surveys of diseases in winter oilseed rape crops since autumn 1976 by ADAS and CSL have provided a quantitative record of their incidence and severity. This database has been only partially exploited to date and there are clearly regional and seasonal differences which, with further understanding of the critical factors responsible for this variation, will be of value in improving decision making on farms. The data presented here supports this statement, with light leaf spot being more important in the north of England and Scotland than in eastern England and the converse applying to stem canker. The experiment also shows the

need for further examination of fungicide time of application as correlation between the amount of disease and final yield are variable where disease incidences are low or moderate. Under great disease pressure, even two full doses of flusilazole plus carbendazim failed to prevent yield loss in a susceptible cultivar compared to a resistant cultivar. The potential for light leaf spot to decrease yield on cv. Bristol grown in Scotland underlines the need to relate disease risk to cultivar choice. The efficacy of the newer azole fungicides was shown to be superior to that of carbendazim. Fungicide resistance reduced its effectiveness in Scotland (Sutherland *et al.*, 1994).

A yield increase of *c.* 0.2 t/ha is required to recover the cost of one full dose of an appropriate fungicide (based on a seed price of £150/t and an azole fungicide cost of £25 per full dose/ha). In Suffolk, cv. Bristol was worth treating but cv. Rocket was not. However in Aberdeenshire, even the more resistant cv. Rocket was worth treating under severe disease pressure. There is a need to improve the method of risk assessment of severe epidemics using diagnostic and forecasting techniques so that crops at risk can be identified. By integrating cultivar resistance and fungicide use there could be a reduction in fungicide requirements.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support of the HGCA (Oilseeds) levy (Oilseeds Project No. OS28) and the help given by our colleagues in doing the experiments.

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Development and control of light leaf spot (*Pyrenopeziza brassicae*) epidemics in winter oilseed rape in the UK

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ABSTRACT

Field trials were done to study development and control of light leaf spot epidemics in winter oilseed rape in different parts of the UK. Oilseed rape debris was applied to crops at Boxworth and Rothamsted in England to provide inoculum for starting epidemics but not at Aberdeen in Scotland. Epidemics were controlled by applying tebuconazole in the autumn and/or spring. At Aberdeen and Rothamsted, light leaf spot epidemics were severe, with 92-100% of plants affected in untreated plots of the cultivar Bristol by March. At Boxworth, the epidemic developed more slowly, with 65% of plants affected by April. Monthly applications of tebuconazole gave the best disease control. At Rothamsted, monthly applications of fungicide gave a yield response of 1.4 t/ha but there were no yield responses to fungicide at other sites.

INTRODUCTION

In the UK, each season an estimated £3-9 million are spent on fungicides to control diseases of winter oilseed rape but losses in excess of £30 million still occur (Fitt *et al.*, 1997). Light leaf spot (*Pyrenopeziza brassicae*) is one of the most important diseases of winter oilseed rape and severe epidemics can cause yield losses of up to 3 t/ha (Gladders *et al.*, 1998). The optimum time for control of the disease is in the autumn (Sansford *et al.*, 1996) but symptoms do not appear until December - February. As a result, growers often either apply fungicides prophylactically or leave crops unsprayed. Epidemics of light leaf spot vary in severity between seasons and surveys have shown that in seasons when severe epidemics were widespread many crops were left unsprayed (Fitt *et al.*, 1997). Conversely, in seasons when little disease occurred many crops received full fungicide programmes. There is a need to develop more accurate methods for predicting severe epidemics of light leaf spot and hence the need for fungicide applications to winter oilseed rape.

A forecasting system is being developed to predict risk of severe light leaf spot, using survey data from ADAS, CSL and SAC (Fitt *et al.*, 1996). The system uses the incidence of light leaf spot on pods in July and environmental and agronomic factors to produce seasonal risk,

initial crop risk and improved crop risk indices. This paper studies the development and control of light leaf spot in field experiments in relation to seasonal risk forecasts for different parts of the UK.

MATERIALS AND METHODS

Field experiments were done at three sites in 1996/97; two were in England (Rothamsted and Boxworth) and one in Scotland (Aberdeen). Experiments were arranged in a randomised split-plot design, with three replicates, cultivars Bristol (NIAB resistance rating 2; Anon., 1997) and Capitol (NIAB resistance rating 8) and ten fungicide treatments. Plot sizes were >50 m^2. Oilseed rape debris was applied to provide inoculum for epidemics; plots at Rothamsted each received half a bale of debris in October (high inoculum level) and the Boxworth site received two thirds of a bale of debris over the whole experiment in November (low inoculum level). Plots at Aberdeen were not inoculated. Disease epidemics were manipulated by applying the fungicide tebuconazole (as Folicur) as a single full dose in the autumn or spring or as a split application in the autumn and the spring (Table 1). Fungicides were applied, by hand-held sprayer or conventional tractor-mounted hydraulic sprayer, in 200-250 litres water/ha.

Table 1. Applications of the fungicide tebuconazole to winter oilseed rape, 1996/97.

Treatment	Fungicide timing (and dose)		
	Rothamsted	Boxworth	Aberdeen
UT	untreated	untreated	untreated
R	Oct-March (1/2)	Nov-May (1/2)	Nov-March (1/2)
A1	Oct (1)	Nov (1)	Nov (1)
A2	Nov (1)	Dec (1)	Dec (1)
A3	Dec (1)	Jan (1)	Feb (1)
A1S	Oct (1/2) + spring (1/2)	Nov (1/2) + spring (1/2)	Nov (1/2)
A2S	Nov (1/2) + spring (1/2)	Dec (1/2) + spring (1/2)	Dec (1/2)
A3S	Dec (1/2) + spring (1/2)	Jan (1/2) + spring (1/2)	Feb (1/2)
S	spring (1)	spring (1)	-
F	flowering (1)	flowering (1)	-

(1) = full dose (250 g a.i./ha) (1/2) = half dose (125 g a.i./ha)

During the season 10 plants/plot were regularly removed and assessed for the presence of light leaf spot. Before the green bud stage of crop growth, plants were incubated in polyethylene bags for 24-48 hours before assessment, to allow light leaf spot symptoms to develop. Incidence was assessed as % plants affected and severity as % leaf area with symptoms on whole plants. Plots were swathed or desiccated 10-14 days prior to harvest and harvested using small plot combine harvesters, with yields expressed at 90% dry matter.

RESULTS

Light leaf spot epidemics were severe at Aberdeen and Rothamsted but slight at Boxworth (Figure 1). At Aberdeen and Rothamsted, light leaf spot first appeared in untreated plots of cv. Bristol in November - December. Disease development was rapid, with 92-100% of plants affected and 12-24% leaf area with light leaf spot by March. At Boxworth, light leaf spot did not appear until January-February and by April the incidence had reached 65% plants affected. Disease severity was still low, with 2% leaf area affected. At all three sites the light leaf spot epidemic started at the same time on the resistant cultivar Capitol as on cv. Bristol (Figure 1). The epidemic on cv. Capitol was severe at Rothamsted, with 82.2% plants affected by March. Disease development was slower at Aberdeen and Boxworth, with 26.7% and 43.3% plants affected, respectively, in March/April.

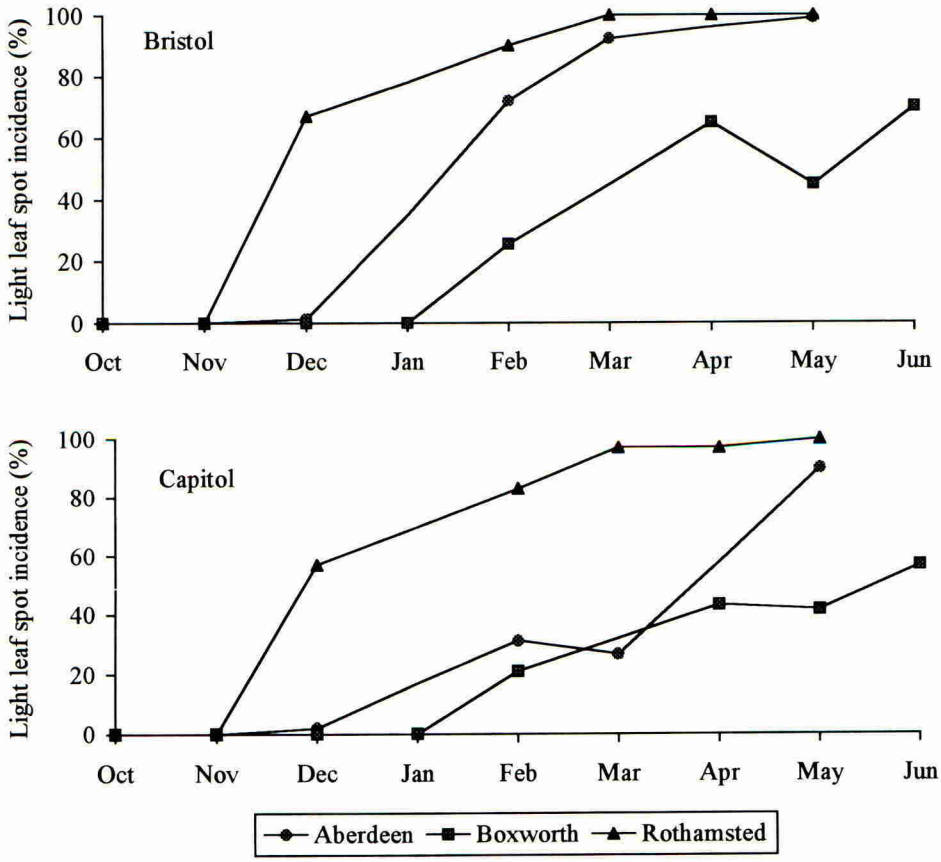

Figure 1. Development of light leaf spot on leaves of untreated plots of winter oilseed rape cultivars Bristol and Capitol at three sites in the UK during 1996/97.

There were large differences in disease incidence between the cultivars Bristol and Capitol, particularly at the Rothamsted and Aberdeen sites, where disease epidemics were severe (Table 2). In April/May the disease incidence in untreated plots was similar for both

Table 2. Effects of timing and dose of tebuconazole on the incidence and severity of light leaf spot in winter oilseed rape, April/May 1997.

Treatment	Light leaf spot incidence and severity					
	Rothamsted		Boxworth		Aberdeen	
	% plants	% area	% plants	% area	% plants	% area
Bristol						
UT*	100.0	17.9	65.0	2.0	98.9	23.7
R	80.0	0.9	0	0.0	73.3	2.3
A1	96.7	5.5	53.0	0.8	100.0	6.9
A2	100.0	11.8	10.0	0.2	100.0	11.0
A3	96.7	8.1	10.0	0.1	100.0	16.7
A1S	93.3	6.3	30.0	1.0	100.0	17.7
A2S	100.0	8.1	0	0	100.0	14.2
A3S	96.7	4.7	0	0	96.7	18.3
S	100.0	7.5	10.0	0.03	-	-
Capitol						
UT	96.7	4.4	43.3	1.0	90.0	3.0
R	26.7	0	26.7	1.1	13.3	0.1
A1	93.3	1.9	10.0	0.1	26.7	0.3
A2	80.0	1.7	3.3	0.01	33.3	0.4
A3	93.3	1.9	0	0	43.3	1.2
A1S	50.0	0.2	3.3	0.01	33.3	0.4
A2S	60.0	1.2	3.3	0.01	30.0	0.4
A3S	50.0	0.2	6.7	0.03	68.9	2.0
S	90.0	2.8	20.0	<0.01	-	-
SED	13.31	5.13	17.43	9.66	8.75	3.81
df	38	38	40	40	42	42

* Treatment codes are explained in Table 1.

cultivars but disease severity was smaller on cv. Capitol. Routine applications of a half dose of tebuconazole decreased disease incidence most. In general, single full dose autumn applications of tebuconazole gave better control of light leaf spot than half dose applications

until mid-March. At Rothamsted, application of a second half dose of tebuconazole in the spring decreased incidence of light leaf spot compared with the full dose application on cv. Capitol, but had little effect on cv. Bristol. At Boxworth, where disease incidence was less, split dose application in the autumn and spring gave better disease control than a single full dose on cv. Bristol but not cv. Capitol. At Aberdeen, where spring fungicides were not applied, full and half dose applications of tebuconazole in the autumn were still giving some control of disease on cv. Capitol in April/May, but were no longer effective on cv. Bristol.

At Rothamsted, cv. Capitol yielded approximately 1 t/ha more than cv. Bristol (Table 3) in all but the routinely sprayed plots. The yield response to routine fungicide treatment compared with the untreated was 1.39 t/ha on cv. Bristol (0.25 t/ha for cv. Capitol). All other treatments yielded less than the routine treatment and there were no differences between autumn or autumn + spring treatments. At Aberdeen, despite the severe disease epidemic, there was little or no yield response; yields of both cultivars were similar and, although fungicide treatment increased yield ($P<0.05$), there were no differences between routinely sprayed plots and other treated plots. Control of the slight disease epidemic at Boxworth produced no yield responses.

Table 3. Effects of light leaf spot epidemics and timing and dose of tebuconazole on the yield of winter oilseed rape, 1997.

Treatment	Yield in t/ha at 90% dry matter					
	Rothamsted		Boxworth		Aberdeen	
	Bristol	Capitol	Bristol	Capitol	Bristol	Capitol
UT	3.96	5.00	3.14	3.23	2.47	2.77
R	5.35	5.25	3.36	3.09	2.91	3.06
A1	4.69	5.27	3.15	3.29	2.73	3.10
A2	4.42	5.44	3.08	3.30	2.90	2.89
A3	4.46	5.25	2.86	3.19	2.91	2.81
A1S	4.76	5.48	3.08	3.49	2.83	2.68
A2S	4.64	5.71	3.21	3.38	2.76	3.06
A3S	4.79	5.47	3.24	3.25	2.41	2.83
S	4.61	5.36	2.83	3.06	-	-
F	4.43	5.54	3.06	3.23	-	-
SED	0.169		0.390		0.169	
df	36		33		42	

DISCUSSION

In October 1996, the light leaf spot forecasting system being developed by Fitt *et al.* (1996) predicted a high risk for Scotland and northern England and a smaller risk for east and south England. The development of a severe epidemic at Aberdeen fitted the prediction for Scotland. The development of a slight epidemic at Boxworth fitted the prediction for east England. However, although the field experiment at Rothamsted received a high level of inoculum, the subsequent development of a severe epidemic, with yield loss, does suggest that in regions where the predicted risk of light leaf spot is small, severe disease epidemics can still occur in individual crops. Growers should not therefore be complacent when a low risk situation has been predicted for their region but should assess each individual crop for risk.

In developing the forecasting scheme, regression analyses on disease survey data suggested that early sowing (before mid-August) and cultivar susceptibility increased the incidence of light leaf spot, but proximity to previous crops did not (Fitt *et al.*, 1996). In Scotland, second successive rape crops have been considered to be at greatest risk from light leaf spot. However, in recent seasons some first rape crops have developed severe epidemics with almost total plant loss (Gladders *et al.*, 1998), although the fields had not grown winter oilseed rape for at least 5 years. If surrounding crops were acting as a source of inoculum for these crops, proximity to previous crops may be more important than was initially suggested by the analyses of survey data.

Results showed that the yield response to control of light leaf spot can differ greatly between susceptible and resistant cultivars. It is important that a forecasting system for light leaf spot provides not only regional risks of severe epidemics in the autumn, but also information on factors such as cultivar susceptibility and sowing date, which affect the risk that severe epidemics will develop on individual crops.

ACKNOWLEDGEMENTS

The work is funded by the Home Grown Cereals Authority, BBSRC, MAFF and SOAEFD.

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Pest and disease control requirements for spring oilseed rape in northern climates

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ABSTRACT

The production of spring oilseed rape met with favour in Scotland because it required few inputs and fitted well within the arable rotation. The crop was, however, particularly susceptible to pollen beetles, *Meligethes* spp., especially as it reached the vulnerable bud development stage at a time when the beetles were most numerous. Trial work showed that the greatest yield benefits were achieved when insecticides were applied at the green bud stage when the beetle population was between 0.5 and 1 beetle per plant. In addition to insect problems, a fungus disease, white blister, *Albugo candida*, was noted for the first time in European oilseed crops in 1996. As control measures had not been tested against this disease in spring oilseed rape, the fungicide metalaxyl was screened as a potential control agent. However, neither seed nor foliar treatments of metalaxyl reduced disease infection and it was concluded that crop rotation was the only effective method currently available for managing this disease problem.

INTRODUCTION

The production of spring oilseed rape has met with favour in Northern Britain because it was heralded as a low maintenance, low input crop which did not require the substantial pesticide inputs commonly made to the winter crop. It also provided a viable alternative to growing spring barley and, in so doing, increased the rotation options for many growers in these areas. Despite these initial claims, it soon became apparent that spring oilseed rape was particularly susceptible to damage by pollen beetles (*Meligethes* spp.), and frequently required an insecticide treatment. Pollen beetles are commonly found in winter oilseed rape and although they occur here in large numbers, the winter crop is able to compensate for damage and consequently few crops require treatment against this pest. Insecticide treatment is only recommended in crops of winter oilseed rape where beetle levels exceed 15 per plant at the susceptible green bud stage, unless the crop is backward or damaged in some way (Lane & Walters, 1994). By contrast, the spring crop reaches the vulnerable bud development stage at a time when the pollen beetles are more numerous. Consequently, spring varieties suffer more heavily from pollen beetle attack as they are less able to compensate for damage. As a result, a lower treatment threshold of 3 beetles per plant has been used for spring rape varieties (Lane & Walters, 1994). This threshold was adhered to by growers in Scotland, but it was soon noted that even where beetle numbers remained below 3 per plant, crops were exhibiting signs of pollen beetle damage. Therefore, it was suggested that this recommendation was not suitable for Scottish crops and that further evaluation of the problem was required.

In addition to potential insect problems, the disease status of spring rape has also recently merited further attention. In the early nineties, few cases of major disease problems were

reported. However, in 1996, significant levels of the fungus disease white blister (*Albugo candida*) were observed for the first time in turnip rape crops in Scotland. The fungal infection is characterised by the appearance of white, chalk-like blisters on the cotyledons, leaves, stems and pods with bleached and thickened areas on the upper surfaces of leaves, together with mycelial growth on the undersides of the leaves. Stems and flowers may also be infected and this can result in white or brown, enlarged and deformed flowerheads and pods, otherwise known as "stagheads", which are the most noticeable symptoms.

This disease has rarely been seen in oilseed rape in Europe, although it has been noted on some brassica weed species, especially Shepherd's purse. White blister, however, is considered an important disease of turnip rape (*Brassica rapa*) and of yellow, or brown mustard (*Brassica juncea*) in Canada and India where it is reported to result in yield losses of up to 60 % when infection levels are severe (Gupta *et al.*, 1991). Not suprisingly, growers finding this disease in the crop were keen to explore control options and agrochemical distributors were quick to seize upon the opportunity to recommend a range of potential fungicides. Products containing metalaxyl have previously been used to control this disease on vegetable brassicas but no information was available regarding it's efficacy against white blister in spring oilseed rape. The aim of this study was therefore two fold: to determine if, and when, pollen beetles should be controlled in spring crops and if it was worthwhile applying control measures against white blister.

METHODS AND MATERIALS

Pollen beetle study

Trials were carried out at Tillycorthie Farm, Undy, Aberdeenshire using the turnip rape *cv.* Kova in 1991 and 1992 and the swede rape *cv.* Puma in 1991 only. The plots were sown in April at a seed rate of 8 kg/ha for the swede rape and 7.5 kg/ha for the turnip rape. All plots received herbicide and fertiliser in accordance with accepted agronomic practice. The insecticide cypermethrin (Ambush C, 100 g/l EC) was applied at a rate of 250 ml/ha using a small plot AZO sprayer calibrated to deliver 200 l/ha. Beetle assessments were carried out every two days from GS 3.1 on twenty, randomly chosen plants from each plot. The plots were treated with insecticide when the mean number of beetles reached; 0.5, 1, 2 and 3 beetles per plant. Due to the dynamics of the beetle population in the *cv.* Kova in 1992, the threshold level of 2 beetles per plant was not attained. The treatments were replicated four times using plots measuring 70 m^2. Plots were harvested in September and seed samples taken for dry matter determination (the seed yield was corrected to 91 % d.m.).

White blister study

Two field trials were undertaken in 1997 at Clinkstone farm, Morayshire. The field used was selected on the basis that it was likely to harbour a high level of white blister inoculum in the soil because of the severe infection of white blister noted in the previous turnip rape crop and because of the frequency with which turnip rape was grown in the rotation. The trials were sown on 30 April at a seed rate of 5 kg/ha in plots measuring 33 m^2.

In the first trial, the effect of seed treatment was investigated using home-saved seed, *cv.* Agena, harvested from a site which was heavily infected with white blister oospores in the previous year. A seed treatment containing metalaxyl (Apron Combi FS, a.i. metalaxyl + thiabendazole + thiram) was applied at a rate equivalent to 5 litres/t seed. Three replicates of treated and untreated seed were sown in a randomised block design.

In the second trial, the cvs Kova, Kulta and Agena were compared for resistance to white blister as part of a larger variety screening trial, each variety replicated twice. An experiment to test the efficacy of foliar applied metalaxyl was superimposed onto this trial. The treatment used was mancozeb + metataxyl (Fubol 58) applied at two different timings; the three true leaf stage of the crop (GS 13) and the yellow bud stage (GS 37). Each plot was divided into three sub-plots, two-sub plots receiving each of the fungicide treatments and the remaining subplot was untreated. The fungicide treatments were applied at 1.5 kg/ha in 200 litres of water using a knapsack sprayer.

The site was monitored regularly throughout the season for the development of disease symptoms. Disease levels were scored visually on a scale of 1 to 9 on 4 June and 28 June. A detailed assessment of the percentage of infected plants was carried out on 6 August on 100 plants, randomly chosen, from each plot.

RESULTS

In 1991, all of the treatments applied to control pollen beetles gave significant yield responses in cv. Kova (Table 1). However, only treatments applied before the beetle population had reached 3 per plant had any significant effect on the yield of cv. Puma. Similarly, in 1992, only the first treatment applied to cv. Kova at GS 3.3, when there was less than 1 beetle per plant, provided a significant yield increase. With regard to the production of healthy pods (Table 2), a greater number were seen on plants treated before the beetles had reached the 3 per plant threshold in cv. Kova, in 1991. In 1992, an increase in pod number was observed when treatments were applied as populations reached 0.5 and 3 beetles per plant.

The white blister infection assessments indicated that using a metalaxyl seed treatment did not reduce the incidence of disease symptoms (Table 3). Likewise, earlier assessments which are not reported here, but made during the trial period, did not detect any difference in disease levels between treated and untreated crops.

Table 1. The effect of insecticide timing against pollen beetles on the seed yield of oilseed rape, 1991-1992.

Mean no. of beetles/plant at time of insecticide application		Seed yield t/ha at 91% d.m. (Date of application) 1991 Kova	1991 Puma	1992 Kova
0.5	GS 3.3	1.48 (17/6)	2.53 (13/6)	1.78 (9/6)
1.0	GS 3.5	1.52 24/6	2.43 19/6	1.69 16/6
2.0	GS 3.6	1.47 27/6	2.59 21/6	- -
3.0	GS 3.7	1.50 01/07	2.38 24/6	1.50 19/6
Untreated		1.28	2.24	1.39
LSD		0.123	0.175	0.389

Table 2. The effect of timing of treatments to control pollen beetle on pod development of oilseed rape, 1991-1992.

Mean no. of beetles/ plant at time of application	Mean no. of healthy pods/plant 1991 Kova	1991 Puma	1992 Kova
0.5	85	60	85
1.0	81	58	65
2.0	84	61	-
3.0	70	59	73
Untreated	56	60	58
LSD	17.4	ns	13.9

Table 3. The effect of seed treatment on the incidence of white blister disease in spring oilseed rape, 1997.

Treatment	% white blister infection
untreated	9.0
metalaxyl + thiabendazole + thiram	10.3

No difference was noted in the incidence of disease levels between the three varieties tested (Fig. 1). Furthermore, neither of the fungicide applications gave effective control of white blister symptoms, although with cvs Agena and Kulta, there was a tendency towards reduced disease symptoms following the later applications made at the yellow bud stage (GS 37).

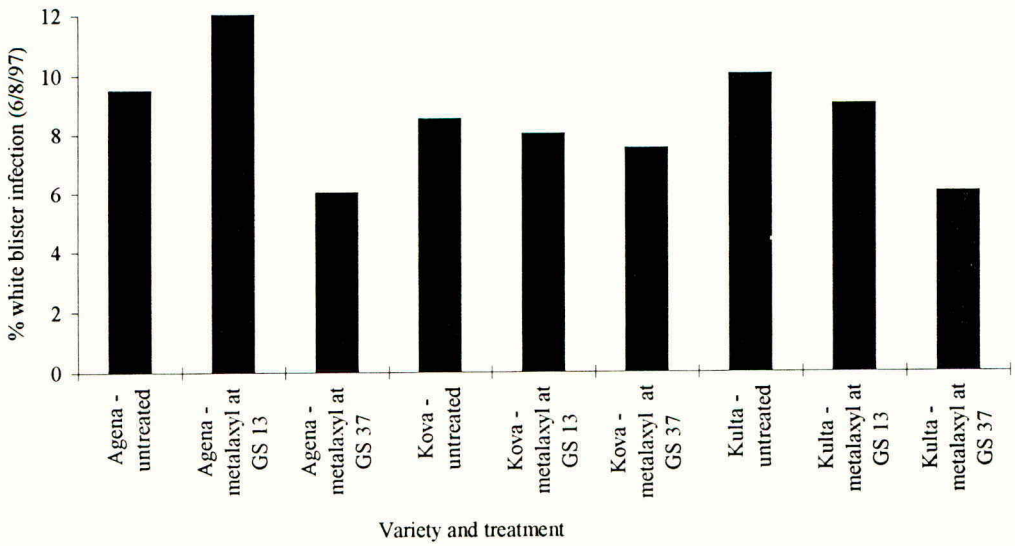

Figure 1. The effect of foliar applied fungicide treatments on the control of white blister in spring oilseed rape, 1997.

DISCUSSION

There is speculation concerning the future viability of spring oilseed rape because of forecasted changes in the price support mechanism. This necessitates detailed re-examination of crop management. The spring crop is particularly vulnerable to pollen beetle attack and it was shown that all insecticide treated plots consistently yielded higher than the untreated plots. Treatments applied at the early bud stage gave significant yield benefits even at beetle populations between 0.5 and 1 beetle per plant in all 3 trials. Treatments applied at a later growth stage, when beetle numbers had risen to 3 per plant, did not result in a yield increase with *cv.* Puma in 1991, or with *cv.* Kova in 1992, and it was concluded that treatment at this pest level was of no benefit. This may be due to the fact that the beetle population reached 3 per plant at a later stage in bud development, by which time the plant may be less susceptible to damage. The results of the healthy pod assessments reinforced the findings that the insecticide treatment was worthwhile and again indicated that the earlier treatments were of most value. For these reasons a treatment threshold of one beetle per plant at the green bud stage is now adopted in Scotland; this is in keeping with the Swedish recommendation which advises treatment at 0.8 beetles per plant (Nilsson, 1987).

It was concluded from the work on white blister that the fungicide metalaxyl did not reduce disease infection on spring oilseed rape. In Canada, where the disease is widespread, resistance to white blister is included in the varietal screening programme, whereas in Europe, this has never been a selection criterion for breeding because white blister has only recently been recognised as a significant problem. Therefore, as there are no fungicides currently available to control white blister and varietal resistance is not yet an option, control must presently rely on preventative measures, including crop rotation, control of volunteers and use of pathogen-free seed.

Proposals to limit the area aid for oilseed rape will result in conventional spring types only being financially viable if production costs are kept to a minimum. These trials have demonstrated that it is not cost effective to apply fungicide treatments to control white blister. Instead, preventative measures, which do not incur extra costs, should be used to minimise the likelihood of disease infection. Farmers attempting to save costs by home-saving seed would be well advised to select from white blister-free crops. By contrast, judicious application of insecticides at the appropriate stage for pollen beetle control will still provide a substantial financial benefit given the high yield penalty if crops are left untreated. The continuing pressure to reduce costs, further emphasises the importance of optimising pesticide timings.

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The effects of lambda-cyhalothrin on the aphid *Myzus persicae*, a vector of turnip mosaic potyvirus, and implications for its control

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ABSTRACT

The effects of lambda-cyhalothrin, a synthetic pyrethroid insecticide, on the peach-potato aphid *Myzus persicae*, were studied using different application rates. These effects were then assessed as a means of reducing the ability of *M. persicae* to transmit the non-persistent potyvirus, turnip mosaic virus (TuMV) within a susceptible crop. All application rates of lambda-cyhalothrin used produced a rapid repellant effect on *M. persicae*. Choice chamber studies, using treated and untreated turnip seedlings, showed how introduced alate aphids avoided the treated plants for up to 7 days after treatment application, whilst laboratory ring tests demonstrated how both apterous and alate aphids vacated the treated sections of leaf discs. When aphids, viruliferous to TuMV, were introduced into these choice chambers, significantly less of the lambda-cyhalothrin treated test plants became infected, when compared to the untreated controls. However, when viruliferous aphids were caged onto treated turnip seedlings, all became infected irrespective of dose rate applied. Aphids that fed on treated virus source plants were shown to acquire and transmit TuMV very rapidly, before being disabled by the treatment. This study emphasises the importance of addressing crop pests as virus vectors within agriculture and demonstrates the potential of using lambda-cyhalothrin as an effective means of controlling the appearance and spread of TuMV within a brassica crop.

INTRODUCTION

Turnip mosaic virus (TuMV), a potyvirus, is found worldwide in both temperate and tropical regions (Jenner & Walsh, 1996). It has a wide host range, infecting 318 species within 43 plant families (Edwardson & Christie, 1991) and is acquired and transmitted by aphids in a non-persistent manner that requires an accessory factor or helper component (Markham *et al.*, 1987). TuMV infects both horticultural and arable brassica crops as well as other important edible crops (peas, rhubarb, watercress, radish, chicory and lettuce), ornamentals (*Abutilon*, stocks and wallflowers) and weed species belonging to 14 different families (Jenner & Walsh, 1996; Shattuck, 1992). Surveys conducted in England by Broadbent (1957) and later in 28 different countries by Thomlinson (1987), showed TuMV to be one of the most economically important viruses of vegetables. This is corroborated by reports of serious crop losses in swede (Thomlinson, 1987), Brussels sprout (Thomlinson & Ward, 1981), white cabbage (Walkey & Webb, 1978) and cauliflower (Thomlinson & Shepherd, 1978). More recently, TuMV has been found infecting winter oilseed rape crops in parts of England and Wales (Hardwick *et al.*, 1994).

Over 40 species of aphids are known to transmit TuMV (Brunt *et al.*, 1990), although *Myzus persicae* and *Brevicoryne brassicae* have been reported as the two most important vectors in the field (Markham *et al.*, 1987). Both of these species have been shown to acquire and transmit TuMV to healthy plants during feeding periods as short as one minute (Markham *et al.*, 1987). This rapid process inevitably allows viruliferous aphids to transmit TuMV to insecticide-treated susceptible plants, before they are disabled.

The use of chemicals that swiftly disrupt the feeding behaviour of insects, or deter them from infesting a treated plant, could help to break the acquisition and transmission cycle between plant viruses and their vectors. This study has evaluated the effect of one such chemical, lambda-cyhalothrin, marketed as Hallmark® and Karate® (ZENECA Agrochemicals), a quick-acting contact and ingested synthetic pyrethroid (Jutsum *et al.*, 1984), on *M. persicae*, and has subsequently explored the potential of using this product to prevent or reduce initial incidence then intra-crop spread of TuMV.

METHODS AND MATERIALS

Test plants

Turnip plants, (*Brassica campestris*), cv. Just Right, were grown from seed individually in 5 cm plastic pots containing a commercial peat-based compost. These were maintained within an insect-proof glasshouse. Earlier sowings that were to be mechanically infected with TuMV (as virus source plants), and those to be used for producing leaf disks, were transplanted into 9 cm pots at the 6-leaf stage.

Origin and maintenance of aphids

Cultures of an R2 clone of the aphid *Myzus persicae* were obtained from ZENECA Agrochemicals, Jealotts Hill Research Station, prior to the start of each assay. These contained both alate and apterous forms. Aphids were maintained on swede seedlings within seed trays.

Virus maintenance

Twenty, four week old turnip plants were mechanically inoculated with a laboratory maintained strain of TuMV, obtained from Horticulture Research International, Wellesbourne. This entailed grinding an infected leaf in a small volume of water using a pestle and mortar. A small amount of this solution was then rubbed onto a young leaf of each of the healthy host plants with fine grade carborundum powder. The inoculated leaves were then washed with tap water. After 2 weeks, the appearance of typical TuMV symptoms indicated that inoculated plants were ready for use as virus source plants.

Treatment applications

Five application rates of lambda-cyhalothrin were used; 5, 12.5, 25, 50 & 100 ppm. These were applied either as foliar treatments, sprayed almost to run-off point and allowed to air dry, or as solutions for leaf dips. Control treatments of tap water and a reference chemical standard of 125 ppm cypermethrin were also used and applied in the same way.

Virus transmission assays

TuMV infected virus source plants were sprayed with all of the above treatments, except the 5 ppm lambda-cyhalothrin. One hour later, one plant was selected from each of the treatments and infested with approximately 500 apterous *M. persicae*. After a 15 to 30 minute acquisition access period, aphids were removed in groups of 20 and each group placed onto an untreated two-leaf turnip seedling, each treatment replicated ten times. This procedure was repeated 3 days after the virus source plants had been treated. Turnip seedlings were then scored for TuMV infection by the appearance of typical yellow-vein symptoms, over the following 2 weeks.

Ring test assays

Using a 9 cm diameter cutting ring, leaf circles were cut from mature turnip leaves. Six replicate circles were made for each of the lambda-cyhalothrin application rates, the 125 ppm cypermethrin treatment and the water only control. Each leaf circle was then dipped to a half-way mark in a solution of its respective treatment. Excess liquid was allowed to drain from the leaf circles before they were placed individually onto a layer of semi-molten agar solution (0.6 %) within 9 cm plastic Petri dishes. After 1 hour, 20 alate *M. persicae* were added to 3 of the leaf circles for each treatment and 20 apterous to the other three. The numbers of aphids on the treated and the untreated parts of the leaves were counted after 3 and 17 hours. Twenty four hours after treatment, all surviving aphids were removed from all of the leaf circles. These were replaced with 20 new alate or apterous aphids, which again were scored on the treated and untreated parts of the leaf circles 3 and 17 hours after application.

Choice test assays

Six small turnip seedlings were sprayed almost to run off for each of the treatments and rates used in the ring tests. One hour later, two seedlings were selected from each treatment and placed randomly in a large perspex insect cage. Approximately 100 alate *M. persicae* were then released into the cage. Twenty four hours later, the number of aphids infesting each of the seedlings was recorded. This choice test assessment was repeated with seedlings 3 and 7 days after treatment.

A second choice test was undertaken with 5 seedlings per treatment, randomly placed around a TuMV infected plant infested with 100 alate *M. persicae*. Seedlings were left within this cage for 4 days before being removed, cleaned of aphids and left for symptom development. The numbers of seedlings within each treatment that became infected with TuMV were subsequently recorded. Two replicate choice chambers were used for this assessment.

RESULTS

Virus transmission assay

A high number of turnip seedlings that received aphids from all of the treated TuMV infected virus source plants became infected within both the 0 and 3 day assessments (Table 1).

Table 1. Total number of turnip seedlings, infected with TuMV after receiving *Myzus persicae* from lambda-cyhalothrin treated virus source plants.

Assay	Virus source plant treatment				
ppm	12.5	25	50	100	water
Day 0	10	10	10	10	10
Day 3	10	8	7	10	10

Ring test assays

Both alate and apterous aphids were shown to rapidly vacate the lambda-cyhalothrin treated areas of all leaves at all application rates. This effect was still occurring 20 hours after aphids had been applied to the leaf disks. The cypermethrin treatment was less effective and no effect was seen in the water treated control (Table 2). A second experiment where aphids were applied to leaf disks that had been treated 24 hours previously, produced similar results (data not shown).

Table 2. Total number of alate and apterous aphids recorded on the treated area of turnip leaf discs per treatment, four and twenty hours after infestation.

Treatment	lambda-cyhalothrin										control		cyperm.	
rate - ppm	5		12.5		25		50		100		water		125	
	4h	20h	4h	20h	4h	20h	4h	20h	4h	20h	4h	20h	4h	20h
Alate	0	0	0	3	2	0	1	0	0	0	30	35	16	10
Apterous	3	5	1	10	2	3	0	0	0	0	22	22	20	15

Choice test assays

Alate *M. persicae* released into the choice chamber one hour after treatments were applied, were found to have avoided the lambda-cyhalothrin treated plants to a greater degree than those treated with water controls and the cypermethrin (Table 3). No aphids were found on the plants treated with 100 ppm and 50 ppm lambda-cyhalothrin. This effect was still evident when aphids were applied to plants treated 3 days previously. However, after 7 days only the 100 ppm lambda-cyhalothrin treatment was still repelling aphids (Table 3). When viruliferous aphids were released into the choice chambers, more of the turnips treated with water and cypermethrin became infected than those treated with lambda-cyhalothrin. Numbers of infected lambda-cyhalothrin treated plants were less following the higher treatment rates (Table 4).

Table 3. Total number of alate aphids recorded on turnip seedlings 24 hours after aphid release in a choice chamber following treatment 1 hour, 3 days and 7 days before release.

Treatment	lambda-cyhalothrin					control	cyperm.
Rate - ppm	5	12.5	25	50	100	water	125
1 hour	13	8	2	0	0	23	27
3 days	45	33	17	7	0	75	44
7 days	48	40	56	34	5	42	62

Table 4. Number of turnip seedlings infected with TuMV within a choice chamber after exposure to viruliferous alate *M. persicae*. (Two replicate tests were undertaken).

Treatment	lambda-cyhalothrin					control	cyperm.
Rate - ppm	5	12.5	25	50	100	water	125
Test 1	2	2	0	0	0	4	3
Test 2	1	0	1	1	0	3	5

DISCUSSION

This study has demonstrated the effects of a range of application rates of lambda-cyhalothrin at deterring both alate and apterous *M. persicae* from colonising treated turnip plants when compared to a cypermethrin and a water only treatment. Laboratory ring tests showed that even rates as low as 5 ppm produced a dramatic repellent effect on *M. persicae* for up to 20 hours after application (Table 2), whilst choice chamber studies showed how rates of 100 ppm were still deterring aphids 7 days after application (Table 3), despite substantial plant growth. These effects were not seen with the cypermethrin and water only treatments and were only apparent following the lambda-cyhalothrin treatments when aphids had an option to feed on plants that had not been treated. We have also been able to show that when TuMV-viruliferous aphids were placed into a similar choice chamber, fewer lambda-cyhalothrin treated plants became infected than those treated with cypermethrin or with water. However, where there was no alternative, aphids probed and fed on lambda-cyhalothrin treated plants (including those treated with the 100 ppm application rate) and were subsequently shown to survive long enough to acquire and effectively transmit TuMV.

The repellent effects demonstrated, suggest that lambda-cyhalothrin may offer an effective method for protecting certain field crops from aphid infestation. Theoretically, ingressing aphids would avoid the lambda-cyhalothrin treated plants and colonise an alternative untreated host. Where aphids are vectoring rapidly transmitted pathogens such as TuMV, this repellent effect could help protect a susceptible crop from initial infection and reduce within-crop spread. However, the fact

remains that outside of the treated crop, susceptible weeds or other untreated crops may become a reservoir for virus and aphid vector. Careful crop management together with the roguing or destruction of these reservoirs, could help reduce the risk of further crop problems.

As already mentioned, non-persistent viruses such as TuMV are acquired and transmitted during very short aphid feeding and probing periods. Results obtained in this study suggest that semi-persistent, aphid-transmitted viruses, requiring longer acquisition and transmission periods, could be controlled by lambda-cyhalothrin. Control of persistent viruses with lambda-cyhalothrin has already been demonstrated (Perrin, 1986). Further laboratory studies and large scale field trials would be needed to investigate this hypothesis and to demonstrate that the results obtained in this study can be reproduced under field conditions.

ACKNOWLEDGEMENTS

The authors thank Dr. John Walsh (HRI Wellesbourne), for providing TuMV infected material. We also acknowledge the BBSRC for enabling this study to be undertaken.

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Eurygaster integriceps in Northern Iraq - strategies for optimal control

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ABSTRACT

Following the Gulf conflict of 1991, the international aid community established a ground based programme for the control of the sunne pest, *Eurygaster integriceps* in Northern Iraq. The programme lasted for 5 years (1993-1997) and saved 130,000 tonnes of wheat on the 700,000 ha treated. Initially, control operations were undertaken by centrally managed teams, but the programme developed to the stage where farmers were trained, equipped and supplied with insecticide to undertake control on their own land. In order to make optimum use of limited resources, control operations were closely linked to the biology and behaviour of the insect. The use of economic thresholds and correct use of equipment and products were designed to reduce adverse environmental impact of large scale pesticide application.

INTRODUCTION

Eurygaster integriceps (Hemiptera : Scutelleridae) is one of a group of insects known as sunne pests. It infests large areas of central Europe and the Middle East, including Northern Iraq. The insects exhibit migratory behaviour, the adults moving from the winter hibernation sites to cereal fields (the invasion area), (Brown, 1962) in spring. After a pre-oviposition period in which they feed on the stems of wheat causing a complete loss of the seed head (known as "white ear"), the females lay up to 200 eggs in batches of 13-14 (Wand, 1995). The nymphs develop through five instars, with the later instars (from the 3rd onwards) feeding on the developing seeds. During feeding, the nymphs inject an enzyme into the seed. Damaged seed, even at levels as low as 2 %, results in wheat flour which is unsuitable for making bread (Lorenz & Meredith, 1988). The insects, now adults, then undertake a northwards migration to the summer aestivation sites. During this migration, the adults feed on wheat which, due to climatic conditions, is still in a doughy state. *E. integriceps* attains pest status only periodically. The latest major outbreak in Iraq began in the late 1980s, after a period of remission since the 1960s. Prior to the 1991 Gulf conflict, control of this pest in Northern Iraq was undertaken as a government task, with areas sprayed by aircraft made free of charge to farmers. The aftermath of the Gulf War and the subsequent establishment of the "safe havens" in Northern Iraq, resulted in the removal of central government staff (Koshy, 1996), this being compounded by the imposition of the United Nations declared "no-fly" zone north of the 36th parallel. These factors made aerial application of insecticides impossible. The problems were further

exacerbated by the effective imposition of a blockade to the region, from both the UN and from the rest of government controlled Iraq (Koshy, 1996). The international aid community, recognising the potential importance of this pest in restricting production of cereals in the region and thus jeopardising food security, established a programme designed to protect the wheat crop from *E. integriceps.*

CONTROL STRATEGY

The control strategy was dictated by the fact that in a ground control operation, as opposed to an aerial spraying operation, the flexibility of response was more restricted. In order to achieve adequate control of *E. integriceps* over the large areas and different terrain and cropping systems of N. Iraq, the control operation was divided into three phases. The phases were closely linked to the life cycle and behaviour of the insect, although in practice they overlapped to some extent. The degree, location and timing of the infestation was monitored using a region-wide insect survey, with the data collected from the survey teams fed back to operational centres where decisions were made as to when to initiate and terminate operations in specific locations.

The first phase of the control operation in spring was directed against the adult insects which had migrated from the primary overwintering sites on the lower slopes of the Zagros mountains and other suitable locations. Decisions for control at this stage of the pest life cycle are based on preventing harvest loss, the economic threshold being 2 adults per m^2; no account was taken at this stage of potential nymphal infestation. This phase of control was largely undertaken in the low hill areas close to the overwintering sites, which are usually the sites of high adult infestation in spring. The farming system in these areas consists of small scattered fields sandwiched within pasture areas; water for spraying is available, although often it is some distance from the cropped fields. However, as the direction and distance of the spring migration is dependant on wind strength and direction, prediction of the sites of initial infestation was difficult.

The second phase was based on control of the nymphs as they developed in the wheat fields. First and second instars are found at the base of the crop and are difficult to control due to inadequate spray penetration into the canopy. Egg parasitism, although found to be low in N. Iraq (Wand, 1995), can play a role in reducing pest infestation. To encourage the survival of these parasites, it is important that the timing of insecticide use is correct. Consequently, control was delayed until third instar nymphs had developed in the field; from this stage on, the nymphs are found on the spikes of wheat, making them an easier target for spray application, especially for low volume and ULV. The economic threshold for control at this stage was set at 5 nymphs per m^2. This phase of the operation was largely undertaken in the plains (areas of major wheat cultivation) where, although individual farm holdings are comparatively small (5-10 ha per farmer), cropping is such that large, contiguous areas of wheat production are often found. Water however, is often in short supply.

The final phase of the control operation was directed at migrating adults as they moved to the summer aestivation areas in June and July. The movement is northwards, taking the new generation of adults into hill and mountain areas. The amount of wheat grown in these areas is low, often in small isolated fields. This results in migrating insects being "funnelled" into

smaller areas, and can result in infestations which are extremely high, 100 adults per m^2 was not unusual. During this phase, no economic thresholds were used, as infestation levels can increase significantly over a short period of time.

In the earlier years of the programme (1993-1995), operations were based on the use of centrally managed teams who were dispatched to areas requiring control operations based on insect survey results. However, control teams are inherently limited in their reach. The highly mobile nature of the insect, together with re-invasion of treated fields from surrounding pasture late in the season, meant that teams were sometimes too late in reaching infested areas. This, together with administrative problems associated with running a large number of control teams and some farmer resistance to this approach, led to the development of the "Farmer Implemented Programme" (FIP), which was initiated on a small scale in 1995, but had extended to over 95 % of the area controlled in 1997. FIP was based on training, equipping and supplying farmers with insecticide, and providing ongoing technical support during control operations. A longer term aim of developing farmer control operations was to provide a degree of self-sustainability in crop protection, so that aid funds could be redirected to other projects within the region.

Environmental considerations

Of paramount importance throughout the programme was the need to minimise the environmental impact of treating large areas of wheat with insecticide. The main considerations were; to minimise operator contamination and consequent poisoning, to avoid contamination of water courses, to avoid drift onto villages, to avoid drift onto non-target areas (particularly pasture areas) and to conserve non-target organisms within and outside the cropping area. These aims were achieved by several means, including chemical choice, correct application rates, appropriate training, protective clothing, correct choice and use of equipment and use of appropriate thresholds.

The main insecticide used throughout the programme was deltamethrin, both as EC and ULV formulations. The advantages of this compound are that it is relatively safe to spray personnel, can be used right up to harvest, and is used in low quantities (typically 10 - 12.5 g a.i. / ha).

A further factor designed to reduce pesticide misuse and consequent environmental impact, was the small charge made to farmers for control operations. Although infestation levels were checked before issuing pesticide or undertaking control operations, the additional requirement of having to pay for control operations was designed to serve as a limitation on pesticide overuse. It could be argued that in an emergency situation, this type of aid should be free at the point of delivery, but the case of pesticide use presents particular challenges. By making a small charge, the farmers themselves were required to make a judgement on whether the benefits accruing from control operations outweighed the financial costs. This also meant that farmers were less likely to overdose or over apply if there was a financial cost. However, setting the charge at the correct level and the administration involved with collecting it, proved the largest non-technical problem. A charge set too low would not have had a significant impact on pesticide use, whereas one set too high would discourage farmers from controlling infestations where control was necessary, and may have also discouraged poorer hill and mountain farmers from undertaking any control operations. In the later years of the programme, especially in 1997, variable pricing was used in an attempt to control pesticide use based on the phases of

infestation. This was partially successful, but presented significant administrative problems. Environmental impact was also reduced by placing emphasis on correct equipment choice, particularly the use of application techniques appropriate to the locality. For example, ULV spraying was not used close to villages or within 50 m of water courses. In these situations, control was effected by use of either hand-held or tractor mounted conventional equipment.

The extent of the control programme, the varying nature of the agricultural production systems and topography, together with environmental implications of pesticide use, meant that the application equipment used was selected to reflect these differences. In addition, timing of control operations was critical, so that high work rate was required. Four distinct types of equipment were selected for the programme. These were; hand-held knapsack sprayers, tractor mounted boom and nozzle sprayers, hand-held low volume machines and vehicle-mounted ULV machines. All of this machinery was imported into the region, together with appropriate spare parts. In addition, local manufacture of the most commonly required spare parts was established. Equipment was distributed to farmers free of charge in FIP areas.

Farmer/control team training

To ensure safe and efficient use of the inputs (both machinery and chemical), and to implement appropriate environmental protection measures, all of those involved in the use of pesticides were trained. The training took various forms, including conventional training courses for spray operators and trainers (lasting up to a week), and training for insect survey teams and administrative staff. In FIP areas, farmers attended a one day course on pesticide use and safety. Also, throughout the duration of control operations, they were supported by trained personnel visiting villages on a regular basis. In addition to these training courses, other training took place at various times throughout the programme, such as a farmer sunne awareness programme (FSAP), run extensively in the early years of the programme, and training directed at mountain farmers to combat infestations during summer migration.

Programme evaluation

At the end of each programme, its effectiveness was evaluated in a number of ways. In order to assess the quantity of wheat saved, sprayed areas were compared to untreated areas. Although this suffers from a number of drawbacks, particularly the difficulty of finding areas which are comparable in all but treatment regime, the estimates are the best that are available for the programme. Further work (not reported here) has directly compared treated and untreated areas, and the results are broadly in line with field results. After each programme, a farmer attitude survey was undertaken to assess their response to the programme. This survey covered aspects such as reasons for not spraying, estimates of harvest yield and losses, problems with control operations etc.. As a consequence of the farmer attitude surveys of 1993 and 1994, the concept of FIP was developed.

RESULTS

Table 1 shows the areas treated for each of the years 1993-1997, together with an estimate (based on field sampling) of the quantity of wheat saved as a result of implementing control operations. A benefit:cost ratio of the programme for each of the years is also shown.

Table 1. Area of wheat sprayed to control *E. integriceps*, 1993-1997 with estimates of wheat saved and benefit:cost ratio.

	1993	1994	1995	1996	1997
Area treated (ha)	50,000	50,000	230,000	240,000	116,000
Wheat saved (t)	10,000	25,000	54,000	25,000	13,400
Benefit:cost	2.4	3.2	3.6	1.9	1.2

The results generally reflect the degree of pest infestation throughout the programme, with the greatest savings of wheat occurring in 1995; although a slightly greater area was treated in 1996, the absolute levels of infestation were lower. In the years 1993-1995, the average saving was about 200 - 250 kg/ha of harvested wheat as a result of spraying. In 1996 and 1997, the average saving per hectare was less than half this at between 100 and 115 kg/ha. The reasons for this can be seen in Table 2, which shows the average levels of infestation and the proportion of field samples with a level of infestation greater than the spraying threshold for nymphs.

Table 2. Extent and size of infestation of *E. integriceps*, 1993 - 1997.

	1993	1994	1995	1996	1997
% area exceeding threshold	27.50	25.90	48.30	37.50	12.50
Average infestation (nymphs/m^2)	4.76	3.50	6.96	4.80	2.01

The data shown is from May of each year, taken from insect survey information for the Governorate of Arbil. The reduced benefit:cost in 1996 and 1997 resulted both from a reduction in infestation when compared to 1995, and from costs associated with training and field monitoring of a large number of farmers during the implementation of FIP. An additional complicating factor in 1997 was the UN agreement with the Government of Iraq to bring in large quantities of food for free distribution under the "oil for food" programme (UN Security Council Resolution 986). This occurred at the time of peak sunne pest infestation and meant that farmers were reluctant to invest additional resources in a crop whose price was undermined by free grain distributions. Many farmers refused, therefore, to control sunne pest as the additional cost and time could not be justified in the light of falling wheat prices.

The attitude of farmers to the control of *E. integriceps* changed significantly over the period of the programme. In the initial years (1993 and 1994), many farmers were reluctant to undertake control operations as they felt that the damage caused by tractors passing through the crop, together with the cost of the control operation, outweighed potential savings. However, continuing educational campaigns and, more importantly, losses suffered by farmers who failed to control where necessary, had an important impact on changing attitudes to the need for control.

CONCLUSIONS

The need to implement a large scale control programme against *E. integriceps* under the extremely unusual political and economic conditions of Northern Iraq during the period 1993-1997, led to the development of a ground based control programme closely targeted to the biology and behaviour of the pest to ensure optimum use of limited resources. Initial control operations in 1993-1995 were based on the use of centrally managed control teams, who were required to control infestations in defined localities based on levels of pest infestation. By 1995, the technical and administrative limitations of a control team approach were beginning to outweigh the advantages. The development of an approach based on farmers undertaking their own control operations was the logical solution. However, this required a large programme to train farmers at village level, to equip farmers with suitable equipment (and the spares backup required), and an efficient pesticide distribution system based on undertaking control at economic thresholds in a timely manner.

Throughout the duration of the programme, over 700,000 ha of wheat were treated for the control of *E. integriceps*, saving nearly 130,000 tons of wheat which would otherwise have been lost from the region. In addition, the training and equipping of farmers to undertake their own control operations means that there is self sustainability in crop protection in Northern Iraq resulting from the programme. Indeed, future inputs to the region (including herbicides as well as insecticides) bought under United Nations Security Council Resolution 986 (oil for humanitarian aid), can be utilised effectively with minimal additional technical support.

ACKNOWLEDGEMENTS

The authors would like to thank the Department for International Development, DFID, (formally ODA) for permission to publish this work.

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Assessing the damage caused by black bean aphid (*Aphis fabae*) on spring beans

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ABSTRACT

A series of four field experiments was undertaken during the period 1993 to 1997 to assess the effect of black bean aphid (*Aphis fabae*) infestations on the yield of spring beans in the UK. Significant infestations (>30 % of stems infested) developed on three experiments, and a low infestation (*c.* 10 % stems infested) on a fourth. Colony size in all experiments was on average <500 aphids stem^{-1}. Single or multiple treatments of pirimicarb applied at a range of growth stages or at different levels of infestation did not result in any significant yield increases. Pirimicarb treatments made at early flowering suppressed virus infection levels in two experiments, but there was no evidence that this resulted in a yield benefit. Implications for the control of *A. fabae* are discussed.

INTRODUCTION

The black bean aphid (*Aphis fabae*) is generally considered to be a potentially serious pest of spring-sown field beans (*Vicia faba*) in the UK. *A. fabae* has a holocyclic life cycle, and overwinters as an egg on spindle trees (*Euonymus europaeus*). Spring migrants developing on spindle fly to bean crops in the early summer. The severity of infestations can vary considerably from year to year (Gould & Graham, 1977), and a forecasting system to predict the level of infestation, principally on spring-sown crops, has been developed, based on the use of overwintering egg counts on spindle trees and catches of alate aphids in the Rothamsted Insect Survey suction trap network (Way *et al.*, 1977, 1981).

Work done in the 1950s and 1960s showed that effective control of moderate *A. fabae* infestations by a single insecticide application made after the main aphid immigration into the crop, but before colonies had started to develop, could result in significant yield benefits. Way *et al.* (1954) found that effective control of moderate aphid populations in small plots (*c.* 10 m x 4 m) gave a yield response of *c.* 265 % (1.3 t ha^{-1}) on crops achieving a treated yield of 2.1 t ha^{-1}. Similarly, further experiments using tractor-mounted equipment found that controlling populations averaging 3550 aphids plant^{-1} increased yield from 0.5 t ha^{-1} to 3.4 t ha^{-1} (Way *et al.*, 1958). However, more recent work (Gould & Graham, 1969) showed that control of aphid populations in the order of 100-3000 plant^{-1} resulted in yield losses of only 0.19-0.75 t ha^{-1}, probably reflecting the fact that the bean varieties grown were taller, set more pods, and hence less prone to aphid damage, than those available in the 1950s.

Spring beans remain a popular break crop in UK agriculture, and variety development has continued apace. Modern varieties tend to be shorter and flower relatively early. This may

alter their susceptibility to damage by *A. fabae*, particularly if infestations develop during the early stages of flowering which may lead to pod abortion, or if smothering colonies develop during pod filling. Current guidelines on economic thresholds for the control of *A. fabae*, based largely on the work of Way & Cammell (1973), suggest that 5 % of plants infested on south-west headlands (possibly rising to an initial infestation of 10 % of plants infested in some areas) indicates a potentially damaging infestation. In view of the change in varieties and crop economics since the 1970s, these guidelines are now likely to be outdated. This paper reports a series of experiments done in the period 1993-97 to assess the damage potential of *A. fabae* on modern spring bean varieties. The primary objective of these was to evaluate the effect on yield of insecticide treatment applied at different timings related to either crop development or to the level of aphid infestation.

MATERIALS & METHODS

Site location

Four experiments were done in commercial spring bean crops over a five year period, three in south Staffordshire and one in Cambridgeshire (Table 1). Other experiments were set up in 1995, 1996 and 1997, but aphid infestations at these additional sites were too low for meaningful assessments to be made.

Table 1. Location and basic husbandry details for experimental sites.

Year	Site	Variety	Sown	Harvest
1993	Codsall, Staffs	Victor	17/02/93	30/09/93
1994	Dunstone, Staffs	Caspar	30/04/94	12/10/94
1995	Wennington, Cambs	Victor	20/03/95	15/8/95
1997	Four Ashes, Staffs	Punch	20/04/97	09/09/97

Experiment design

Experiments in 1993, 1994 and 1997 were of a complete randomised block design, replicated four times; the 1995 experiment, a 5 x 5 latin square design. Plot size was dependent on tramline spacings at individual sites, but was usually 20-24 m x 4 m except in 1995 when 10 m x 2 m plots were used.

Treatments

The insecticide used for all treatments was pirimicarb applied at 140 g a.i. ha^{-1} in 200 to 400 litres of water ha^{-1}. The higher water rates were used for late flowering and post-flowering treatments. Treatment timings in the 1993 and 1994 experiments were as follows:

A - untreated (no aphicide)
B - fortnightly from first aphid invasion to end of flowering.
C - at first flower.
D - at mid-flowering (50 % of buds flowering or flowered).
E - at end of flowering (all flowering finished).
F - at 5 % stems infested with aphids.
G - at 10 % stems infested aphids.
H - at 20 % stems infested with aphids.

In the 1995 and 1997 experiments, only treatments A, B, C, D and E were included in the experiment designs..

Assessments

Aphid counts were made immediately prior to all treatments on untreated (Treatment A) plots and on plots about to receive treatment. Once a treatment had been applied, previously treated plots were assessed on subsequent visits. Twenty stems per plot (i.e. not whole plants unless they had only one stem) were assessed to determine the percentage of stems infested. The level of infestation on each stem was also scored using the following system:

0 = no aphids
1 = 1 to 10 aphids/stem
2 = 10 to 100 aphids/stem
3 = 100 to 500 aphids/stem
4 = 500 to 1000 aphids/stem
5 = 1000 to 10000 aphids/stem
6 = >10000 aphids/stem

Crop growth stages were assessed using the key described by Knott (1990). Total yield of all plots corrected to 85 % d.m. was assessed by taking a single cut through the middle of each plot using a plot combine.

In 1993 and 1995, virus-like symptoms (leaf chlorosis and leaf roll) developed in the experiments. As the severity of the symptoms appeared to be associated with insecticide treatment timing, a visual estimation of the apparent level of virus infection in terms of percentage plants infested was made in each plot post-flowering.

RESULTS

Aphid infestation

Peak aphid infestations on untreated plots occurred during the mid- to end of flowering period on all experiments (GS 204 to 205). Full details of the timing and size of peak infestations are given in Table 2. Significant infestations (>30 % stems infested at the aphid peak) developed in 1993, 1995 and 1997. However, colony size was consistently low in all the experiments, with only isolated plants developing extensive smothering aphid colonies.

Table 2. Timing and size of peak aphid infestations on untreated plots on all experiments.

Year	Peak date	Growth stage	% stems infested	Max. colony size (aphids stem^{-1})
1993	30-Jun	205	30.0	500
1994	25-Jul	205	11.3	500
1995	06-Jul	205	56.0	-
1997	02-Jun	204	35.0	100

Observations on virus infection

In 1993 and 1995, symptoms 0f virus infection (principally leaf rolling, but also some leaf chlorosis) became apparent towards the end of flowering and were very obvious once flowering was complete. Symptoms included chlorosis and rolling of young leaves, chlorotic vein-banding of leaves of all ages, and chlorotic blotches and mottling on leaves of all ages.

The levels of overall virus infection (all symptoms) for both the 1993 and 1995 experiments are given in Table 3. In 1993, there were significant differences between treatments. Lower levels of virus were found in plots receiving a single insecticide treatment at early flowering (Treatment B, C and F) or an early flowering treatment as part of a routine programme (Treatment B). Single applications made towards the end of flowering (e.g. Treatments E and H) had virus infection levels similar to untreated plots (Treatment A). In 1995, the early flowering application (Treatment C) had the lowest level of infection, but this was not significantly different from the untreated infection level.

Table 3. Percentage plants showing virus symptoms post-flowering in 1993 (28 July) and 1995 (21 July). Data were angular transformed for analysis, transformed means presented.

	Year	
Treatment	1993	1995
A (untreated)	17.7	41.0
B (routine)	8.4	35.5
C (first flower)	9.2	33.8
D (mid-flower)	11.2	33.5
E (end flower)	13.6	43.0
F (5% infested)	7.6	-
G (10% infested)	13.2	-
H (20% infested)	14.9	-
SED	1.78	3.90

In 1993, diagnostic tests on plant samples were done, but the presence of virus could not be confirmed due to the advanced state of growth.

Effects of aphid infestation on yield

The results of the yield assessments for all experiments are given in Table 4. Although there was considerable variation in yield potential between the four experimental sites (untreated yield ranged from 3.4 to 7.4 t ha^{-1}), no significant effect of insecticide treatment on yield was recorded in any of the experiments. Significantly lower yields were recorded for Treatment E plots in 1993. However, this was due to poor crop growth and a combine blockage at harvest, and was not related to aphid infestation. There was no evidence that virus infection influenced the yield of any of the plots in 1993 or 1995.

Table 4. Effect of treatment on yield (t ha^{-1} @ 85 % d.m.).

	Year			
Treatment	1993	1994	1995	1997
A (untreated)	7.4	5.2	3.4	5.9
B (routine)	6.9	5.4	3.7	5.8
C (first flower)	7.0	5.3	3.2	5.6
D (mid-flower)	7.1	5.2	3.7	6.1
E (end flower)	5.7	5.2	3.2	5.9
F (5% infested)	7.0	5.2	-	-
G (10% infested)	6.9	5.3	-	-
H (20% infested)	6.9	-	-	-
S.E.D. (p=0.05)	0.70	0.11	0.22	0.41

DISCUSSION

The infestation level of *A. fabae* required to justify control measures as suggested by Way & Cammell (1973) of between 5 and 10 % of plants infested was thought to cause *c.* 4 % yield loss. At current (1998) UK prices (feed beans at £75 t^{-1} and pirimicarb insecticide cost of £12.50 ha^{-1}), a yield benefit of 3.5 % on a crop yielding 5 t ha^{-1} is required simply to cover the insecticide cost, regardless of application costs and wheeling losses. Thus the economics of control at current prices compared to the 1970s are not dissimilar. However, on the basis of the experiments reported here, it is likely that modern spring bean varieties can tolerate higher infestations in terms of percent plants infested than those grown in the 1970s, as infestations of up to 56 % of stems infested did not result in significant yield benefits (Table 2 and Table 4). Nonetheless, the issue of colony size is clearly important - where large, smothering colonies

develop on modern short-strawed varieties, the impact on the yield of affected plants could be significant. Way *et al.* (1958) found a curvi-linear relationship between grain yield and log number of aphids plant^{-1}, and further investigation of this relationship on modern varieties would help to clarify the true current damage potential of *A. fabae*. In the meantime, current advice could be modified to ensure that treatments are only made where smothering aphid colonies appear to be developing.

The impact of virus diseases on spring bean yields could, in theory, be significant as some viruses are known to cause serious yield losses in infected plants, particularly when infection occurs early. The observations made during the 1993 experiment strongly suggested the presence of an aphid-borne virus such as bean yellow mosaic virus (BYMV), transmitted *by A. fabae* and *Acyrthosiphon pisum* (pea aphid) or bean leaf roll virus (BLRV), transmitted *by A. pisum* only. *A. pisum* was present in low numbers in the 1993 experiment. The weevil vectors of other common bean viruses such as broad bean stain virus (BBSV) and broad bean true mosaic virus (BBTMV) would not have been controlled by pirimicarb. However, the incidence of virus in commercial spring bean crops is usually very low, so it is unlikely that aphid control specifically to reduce virus infection would be required in the vast majority of crops.

ACKNOWLEDGEMENTS

This work was funded by the PGRO Pulse Levy.

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The economic impact and evaluation of control strategies for the reduced-rate use of aphicides against winter wheat aphids in the UK

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ABSTRACT

A cyclical variation in levels of grain aphids has been observed that may assist strategic decision making from season to season. Outbreak years have been shown to be followed by troughs of low incidence, with high natural enemy levels, causing a gap of several years before the next major occurrence. Experiments have identified where doses of cereal aphicides may be reduced to one third of the recommended label rate where action is taken as aphid numbers reach threshold levels and a moderate level of natural enemy activity is observed. Economic responses to reduced rates of application from 33 experiments over seven seasons have been similar at all but one site.

INTRODUCTION

Numbers of the grain aphid (*Sitobion avenae*) and the rose-grain aphid (*Metopolophium dirhodum*) occurring in summer in English wheat crops have been monitored by an annual survey of 60 fields since 1976. Initially, assessements were only made at flowering, but from 1981 the fields were assessed at three growth stages; the end of ear emergence (GS 59), flowering (GS 61) and watery ripe (GS 71). Four peaks of infestation have occurred during the course of the survey, each followed by a period of reduced incidence before numbers started to increase again towards a new peak.

Prevailing temperatures from May to July accounted for none of the observed variation in incidence. Surprisingly, aphid outbreaks were not consistently associated with warm summers. Numbers of aphids found at GS 59 accounted for 18 % (P = 0.090) and numbers at GS 61 for 37 % (P = 0.010) of the variation recorded at GS 71; these relationships were not improved by taking account of prevailing temperature.

Experiments on the development and control of naturally occurring aphid populations have been conducted throughout the period. The results from 61 closely monitored field

experiments conducted between 1987 and 1996 have been utilised to cost the damage caused by the fluctuating national aphid population and to identify possible causes of this fluctuation for further investigation. The levels of aphid incidence found on untreated control plots in these experiments closely follow the national trend in years of low to moderate incidence, but exceed it at GS 71 in years of higher incidence when aphicide application to more heavily infested fields decreases national population levels.

MATERIALS AND METHODS

The survey was conducted on a random selection of farms stratified on a regional basis to reflect the proportion of the national wheat crop grown in that region. Each field was visited on three occasions; at GS 59, GS 61 and GS 71. Where aphicides were applied in response to high aphid numbers, the field was replaced by another, untreated field, where available. On each occasion 100 tillers were examined, taking five tillers at random from each of 20 points on a diagonal across the field, and recording the numbers of aphids found.

The protocols for the grain aphid experiments altered over time (Oakley & Walters, 1994; Oakley *et al.*, 1996) but a core element was maintained throughout the series. Untreated control plots were monitored for aphids and their natural enemies at a range of growth stages including GS 59, GS 61 and GS 71. Treatments with pirimicarb at 0.14 kg a.i./ha at GS 61 was included in all studies. Dimethoate was also applied at 0.34 kg a.i./ha at GS 61 up to 1989, after which it was replaced with alpha-cypermethrin at 15 g a.i./ha to reflect the changing pattern in aphicide usage. All studies were taken to yield.

RESULTS

Figure 1 shows the results of the survey since 1981 in terms of the mean numbers of aphids found per head each year at the three growth stages.

On analysis of the yield responses in the aphid control experiments compared to the measured aphid levels, a highly significant regression ($p < 0.001$; $R^2 = 87.5$ %) was found between the mean aphid numbers recorded on untreated control plots at GS 71 each year and the mean response to aphicides applied at GS 61. The regression equation obtained was:

$$\text{yield response} = -0.0009 + 0.0338\ (\pm 0.0045)\ \text{t/ha}$$

for each aphid found per tiller at GS 71. This relationship was used to cost the potential loss from summer aphid infestations as indicated by aphid infestations at GS 61 on the experimental sites as compared to the national loss indicated by the survey (Table 1). The difference between the two figures gives an estimate of the return from the aphicide treatments applied to crops each year.

The annual wheat area in England fluctuated between 1.63 and 1.93 million ha during the period. If a mean area of 1.8 million ha and crop value of £100 /tonne is assumed, the

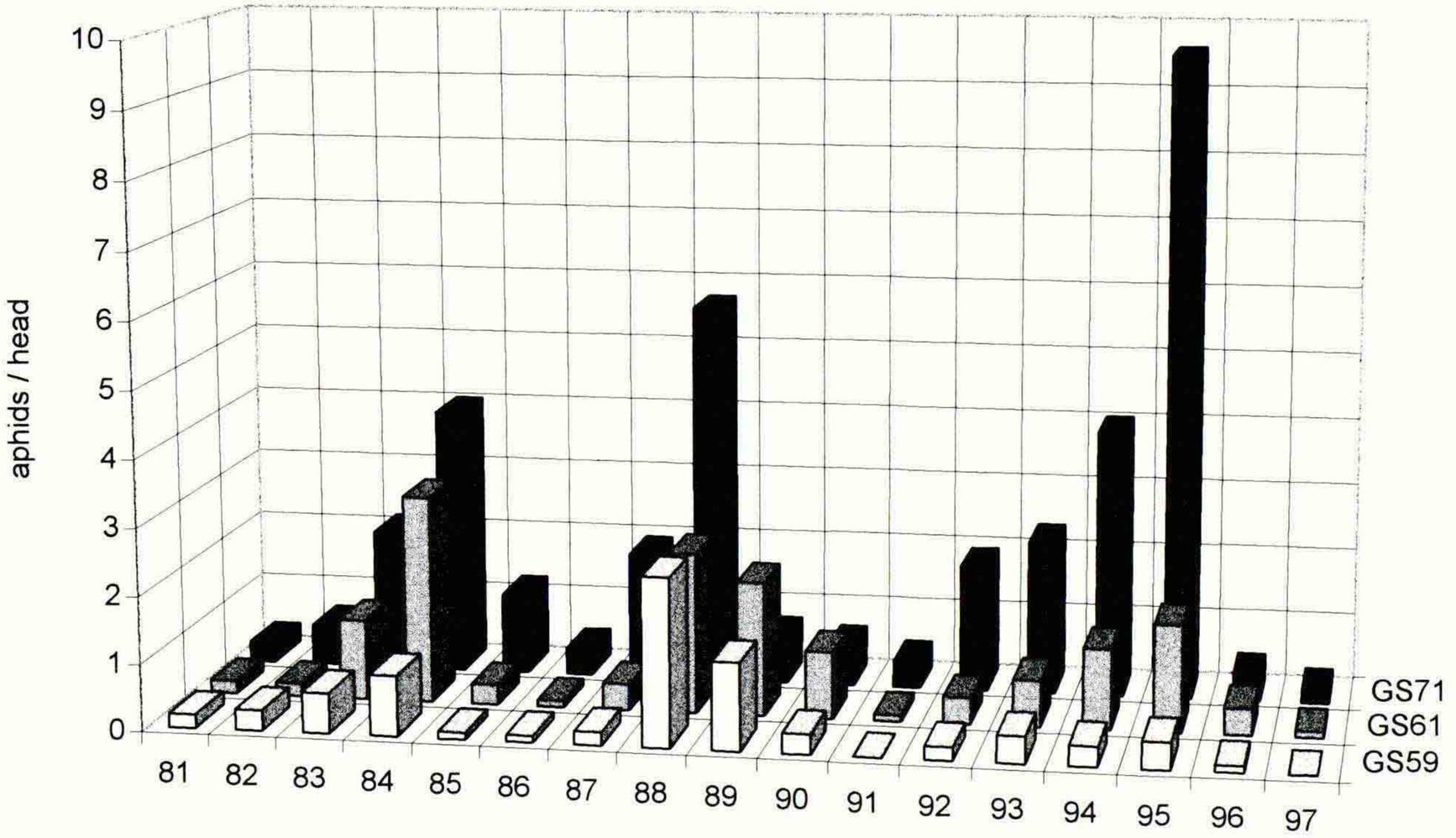

Figure 1. Annual aphid incidence at three growth stages in UK winter wheat crops, 1998–1997.

annual mean potential recoverable yield loss due to summer aphid attack in wheat can be estimated at £39 million (range £5 - 191 million) and the actual recoverable yield loss left after treatments are applied, at £17 million (range £2 - 58 million). The cost of aphicide application varies according to the treatment selected and whether applied alone or in tank mix but averaged £10/ha during the period. This gives a treatment cost equivalent to 0.10 t/ha below which potential yield losses can not be economically pursued.

Table 1. Estimated potential and actual preventable yield losses from summer aphid attack to winter wheat in England, 1987 to 1996.

Year	Potential yield loss (t/ha)	Actual yield loss (t/ha)
1987	0.06	0.06
1988	0.22	0.19
1989	0.03	0.02
1990	0.04	0.02
1991	0.10	0.01
1992	0.36	0.06
1993	0.05	0.08
1994	0.17	0.14
1995	1.08	0.32
1996	0.03	0.01
Mean	0.21	0.09

Years of high aphid incidence were characterised by lower than average incidence of natural enemies, but the relative importance of the natural enemies associated with years of lower incidence varied from year to year. The years following a peak in infestation were generally characterised by high numbers of coccinellids and syrphids emerging from hibernation at a relatively early stage in the spring colonisation phase of aphids. The relatively high predation rates were thought to be responsible for the decline in aphid populations between GS 61 and GS 71 observed in both 1989 and 1996. In 1989 the combined effect of the natural enemies resulted in a natural control of overwintered aphid infestations, giving a worthwhile yield response to treatments applied at the boots swollen growth stage (GS 45) at three of six sites, so that no yield responses were obtained to sprays applied at GS 61 (Oakley & Walters, 1994). In 1996 no yield responses were obtained at four of five sites. At the fifth, where a small yield response to pirimicarb was obtained, a spray of alpha-cypermethrin gave a significant reduction in numbers of natural enemies and caused aphid numbers to resurge above the levels on untreated control plots (Oakley, 1997).

Reduced rate aphicides were compared to full dose rates in the experiments conducted from 1990 (Oakley *et al.*, 1996) of which 33 sites were completed between 1990 and 1996. At only one site (Boxworth 1995) were aphid numbers above the 66 % of tillers

infested action threshold at GS 61 when treatments were applied, but at a further 16 of the 33 sites, economic yield responses were obtained to some of the treatments. The responsive sites were characterised by aphid populations on untreated control plots continuing to rise between GS 61 and GS 71 and the unresponsive sites by aphid numbers remaining static or falling between these growth stages. The proportion of responsive to unresponsive sites varied in line with the overall survey results for the year. A summary of the yield responses and profitability of treatment according to the responsiveness of the site (Table 2), highlights the need to predict the outcome of aphid population development in deciding the need for treatment.

Table 2. The yield responses obtained and profitability of treatment of 30 reduced rate aphicide experiment conducted between 1990 and 1996 grouped according to the responsiveness of the site.

	Treatment			
	pirimicarb		alpha-cypermethrin	
	full	reduced	full	reduced
All 17 responsive sites				
Yield response t/ha	0.61	0.46	0.48	0.40
Profit £ /ha	50.24	42.13	44.46	38.39
16 responsive sites below threshold at application				
Yield response t/ha	0.39	0.32	0.41	0.31
Profit £ /ha	27.93	28.81	36.74	29.56
16 unresponsive sites				
Yield response t/ha	-0.14	-0.09	-0.09	-0.09
Profit £ /ha	-24.50	-13.00	-12.95	-10.57

The results have been shown with and without the Boxworth 1995 data included as this was a very exceptional site, with yield responses of up to 4 t/ha, and, was the only one with aphid numbers above the conventional action threshold at the time of treatment.

DISCUSSION

The yield loss estimates based on the survey results would suggest that in crude terms the strategy adopted in the UK for summer aphid control management is about right with a potential mean yield loss of 0.21 tonnes/ha at the economic response aphid threshold, across a ten year period. However, an examination of the responses from the experiments suggests that much further improvement is possible if fields below threshold at GS 61, but where numbers will subsequently rise above economic levels, can be identified in time for a reduced rate of aphicide to be applied at GS 61. The results confirm that providing that the odd field where exceptionally high aphid infestations may develop can be identified, reduced rates of application as suggested by Poehling (1987) may be used widely. Seasonal influences have been shown to be important in aphid population development and not linked to subsequent temperatures, so that a seasonal forecast of potential for aphid build-up could be developed if the underlying factors influencing aphid population dynamics were better understood. Detailed modelling work of aphid population dynamics to identify parameters to necessary for the decision making process, is underway at CSL.

The MAFF Pesticide Usage Survey of arable crops (Thomas *et al.*, 1998) takes place in alternate years. Ignoring 1994 when an outbreak of wheat blossom midge (*Sitodiplosis mosellana*), distorted pesticide usage, treatment cost from 1988 to 1996 varied between £1.9 million and £6.1 million per annum at 1996 prices, giving a mean annual cost of £3.4 million. This would suggest that the pesticide usage is generally profitable, yielding an annual return of around £18 million. The variations in usage from year-to-year do not fully reflect the aphid incidence pattern, again suggesting that improvements in the decision making strategy adopted are possible.

ACKNOWLEDGEMENTS

This work was funded by the Ministry of Agriculture, Fisheries and Food.

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The within-field spatial and temporal distribution of the grain aphid (*Sitobion avenae*) in winter wheat

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ABSTRACT

The within-field spatial distribution of the grain aphid (*Sitobion avenae*) was investigated by intensively sampling a field of winter wheat during the spring and summer of 1996 using a 9 x 7 sampling grid comprising 63 locations at 30 m intervals. The data were described using Taylor's power law and by SADIE statistics in order to identify spatial pattern. Analysis demonstrated generally weak spatial association and some edge effects, although there was a significant within-field dependence of yield on aphid population parameters. The implications of these findings in relation to the development of pest management strategies are discussed.

INTRODUCTION

The infestation of cereals by aphids and the subsequent effects on yield, have been studied in detail by many workers (Dixon, 1987). Aphid populations are often assessed by counts which are randomly located within the study area, which allows the description of mean-variance relationships, but investigations which describe and map within-field distributions are limited. Recent advances in statistical methodology utilising Spatial Analysis by Distance IndiciEs (SADIE) allow the description of two-dimensional spatially referenced data sets (Perry *et al.*, 1996), which provides an opportunity for a detailed analysis of within-field aphid distributions. The way in which aphid populations develop within a field and, in particular, the persistence of discrete sub-populations could influence the development of, for example, precision farming approaches to aphid pest control, the optimisation of scouting crops for aphid infestations and the enhancement of biological control strategies. This paper describes a study which investigated the spatial distribution of the grain aphid (*Sitobion avenae*) within a field of winter wheat during the spring and summer of 1996. The effect of aphids on within-field variations in yield, and the effect of N availability on aphid population dynamics are also reported. The findings from this study are discussed in relation to their relevance to the development of pest management strategies.

MATERIALS AND METHODS

A field of winter wheat (250 m by 180 m, near Wimborne, Dorset, UK) was sampled intensively on six occasions during 1996 between 7 June and 12 July. A grid comprising 63 sampling locations in a 9 x 7 rectangular grid at intervals of 30 m was positioned within the crop. At each location, sampling was done to record aphid numbers, yield per ear and grain N. These data are part of a larger study which included sampling at three spatial scales and recording other parameters including natural enemy numbers and weed cover at each spatial location (Holland, Winder and Perry; unpublished).

Aphid numbers and species were recorded at each location by the inspection of five tillers. The five tillers were marked with black tape and each week counts were done on these tillers on six occasions. Once the crop had reached maturity, yield was measured at each sampling location by harvesting a $0.1m^2$ area of the crop. The number of ears within each $0.1m^2$ quadrat was recorded and the sample was then threshed, the grains weighed and moisture content determined. The grain was analysed for % N by standard Kjeldahl extraction as an assay to determine nitrogen availability to ear feeding aphids (S Duffield, pers. comm.).

Using these data, average aphid densities, yield (corrected for moisture content) and % N values were calculated for each of the 63 locations. Regression analysis using SPSS for Windows determined relationships between aphid days (Ruppel, 1983), peak aphid density, yield and % N. The data were represented visually by surface mapping using Surfer for Windows.

RESULTS

All results refer to *S. avenae* which was the only species present within the crop in significant numbers. Initially, whole field populations were 0.18 aphids shoot^{-1} and they reached a maximum of 3.82 aphids shoot^{-1}, although there were considerable within-field differences in populations (Table 1, Figure 1). The parameters b and $\log_{10}a$ of Taylor's power law were estimated as 1.5 and 0.68 respectively, which are typical values for insect populations and indicate variance heterogeneity (Taylor *et al.*, 1978).

Table 1. SADIE index of aggregation (I_a). Values of I_a exceeding 1 indicate spatial aggregation. Mean aphid density (m, aphids shoot^{-1}), variance (s^2), and minimum and maximum recorded densities (aphids shoot^{-1}) calculated for whole field. Values for each date calculated from 63 counts from 9 x 7 sampling grid.

	I_a	m	s^2	min	max
7 June	1.00	0.18	0.31	0	4.0
14 June	1.04	0.22	0.22	0	3.0
21 June	1.14	0.58	0.53	0	2.4
28 June	1.31	1.76	11.5	0	20.4
5 July	0.89	1.08	4.1	0	10.4
12 July	1.00	3.82	22.4	0	23.4

7 June

0.6	0.0	0.0	0.0	0.0	0.2	0.0
0.2	0.2	0.0	0.0	0.0	0.0	0.2
4.0	0.0	1.2	0.0	0.0	0.2	0.0
0.0	0.0	0.0	0.0	0.2	0.0	0.0
0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	1.0	0.2	1.0
0.0	0.0	0.4	0.0	0.0	0.2	0.0
0.0	0.0	1.0	0.0	0.0	0.2	0.0

14 June

0.4	0.0	0.4	0.0	0.0	0.4	0.0
0.6	0.2	0.2	0.6	0.0	0.0	0.0
0.0	1.2	0.0	0.4	0.0	0.0	0.0
0.0	0.8	0.0	0.0	0.0	0.0	0.2
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.4	0.0	0.0	0.2	0.4	0.0	0.0
0.6	0.0	0.0	0.0	0.0	0.6	0.0
0.0	1.6	0.0	0.2	3.0	0.2	0.0
0.2	0.2	0.2	0.8	0.0	0.0	0.0

21 June

0.0	0.0	2.4	1.4	0.4	0.0	0.0
0.4	0.0	1.6	0.6	0.0	0.0	0.4
0.0	2.4	1.6	0.4	1.2	1.0	0.4
0.2	0.2	0.0	1.0	1.0	2.0	0.6
0.0	0.4	0.0	0.2	0.0	0.6	1.2
0.0	0.0	1.0	1.0	2.0	0.2	0.2
0.4	0.0	0.0	0.0	1.0	0.0	2.0
0.2	0.0	0.0	0.0	0.2	0.0	2.4
0.0	0.4	0.6	2.2	0.0	1.0	0.0

28 June

0.8	0.2	3.6	0.4	0.0	4.6	0.0
1.2	1.6	1.8	0.6	0.0	14.2	0.2
1.2	0.6	1.0	0.0	3.2	4.2	1.2
4.0	0.4	0.0	0.0	0.0	0.0	20.4
0.6	0.0	0.8	0.0	1.6	5.4	2.8
1.8	0.2	0.6	1.2	0.0	0.2	1.8
0.4	0.2	0.0	1.6	0.0	10.4	3.2
1.0	0.0	3.2	0.0	0.4	0.0	1.2
0.0	0.0	0.6	1.4	1.8	0.2	2.8

5 July

4.4	2.2	0.0	0.0	0.0	1.4	0.0
0.0	0.0	0.2	0.2	0.0	0.0	0.6
7.4	0.2	0.2	0.4	0.0	0.0	0.4
3.6	0.2	0.0	0.4	2.2	7.0	0.2
0.4	1.0	0.0	0.0	2.2	0.2	4.2
0.6	0.6	0.0	0.0	0.0	0.0	0.2
0.4	1.8	0.0	0.0	0.0	5.2	0.6
10.4	0.4	0.2	0.2	1.4	0.2	3.2
0.0	0.0	0.2	0.0	1.8	1.4	0.0

12 July

5.8	12.0	0.8	0.6	0.6	4.8	8.2
0.8	2.6	1.2	0.4	0.4	0.6	9.6
7.6	4.2	3.0	7.2	2.0	3.6	6.6
14.4	1.2	1.2	0.2	0.4	2.4	1.2
1.0	7.2	0.4	0.0	0.4	15.2	9.0
7.8	6.4	1.2	0.0	0.4	4.2	21.4
8.4	1.0	1.6	0.4	1.4	16.4	1.0
9.0	2.2	0.0	1.8	0.2	1.4	1.4
0.0	0.0	3.8	4.4	2.2	1.4	2.2

Figure 1. Spatial representation of aphid counts recorded on 6 occasions during 1996. Each value represents the mean aphid density (aphids shoot^{-1}) recorded at that location. Values circled are those which are higher than the mean for the field.

At the large scale, index values indicated that, apart from marked edge effects, spatial pattern was absent on the first, fifth and sixth sampling dates. On occasions two, three and four there was evidence of increasing aggregation; values of the spatial index I_a were increasing and larger than unity. However, even though some spatial pattern was present over occasions two to four, there appeared to be little consistency apart from the tendency for higher aphid densities to be observed at the field edges (Figure 1). The centre of the field had relatively smaller counts throughout most of the season. When the aphid data were represented as total aphid days, the within-field distribution over the whole period was clearly evident, with relatively large aphid numbers being evident along one field margin, and relatively small populations in the centre of the field (Figure 3).

The aphid populations that developed within the field had a measurable effect on grain yield. A significant negative relationship between aphid days and yield ear^{-1} was observed (Figure 2), indicating that observed within-field differences in aphid populations were sufficiently large to affect yield. A significant negative relationship between yield ear^{-1} (Y), and peak aphid density shoot^{-1} (P), was also observed ($Y = 1.3 - 0.2\log_{10}P$, $r = 0.29$, $P<0.05$). Mapping of yield shows some correspondence with the associated map of aphid days, with generally higher yields within the centre of the field where aphid numbers were fewest (Figure 3). No relationship between aphid populations, expressed either as aphid days or as peak aphid density, and grain nitrogen was detected, and the map of % N indicates no correlation with aphid days, even though there was within-field variation in % N (Figure 3).

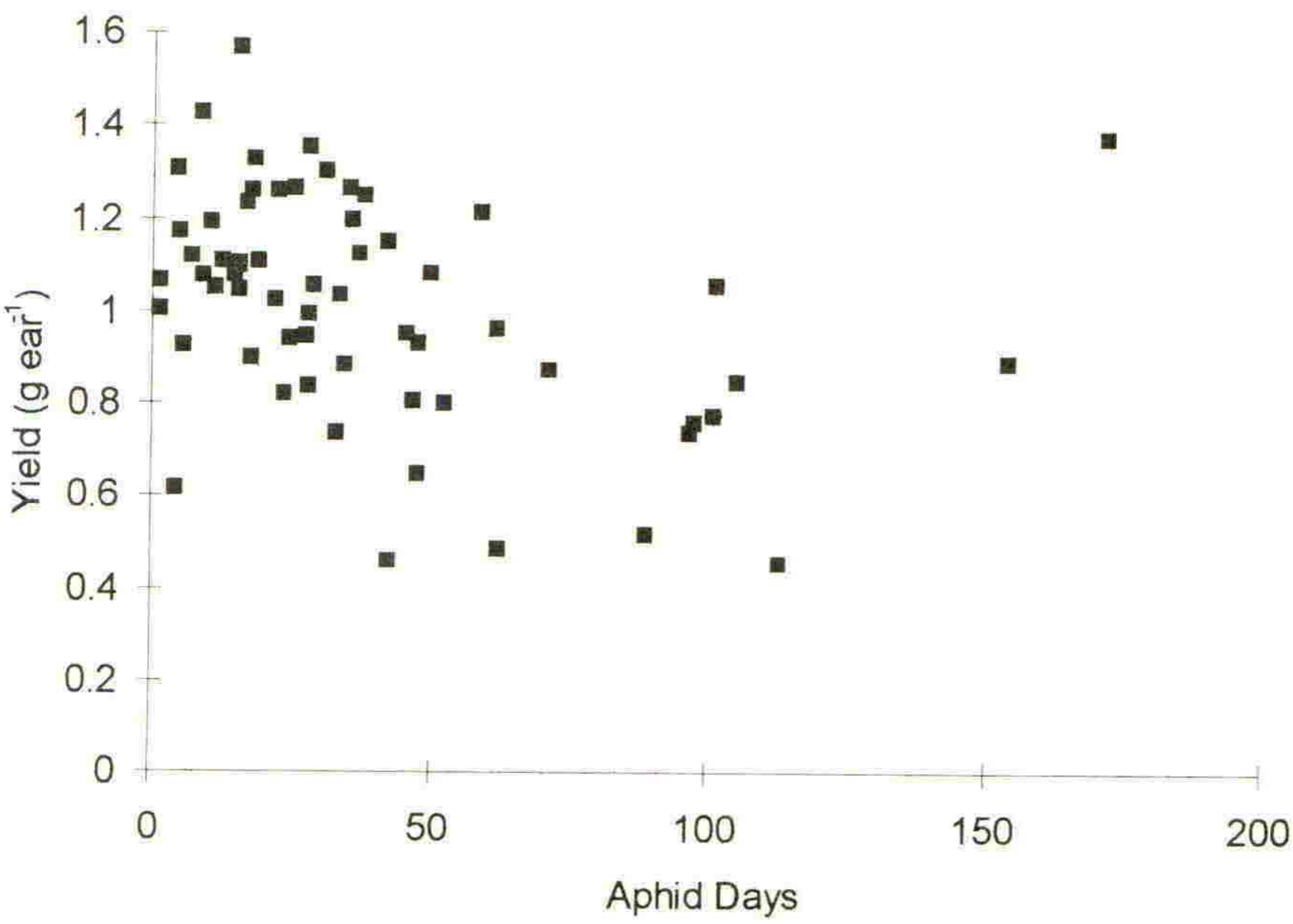

Figure 2. Within-field regression relationship between aphid days (A) calculated using the method of Ruppel (1983) and yield (Y, g ear^{-1}). The regression relationship describing these data is $Y = 1.31 - 0.2\log_{10}A$, $r = 0.34$, $P<0.01$.

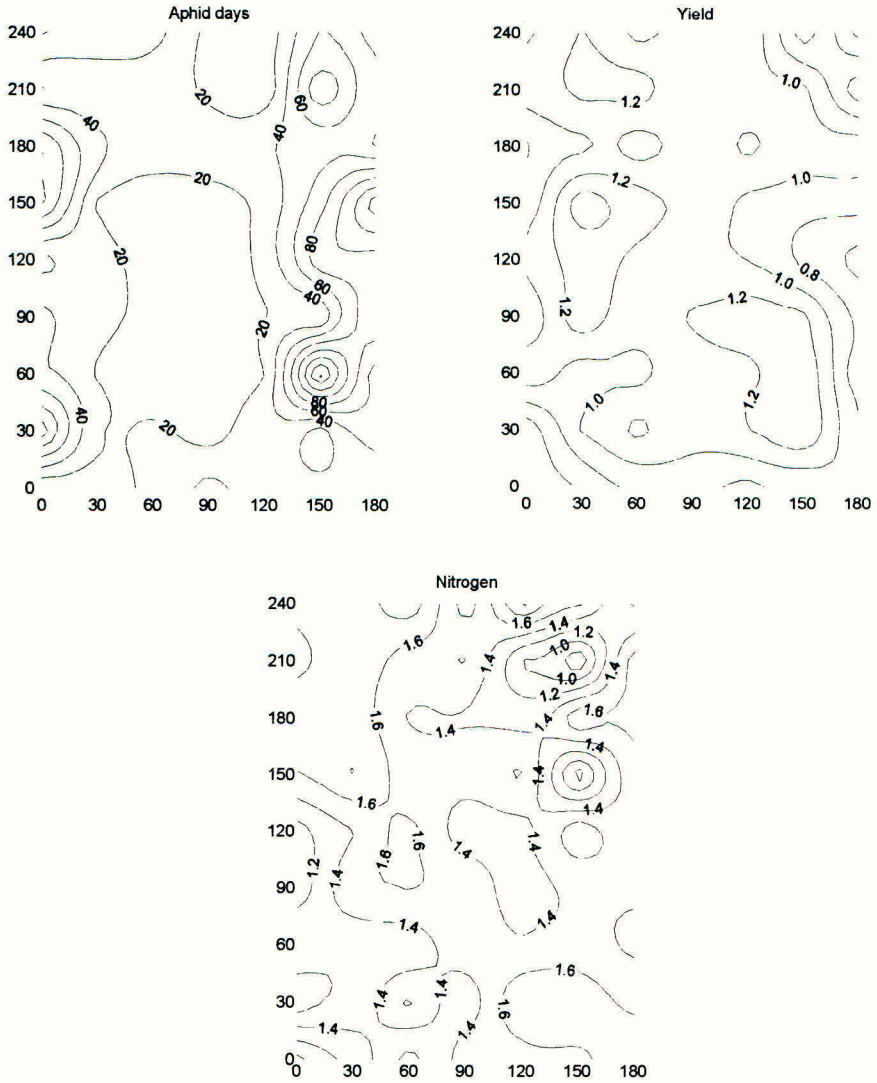

Figure 3. Maps representing within-field distributions of aphid days (Ruppel, 1983), yield (g ear^{-1}) and % N in grain. The x and y axes on each map are in metres.

DISCUSSION

This study demonstrated sufficient variability of within-field aphid populations to result in yield reduction. However, characterisation of the within-field distribution was difficult because there was little consistency in spatial pattern between weeks. Aggregation was largely ephemeral, probably due to the high aphid mobility. Sunderland *et al.*, (1986) have shown that up to 90 % of the aphid population shoot^{-1} fall to the ground each day, although their subsequent dispersal is poorly understood. There was evidence of higher densities towards the edge of the area sampled, extending from 30 m to 60 m into the field; the effect was most evident when aphid data were measured in units of aphid days, due to its integrating effect. Edge effects have been attributed to the influence of windbreaks on the distribution of aphids (Lewis, 1967) or the shelter given to aphids once they have entered the crop (Taylor, 1962). They may be characteristic of a specific field, given its topography, although long-term studies would be needed to confirm this. Additionally, knowledge of the consistency and magnitude of edge effects could improve the precision of pest incidence counts used in decision making during integrated crop management and lead to the selective spraying of 'at risk' areas. Nitrogen has been shown to increase the development of cereal aphid populations in the laboratory (Dixon, 1987) although in this study and in others (Zhou & Carter, 1991) no consistent effect has been demonstrated for *S. avenae* in the field. This indicates that other factors have a much stronger influence on grain aphid population dynamics.

ACKNOWLEDGEMENTS

The authors thank Mr A Sanders of Crichel Farms LTD for generously providing the field site and the many staff and students of The Game Conservancy Trust and Bournemouth University who helped with field and laboratory work. IACR-Rothamsted receives grant aided support from BBSRC.

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Spatial modelling of slug populations in arable crops

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ABSTRACT

Slug behaviour and population dynamics may be predicted by weather, soil characteristics, crop and weed distribution. Spatially explicit simulation models have been developed to examine slug population dynamics in arable fields. The model, using life history parameters for *Deroceras reticulatum*, predicts that juvenile slugs should be found in "hotspots". The mature slug distribution, however, is more scattered. The hotspots of juvenile slugs produced by the model appear to be borne out by an intensive study of the distribution of *D. reticulatum* and *Arion intermedius* in a uniform field of winter wheat in 1997-98, using a spatially structured sampling grid. A scattered distribution of mature individuals of *D. reticulatum* was also found in this study, but mature *A. intermedius* were shown to be distributed in a larger hotspot, of approximately 40 m diameter, than was predicted by the models.

The impact of slug populations in hotspots on crop growth can be measured in terms of leaf area index and biomass. The model can predict crop damage across a range of scenarios, from the worst case (i.e. within a hotspot) to the best. The model predicts crop damage for whole fields, with just a simple set of input parameters. Thus, using simple field data, the model will be of use in producing forecast advice for farmers about the risk of slug damage and the best control strategy to use.

INTRODUCTION

Slugs are one of the most serious pests to growers of arable crops. Molluscicides used in arable crops (mainly winter wheat and oilseed rape) cost around £10 million per year (Garthwaite & Thomas, 1996). This figure does not include application costs. The cost of slug damage in wheat was estimated by ADAS to be 0.22 % of the value of the crop (Port & Port, 1986), and with an estimated area of over two million hectares of wheat grown in the UK (Davis *et al.*, 1993), this costs over £2.7 million per year (7 tonnes/ha at £85/tonne). In surveys in the 1980s, slugs were perceived by farmers and consultants to be the most troublesome pests of wheat crops (Glen, 1989). This may be due in part because of the difficulty in predicting damage and the need for control measures (Glen *et al.*, 1993). Most of the damage is caused by *Deroceras reticulatum* and *Arion* spp. (Glen, 1989; Glen *et al.*, 1992).

There is evidence that the distribution of slug populations in arable fields can be patchy (Hunter, 1966; Airey 1984), and this is believed to be due mainly to the deposition of eggs in masses, the strict moisture requirements of slugs, their low dispersal potential and patch loyalty behaviour. However, more powerful statistical techniques have recently become available for analysis of spatial distribution patterns (Perry, 1995; Korie *et al.*, in press). Bohan *et*

al. (1997), used these new techniques to investigate the spatial distribution patterns of two pest species, *A. intermedius* and *D. reticulatum*, in a uniform arable field at IACR-Long Ashton, in March 1997. These species were found to have markedly different spatial distributions, suggesting a complexity of spatial organisation in slugs that has not previously been reported and that there are differences between the spatial organisation of *D. reticulatum* and *A. intermedius* populations.

Current practice for control of slug damage is normally to broadcast molluscicide pellets over the whole field. However, if hotspots of slug abundance can be identified, molluscicide costs can be reduced by treating only these patches. Predicting the location of these patches will be difficult due to the lack of correlation between environmental variables and slug hotspots (Airey, 1986). However, it is likely both that the grower will be aware of local slug hotspots on a field scale and that appropriate sampling could identify treatable patches. The simulation model is important because it may add to this information by predicting the long-term magnitude of hotspots, thus allowing risk assessments of slug damage to be made for effective slug control.

This study has taken two approaches. The first is the development of a simulation model in order to make long-term predictions and to interpret field data to make contemporary assessments of slug distributions both within hotspots and outside them. The second is a detailed assessment of spatial patterns of slug distribution over a full year in an arable field at Long Ashton. Additional field data are being collected from other sites to validate the simulation model.

MATERIALS AND METHODS

The model developed is an individual-based model, simulating the behaviour and life-history of each slug in the population. It is also spatially explicit, currently modelling an area 40 m x 40 m. Each slug in the modelled space moved or remained stationary on a daily basis, remaining in an area if it was considered "suitable", according to a loose set of environmental qualities such as soil moisture and presence / absence of food and shelter.

Life history changes were made on a weekly basis using a probabilistic approach, each individual having a particular chance that it will grow or reproduce (based on age) and die (based on a measure of environmental stress). The life history parameters used were for *D. reticulatum* (where available). One restriction of simulation models of slug populations is the paucity of basic life history information in the literature. Nevertheless, data for growth were available in South (1982) and Prior (1983), experiments on slug fecundity had been performed by South (1982), and survivorship information was derived from Pearl and Miner (1935).

A sub-model, simulating weather conditions for the Bristol region gave the model its seasonality. This stochastic model generated values on a daily basis for temperature, precipitation, solar radiation and wind. These values in turn, were used to calculate soil moisture. The output of this environmental model was used to assess suitability of conditions for slug activity. Thresholds for temperature and soil moisture were established according to Young *et al.*, (1991) - if these thresholds were not met on any particular day, then slugs would not be active, and thus would not grow, reproduce or move.

A sub-model, simulating crop growth was also incorporated. This was important for the calculation of soil moisture (through evapotranspiration), but it also produced shelter and food

for the slug population. Ultimately, the potential for crop damage will be incorporated into this sub-model.

The whole model can be run with any starting population for any number of weeks. For the simulation runs in this study, the model was run for one year to produce an initial slug population. It was then run for thirteen weeks to get a population for mid-spring, then for a further three six-month periods (twenty-six weeks), giving populations for mid-autumn, and then spring and autumn for a second year.

The field site chosen was a flat, homogeneous field of winter wheat sown in autumn 1996 following non-inversion tillage to incorporate residues of the previous crop of oilseed rape (Field 54, Island Orchard, Long Ashton Research Station). Spatial sampling was done on six occasions from March 1997 to March 1998. For efficiency, the sampling was conducted using a geometric series of 4 spatial scales, at 25 cm, 1 m, 4 m and 16 m. These spatial scales were nested within each other producing a regular series of four sampling grids (Figure 1, Bohan *et al.*, 1997) across the field. On each sampling occasion, the precise location of each sampling grid within the larger grid, was determined by random numbers from a uniform distribution. The sampling for slugs was conducted using a soil-flooding technique, as described by Glen *et al.*, (1992) modified from South (1964) and Hunter (1968). 25 cm x 25 cm samples of soil were removed to a depth of 10 cm, from each sampling point. The individual soil samples were then transported to the laboratory where they were slowly flooded over the course of nine days. The flooding samples were checked every day for slugs, which were identified and weighed. The number and fresh weights of slugs, per sample, was then analysed using Genstat and the Spatial Analyses by Distribution IndicEs (SADIE) algorithm to detect spatial patterns (Perry, 1995) and the SADIEA algorithm to detect associations and changes in spatial patterns (Korie *et al.*, in press).

RESULTS

Population model

The slug populations that resulted from the simulation model have been divided into three groups - eggs, juveniles and adults. For this study, juveniles are defined as individuals which have not yet reproduced, and physiologically have a faster growth rate than the adult group (Abeloos, 1944; South, 1989).

The patterns of population sizes seem to show overlapping generations (Figure 1). The first generation has a peak of juveniles in autumn from eggs laid in mid-spring, which overwinter as immature adults, laying eggs again by spring. The second generation arises from eggs laid in about November, which overwinter, (and are somewhat resistant to cold temperatures, see Carrick, 1942), hatch into a small population of juveniles in late spring, which then mature into egg laying adults by the end of autumn. As can be seen from Figure 1, the first of these generations is larger than the other (mainly due to losses of overwintering eggs in the second generation), thus adults are most abundant in spring, whereas juveniles are more abundant in autumn than in spring.

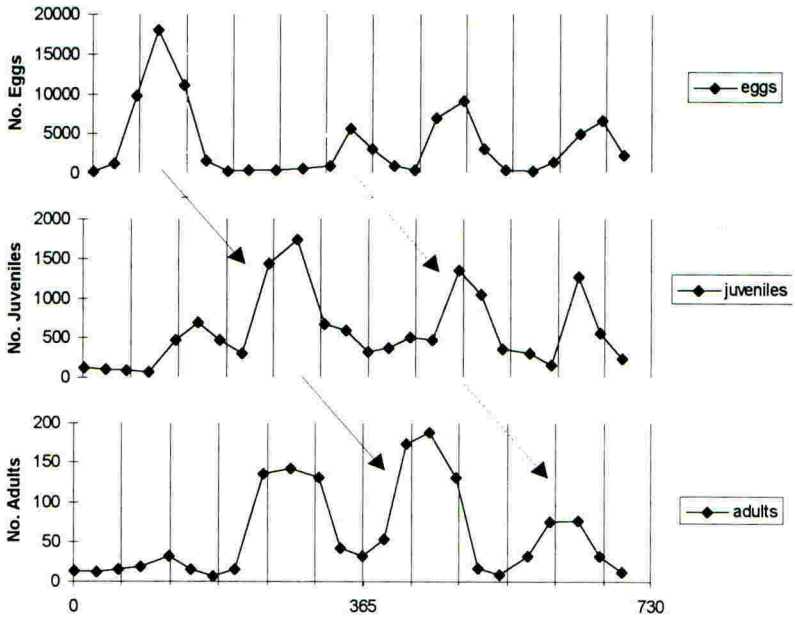

Figure 1. Graph showing the two generations of slugs over the course of a two-year simulation. The arrows indicate the progression of the overlapping generations. Note the ten-fold difference in the numbers of slugs in each graph.

The predicted distribution maps for the juveniles in both autumns as simulated by the model are shown in Figure 2. Only the autumn period is shown as this is the time that juveniles were most abundant. More variation appears in adult distribution between years than with juvenile distributions.

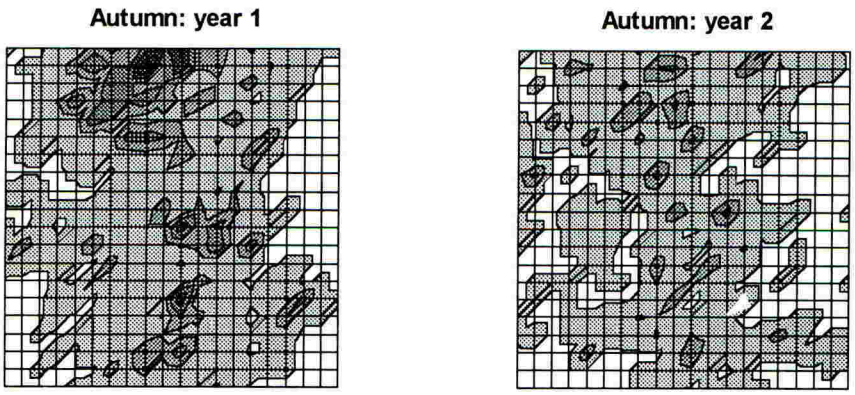

Figure 2. Maps showing the distributions of juvenile slugs in an area of 40 m × 40 m in the autumn of both years of the simulation. The contour lines are in steps of five individuals per m^2.

This pattern was not seen for adult slugs (Figure 3). Again, only the season in which the age class was most abundant is shown; in the case of mature slugs, this was spring. There were no obvious similarities between adult distribution from year to year, and they seem to be more evenly spread over the modelled area, in comparison to the more clumped distribution of the juveniles.

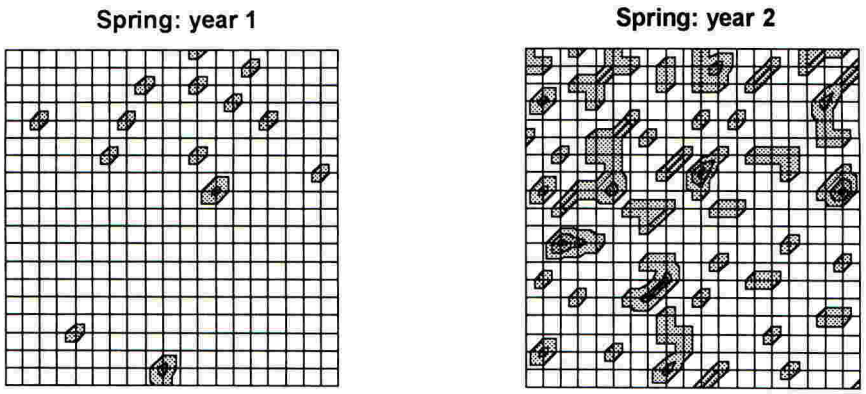

Figure 3. Maps showing the distribution of mature slugs in an area of 40 m × 40 m in the spring of both years of the simulation. The contour lines are in steps of one individual per m^2.

Field population study

The distribution of juvenile slugs in the arable field in March 1997 was found to conform to the spatial distribution predicted by the population model. For example, juvenile slugs of both *A. intermedius* and *D. reticulatum* were found in discrete patches, or hotspots, across the 1m sampling grid (Figure 4). These juvenile patches were probably formed from groups of individuals hatching from egg batches laid by adult slugs in the preceding autumn/winter. The distribution of mature stages of *D. reticulatum* also appeared to conform to the model output, in that their distribution was spatially random and extremely dynamic, from sampling to sampling, across the 16 m spatial grid. The distribution of *D. reticulatum* juveniles also appeared to be spatially dynamic throughout the year. The distribution of *A. intermedius* was less well described by the model; a distinct and stable patch structure, of approximately 40 m diameter, was noted for both juvenile and adult *A. intermedius* on each sampling occasion. This patch did not correspond with any obvious environmental variables measured during the period of the study, but could perhaps be related to the suitability of that particular area of the field for survival in more extreme conditions, before the start of the study. The difference between the model prediction and the observed spatial pattern of *A. intermedius* can be readily explained by the reliance on *D. reticulatum* population parameters in the model.

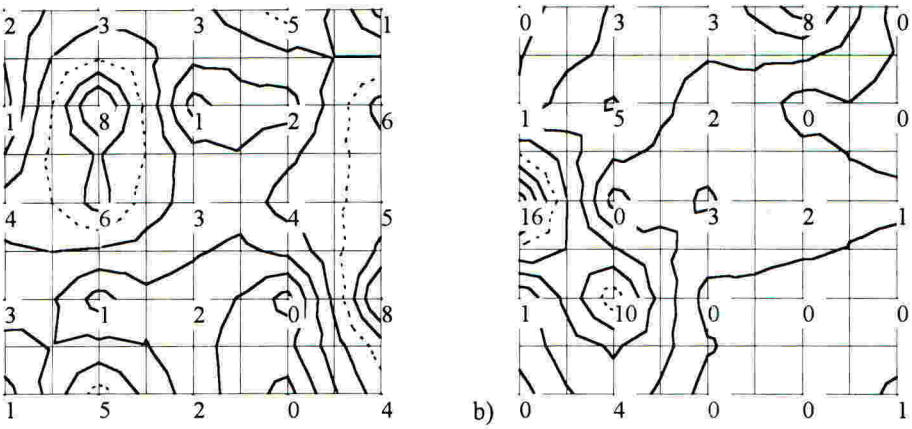

Figure 4. Maps showing the distribution of a) *A. intermedius* and b) *D. reticulatum* juvenile slugs in a grid of 5 × 5 sampling points, spaced 1 m apart, in March 1997. The contour lines are in steps of one individual in a) and two individuals in b).

DISCUSSION

The simulation model shows that using published life-history data for the field slug, *D. reticulatum*, the characteristics of the population dynamics of this important pest species in the field can be reproduced with a fair degree of accuracy. Hunter and Symonds (1971) present evidence for two distinct but overlapping ("leap-frogging") generations in field populations of *D. reticulatum* that correspond to the population dynamics presented here. However, it is important to note that *D. reticulatum* of most age classes may be found at any time of year (Haynes *et al.*, 1996).

The spatial model predicts that juvenile slugs, because of their lower potential for movement, tend to be found in a patchy distribution, with these "hotspots" centred around the positions of the egg masses from which they hatched. A gravid slug may lay several egg masses in a short period of time, if conditions are suitable (Duval, 1972; Rollo *et al.*, 1983; South, 1965), leading to an increase in juvenile density when the eggs hatch. Slugs in general do not disperse over long distances. Despite their potential to do so, the net dispersal distance may not be great, primarily because of their tendency to make circular foraging patterns (Duval, 1972; Rollo & Wellington, 1981) and their homing behaviour (Duval, 1972; Rollo & Wellington, 1981). Juveniles, being smaller, disperse even less than adults, maintaining the hotspot structure. Adult *D. reticulatum* disperse further than juveniles and have had a greater time to disperse over the landscape. For this reason, the correlation of their location with where they hatched, has diminished. *Arion intermedius* is less surface active and, thus, moves over shorter distances than *D. reticulatum*. This restricted movement together with its longer, annual life cycle compared to *D. reticulatum*, may account for the observed differences between *A. intermedius* and *D. reticulatum* in spatial patterns throughout the year on the study site at Long Ashton.

One of the restrictions of simulation models of slug population dynamics is that much of the data that can be obtained from the literature pertains to laboratory experiments, thus, there is

little information on how environmental variables influence life histories. Further work is clearly needed to increase the accuracy of predictions based on these models.

Although still at an early stage, the model can be used to assess the efficacy of control measures. Currently, field-wide treatments with molluscicides may be only sufficient to give adequate control of slugs outside of the hotspots, leaving behind foci of slugs that can then damage the crop. Conversely, the treatment may be sufficient to control slugs in the hotspot, and therefore be in excess of what is required over the majority of the field. The analysis presented here is an important step towards the prediction of slug problems in arable crops and, thus, the level of control required both within and outside the hotspots that will be needed to reduce slug damage to an acceptable level.

ACKNOWLEDGEMENTS

This paper is part of a joint project on slug population dynamics funded by the Ministry of Agriculture, Fisheries and Food. IACR receives grant-aided support from BBSRC.

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