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FORECASTING THE ABUNDANCE OF ORANGE WHEAT BLOSSOM MIDGE IN WHEAT

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ABSTRACT

Potential systems for forecasting the abundance of orange wheat blossom midge in wheat crops were compared at five sites in England in 1994. Weekly soil sampling to monitor pupation was compared with use of water traps, direct adult counts and assessment of egg numbers in crops. Monitoring of pupation gave a week's advance warning of adult midge activity, allowing assessment methods to be targeted at crops at susceptible growth stages. Water traps caught sufficient numbers of midges to give an indication of incidence. Direct counts of midges during the day gave a quick classification of midge abundance in the field and indicated accurately those fields in which few eggs were laid.

INTRODUCTION

A widespread outbreak of the orange wheat blossom midge (*Sitodiplosis mosellana*) occurred in Britain in 1993, but was noticed too late for effective action to be taken. Analysis of the grain samples taken for the Home-Grown Cereals Authority's Cereal Quality Survey showed that all but 2 of the 392 samples examined had been affected to some extent. More than 10% of grains were damaged in 21% of the samples and a further 29% had from 5 to 10% of grains affected. These levels represent the degree of infestation that warranted control measures to protect yield alone for feed crops, and quality premia for seed and milling

crops respectively (Oakley, 1994). The high numbers of larvae returning to overwinter in the soil in 1993 were thought to pose a significant threat of further damage in 1994.

The recommended products available for use in Britain are known to control adult midges, their eggs, and/or the larvae hatching from them. They are of short persistence, so need to be applied within the narrow window between the arrival of adult midges in a crop and the emergence of larvae from the eggs (Elliot, 1988). Larval diapause in the soil can be protracted with 10 to 95% of larvae pupating in any one year, according to prevailing soil temperature and moisture. Adult midges can emerge over a six-week period and fly some distance to lay eggs in crops at the susceptible ear emergence stage. The number of midges migrating to a crop, and the number of eggs laid within it, depend on the number of nights within the midges' one-week life-span that suitable weather conditions for flight occur during their active period at dusk. Even where large numbers of larvae have overwintered in the soil the amount of damage inflicted on a particular crop depends on the proportion pupating, the degree of coincidence between midge flight and the susceptible growth stages, and on the suitability of the weather at that time (Basedow & Gillich, 1982). It is very difficult, therefore, to anticipate in advance which crops may need protection, and when protective sprays would need to be applied. The only reliable way of making this decision is to inspect crops at dusk on evenings suitable for flight to assess the numbers of midges present. In the hour before dark, midges can be easily seen, identified and counted. At other times they can be difficult to find and count when resting within the crop canopy.

There was a need therefore to predict whether significant numbers of midges would emerge in 1994, to identify those crops which would be at the greatest risk of attack. A series of experiments designed to provide the information to develop a warning system was initiated at five sites in 1994. Additional pupation monitoring was carried out at five other sites (funded by DowElanco, the Chalkland Cereal Centre and ADAS) to provide additional information for use in forecasting damage.

MATERIALS AND METHODS

Experimental sites were established at ADAS Research Centres in Hampshire, Warwickshire and Cambridgeshire (2) and at a farm in the Yorkshire Wolds which had suffered damage in 1993. At each site a 'source' area was identified which had contained a midge damaged crop in 1993. No insecticides were applied to the source areas during the course of the experiment. A winter wheat crop was monitored for midge abundance at each site. Where the source field was cropped with wheat again in 1994, at Boxworth, Cambs and Grindale, Yorks, these crops were monitored; at the other sites a first wheat crop in an adjoining field was used. A chemical evaluation experiment was sited in each monitored crop. These experiments compared the control of midge larval attack given by different types of insecticides applied at GS 55, 59 or 63 to the monitored crops. The results of these experiments will be reported elsewhere.

Pupation monitoring

Soil samples were taken from the source fields each week from 9 May onwards. Bulked samples were taken with a trowel to 75 mm depth to give a minimum sample size of 4

kg on mineral soils or 2 kg on organic soils. The soil samples were washed through 4-mm diameter top sieves onto a 300μ-diameter bottom sieve. The contents of the bottom sieve were then placed in a saturated solution of magnesium sulphate and the floating organic matter removed, dispersed on filter papers and examined under a low-power binocular microscope. The immature stages of *S. mosellana* were removed and classified as cocooned larvae, free larvae, parasitised larvae, neonate pupae or sclerotised pupae

Water trapping

Five 260 mm diameter yellow water traps (Ringot, France, as used by Barr & Lescar, 1985) were either positioned along a headland adjacent to the monitored field, or, where a separate source field was monitored, along the headland of the monitored field adjacent to the source field. The traps were placed at 30 m intervals, 100 mm below the top of the crop canopy and filled with water plus a little wetter. They were emptied weekly and the number of male and female *S. mosellana* counted.

Crop parting

The number of adults *S. mosellana* within the plots was assessed by parting each plot at five points at GS 55, 59 and 63, and counting all midges disturbed. At each point both hands were thrust into the crop and then separated to disturb 10-12 wheat tillers. The number of midges disturbed were observed for 10 seconds after each parting.

Egg numbers

The number of eggs on ten wheat heads in each untreated control plot at GS 55, 59 and 63 was counted using a low-power binocular microscope.

RESULTS

The weekly soil sampling results (Figure 1) showed variations in pupal development between sites which were reflected in adult activity and oviposition. At Drayton in Warwickshire two phases of pupation were recorded, producing flushes of adult emergence from 23 May. Windy weather inhibited flight and egg laying in the monitored crop. Peak activity of 10 midges per trap week was detected in the week ending 20 June by which time the crop had reached the flowering stage. At Grindale in North Yorkshire there were also two phases of pupation. Adult midges emerged from 23 May, well before any crops in the area reached the susceptible growth stages. Emergence continued through to the end of June. A peak of adult activity of 4 midges per trap week from 7 to 14 June coincided with the early ear emergence stage of the monitored crop. At Boxworth in Cambridgeshire there was only one phase of pupation, resulting in a sharp peak of adult midge activity of 99 midges per trap week from 7 to 14 June, coinciding with the ear emergence stage of the monitored crop. At Arthur Rickwood in Cambridgeshire there were two phases of pupation. A more extended period of adult activity averaging 15 midges per trap week was recorded from 31 May to 14 June with ears starting to emerge in the monitored crop in the second half of this period. At Bridgets in Hampshire pupation developed in three phases corresponding to fluctuations in soil temperature above 13°C at times when the soil was moist. The third peak of adult

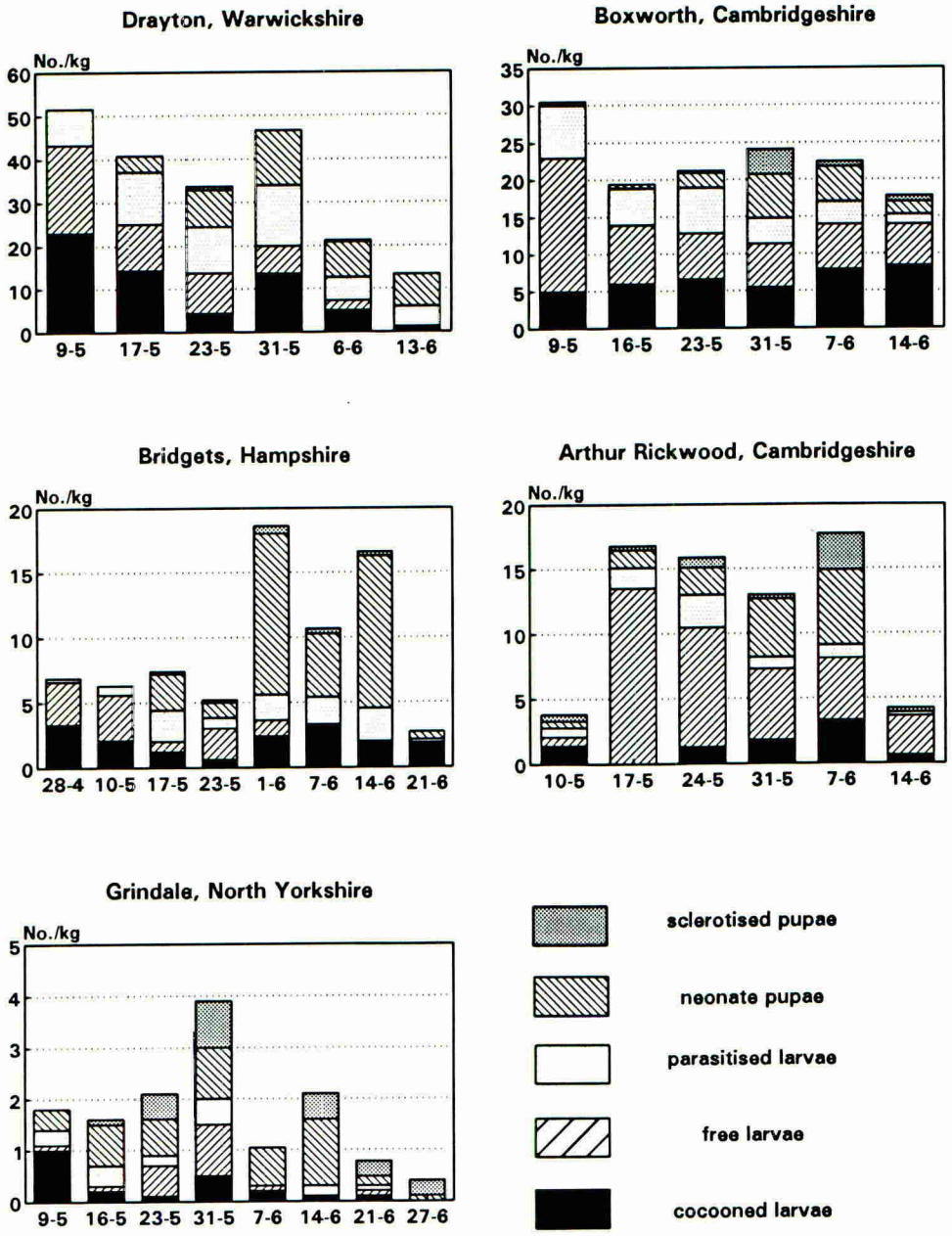

FIGURE 1. Numbers of different stages recovered from pupation monitoring samples.

activity coincided with the susceptible stage of the monitored crop, but windy weather inhibited flight for a week after the main midge emergence period so that peak activity of 101 midges per trap week was recorded from 21 to 27 June.

The numbers of eggs found ranged from 0.3 per ear at GS 63 at Drayton to 6 per ear at Boxworth at GS 59 and 63. The majority of eggs were laid in the latter half of the ear emergence period. The late flush of midge activity at Bridgets after GS 63 did not result in a higher level of attack than indicated by egg counts at GS 63.

DISCUSSION

Monitoring of pupation gave a good indication of midge emergence and, when linked to outlook weather forecasts, of midge activity within crops. The adult midge activity recorded by both the water traps and crop parting observations was closely linked to both the growth stage of the crop concerned and the prevailing weather. The results will be analysed in relation to meteorological records taken at the sites and the development of the attack in the crops in a future paper. Pupation took place in flushes corresponding to periods of higher soil temperature when the soil was moist. More flushes of pupation were recorded on sites with lighter soils (Arthur Rickwood, Bridgets and Grindale) which may be quicker to warm and easier to wet. On the heavier soil types (Boxworth and Drayton) pupation was triggered less frequently and at Boxworth a smaller proportion of the larvae pupated. Early indication of fresh larval reactivation was provided by the numbers recovered from the surface layer as more larvae migrated from greater depth.

Emergence of adults from sclerotised pupae was rapid when soils were warm and moist, but was delayed when conditions were unfavourable. Adult longevity appeared to be variable. In cool, windy conditions, when midge flight was frequently inhibited, longevity appeared to be extended to about two weeks. Pivnik & Labbé (1993) quote a mean life span of 6.6 ± 0.6 days for adult midges kept in the laboratory under favourable conditions for oviposition. It seems probable than when conditions inhibit flight in the field the adult midges can conserve energy and extend their life span.

The pupation monitoring carried out at a further five sites for advisory purposes, gave broadly comparable results to those found at the experimental sites and confirmed the variations in pupation observed in relation to soil type and area. The advanced warning of midge activity given by pupation monitoring was found to be of value in directing scouting efforts. It also gave a indication of the level of parasitisation by cynipid wasp parasitoids, which are the principal natural enemies (Affolter, 1988). Other trap systems were tried elsewhere for field monitoring purposes, but sticky traps were generally found to catch too few adult midges to indicate activity in an area. Water traps caught large numbers of midges, with the numbers caught reflecting the activity in the crop and seem to represent a better alternative for monitoring activity on a field-to-field scale. Day time crop parting gave a reasonable indication of midge activity enabling more fields to be examined than with observations of ovipositing midges at dusk. Further calibration of numbers is required before threshold levels can be set for this method. Egg monitoring was a very laborious process, and provided little additional information to that derived from simpler observations of adult activity or numbers. Pivnik (1993) has investigated the possibility of using pheromone traps

to monitor midge activity by using traps baited with virgin females. The need to supply a fresh 'bait' midge every evening precludes the current use of this method, but his results demonstrate the possibilities were the pheromone to be identified and synthesised.

A survey of 44 ADAS crop consultants confirmed that difficulties in assessing midge risks in the field in 1994 were mainly at lower levels of incidence, where confusion of midges with other insects was more probable. In areas where 10 - 20 % of fields were sprayed it was considered that 14% of the sprayed fields had above threshold numbers of midges, whereas in areas where more than 50% of the fields were sprayed 66% of the sprayed fields had above threshold infestations. A clear need for improved field assessment methods was identified.

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EVALUATION OF THE USE OF BAITED TRAPS TO ASSESS THE RISK OF WIREWORM DAMAGE TO POTATO

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ABSTRACT

In 1993, the commercial use of a simple cereal-baited trap for detecting wireworms in fields intended for potato production and predicting the level of damage was evaluated. Traps were placed in 42 potato fields in northern, western and south-western England with a perceived risk of wireworm attack. Wireworms were detected at 17 sites. At 16 of these sites, and at a further 9 sites where no wireworms were detected, assessments were made at harvest to determine the level of wireworm attack. Assessments indicated no clear relationship between bait trap catches of wireworms and subsequent damage to potato tubers. These results are compared with published data on the relationship between wireworm damage to potato and the traditional soil-core method of sampling for wireworms.

INTRODUCTION

Wireworms (*Agriotes* species) are the soil-dwelling larvae of click beetles (Coleoptera:Elateridae) and are locally important pests of arable crops in the U.K. Their natural habitat is grassland, but when infested grass fields are ploughed, arable crops planted subsequently can be attacked. Wireworms take several years to reach maturity, so attacks can occur up to four years after the grass was ploughed. Potatoes are particularly prone to damage, as wireworm feeding on the tubers results in deep, narrow holes which seriously reduce the quality (although not the yield) of the crop; economic damage can occur at low wireworm population densities (Gratwick, 1989). In northern and western England, it is common practice to plant potatoes after grass, and it is therefore in these areas that wireworms are a particular problem.

Insecticides used for the control of wireworms on potato in the U.K. (ethoprophos and phorate) are relatively expensive and need to be applied at planting. Not all grass fields are infested with wireworms, and therefore it is common practice to make a pre-planting assessment of the risk of wireworm attack. The soil-sampling techniques usually used were developed nearly 50 years ago (Salt and Hollick, 1944; Cockbill *et al.*, 1945). These are labour-intensive, time-consuming, and involve laboratory processing of samples. The relatively high threshold for detection using soil cores (*c.* 62,500 wireworms/ha) means that there is no prospect of developing an economic threshold with these methods as potentially damaging wireworm populations may be missed. To address these problems, a pilot study was carried out in 1992 to evaluate alternative methods of wireworm detection using food baits, a technique widely tested in the USA (e.g. Ward & Keaster, 1977; Toba & Turner,

1983; Jansson & Lecrone, 1989). The results of this work (Parker, 1994) indicated that a simple cereal-baited trap could be used to detect wireworms in the field without the need for large-scale soil-sampling and laboratory processing. This paper describes the evaluation of this baited trap technique in commercial potato fields as a practical tool for detecting wireworms and predicting the subsequent level of attack to the potato crop.

MATERIALS AND METHODS

Site selection

Suitable fields were identified on the basis that they were being planted with potatoes in 1993, and should ideally have been in long-term grass (more than seven years) immediately prior to the potato crop. Fields one to three years after grass were also considered suitable providing no treatment that could have affected wireworms had been applied to the arable crops preceeding the 1993 potato crop (Table 1).

Trap preparation

The traps were made out of 350 ml straight-sided plastic pots (Medfor Products, Fleet, U.K.). The sides and bottom of the pots were perforated with a total of 30 x 4 mm evenly-spaced holes. Each pot was closed with an unperforated plastic lid. Each trap was filled with a bait consisting of a 1:1 wheat:barley seed mix (*c.*120 ml of each) mixed with coarse vermiculite and 'Lytag' (pulverised fuel-ash) as water-retentive media. The traps were filled and stored dry, but just prior to use, they were submerged in water for up to 36 hours to allow the grain to take up water. This helped speed the germination of the grain in the field.

TABLE 1. Summary of trapping site locations, grass history, number of sites where wireworms found and number of sites assessed for damage.

		Grass	duration	history	(years)	No. with	No. assessed
County	No. sites	Mean	Median	Min	Max	wireworms	for damage
Cheshire	8	22.3	20.5	5	50	2	3
Devon	3	34.0	50.0	2	50	0	1
Hampshire	2	-	-	-	-	0	1
Herefordshire	5	28.0	30.0	2	50	3	4
N Yorkshire	4	17.0	7.0	4	50	3	4
Shropshire	5	8.0	10.0	5	10	2	3
Somerset	11	25.9	8.0	1	>100	7	8
Staffordshire	1	1.0	1.0	1	1	0	1
Warwickshire	3	18.7	25.0	6	25	0	0

Trap placement and recovery

Traps were set out in the field during March and April immediately prior to the planting of the potato crop. Trapping was carried out pre-ploughing wherever possible, but in practice fields had often been ploughed prior to the trapping period. Ten traps per field were used, spaced out in a W-pattern across the field. Each trap was placed upright in the bottom of a 9 cm deep hole, which was backfilled with field soil and the trap location clearly marked. At the

end of a two week period, the traps were recovered and small collar of soil from around the sides of the trap taken for examination, as the pilot work had indicated that a significant number of wireworms could be found just outside the trap. All traps were examined by hand in the laboratory for the presence of wireworms either inside the trap or in the surrounding soil collar.

Damage assessment

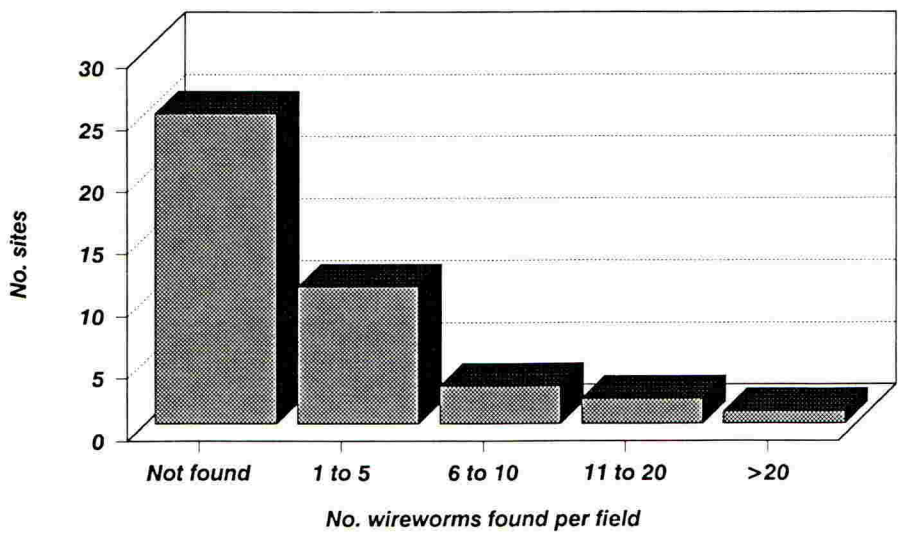
Just before harvest, 16 of the fields where wireworms had been detected plus a further nine fields where no wireworms were found (Table 1) were re-visited and a 'commercial' assessment made of the level of wireworm damage to the crop. This was done either pre-harvest by examining 10 tubers from around each of the trap sites (100 tubers in total), or post-harvest by examing 100 tubers selected at random from the potato store. The majority of the fields had not been treated for wireworms; a separate record of damage was kept for those fields which had received treatment (ethoprophos granules broadcast at 60 kg product ha^{-1} or phorate granules applied in-furrow at 34 kg product ha^{-1}).

RESULTS & DISCUSSION

Trap catches

Wireworms were detected at 17 out of the 42 sites sampled. This detection rate (40%) compares favourably with the 23% detection rate achieved using soil-cores in 108 commercial potato fields sampled during 1989 to 1991 by ADAS. This provides additional circumstancial evidence to confirm the findings of Parker (1994) that the use of baited traps is at least as effective (and in some cases better) than soil cores at detecting wireworms. The distribution of trap catches is shown in Figure 1.

FIG. 1. Frequency distribution of wireworm trap catches (number of wireworms found inside and outside traps pooled).

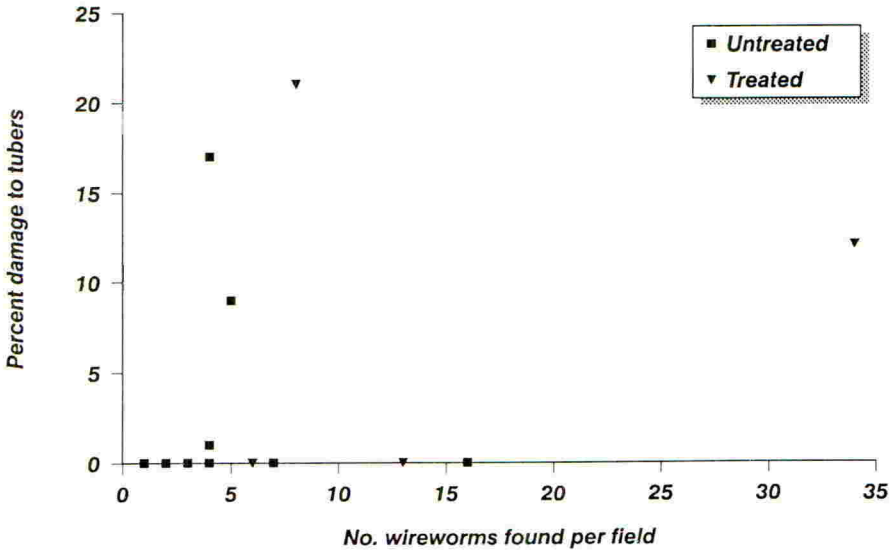


The majority of wireworms were found just outside the traps, with only 8% of the insects found inside the trap. This contrasts with the pilot work (Parker, 1994) where *c.* 50% were found in the traps. Compared with 1992, the 1993 traps were over-filled resulting in a very tight mass of germinated grain and vermiculite filler inside the trap by the end of the trapping period. This made it physically difficult for wireworms to enter the trap and probably accounts for the low number of wireworms found there. The design of the trap system has subsequently been altered, and preliminary evidence from work done in 1994 has indicated that more wireworms do enter the trap.

Damage assessment

No damage was found in any of the fields where wireworms had not been detected. Although this is good evidence that signifcant wireworm infestations were not being missed by the trapping technique, conversely damage was found in only five of the 16 fields where wireworms were detected pre-planting. It is therefore possible that wireworms were undetected but did not cause noticeable damage. Of the five attacked fields, two were treated (ethoprophos at 60 kg product ha^{-1}) and three were untreated. The highest level of damage (21% tuber damage) was recorded at a treated site. The relationships between the pre-planting trap catches and subsequent damage in treated and untreated crops are shown in Figure 2.

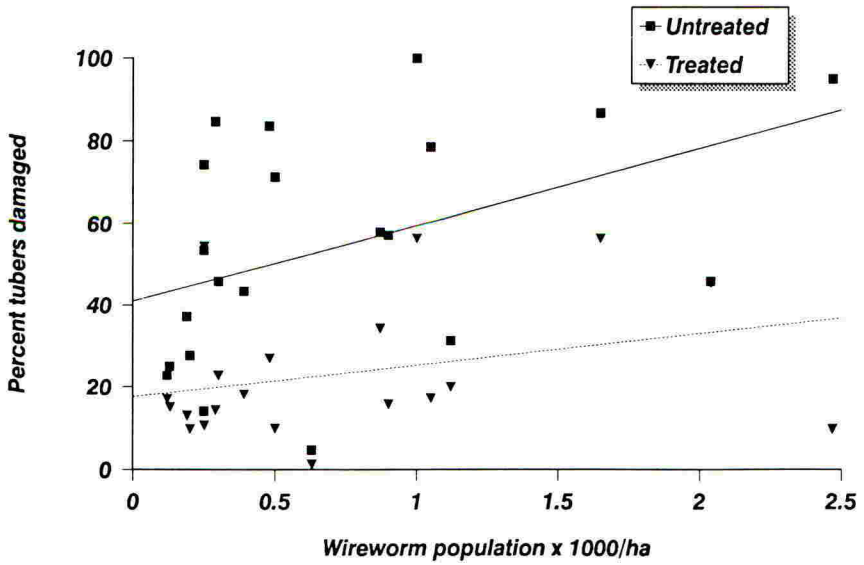
FIG. 2. Relationship between percent wireworm damage to tubers at harvest and pre-planting catches of wireworms by baited traps (see text for treatment details).



The regression of wireworms trapped on percent damage was not significant for untreated fields (% damage = 2.76-0.064 trap count; t = -0.14, p = 0.89). The data set was too limited to justify regression analysis for treated fields, but the data points are shown on Figure 2. Although it could be argued that the low level of damage recorded (<21% in all cases) was due to the relatively insensitive sampling method, the results are confirmed by unpublished data from small plot work (carried out by W E Parker) which indicate that relating wireworm catches at baits to subsequent damage levels to tubers is unlikely to be feasible.

The damage assessment data obtained in 1993 can be usefully contrasted with data abstracted from previous work on wireworm control, where even at relatively low population densities (assessed using soil core sampling methods), untreated damage of >20% tubers attacked was usual (Figure 3, data mainly from Hancock *et al.*, 1986 and Parker *et al.*, 1990). With this more extensive data set, the relationship between the level of damage without treatment and the pre-planting wireworm population is significant (% damage = 41.0 + 18.7 wireworm population; t = 2.12, p = 0.047), but the percentage variance accounted for is low (R-sq. = 14.9%). The intercept on the Y-axis (i.e. no wireworms found) is at 41% damage (Figure 3), clearly unacceptable for predictive purposes. These data also illustrate the difficulty of obtaining 100% control of wireworm damage on potato using currently available insecticides, even where population densities are apparently low. The tendency is for relative control to be greater at higher wireworm densities (Figure 3), but absolute levels of control are usually in the range 10 to 20% of tubers attacked ('treated' regression line: % damage = 17.7 + 7.76 wireworm population; t = 1.36, p = 0.19, intercepting the Y-axis at 17.7 % tuber damage).

FIG. 3. Relationship between percent wireworm damage to tubers at harvest and pre-planting wireworm counts made using soil cores (treatment = ethoprophos at 60 kg product ha^{-1}).



CONCLUSIONS

This work has helped to confirm that in terms of detecting wireworms, the trap system is at least as effective as traditional soil sampling methods. The cost of baiting is also less as sampling time is reduced by at least 40% (Parker, 1994), and laboratory processing (costing growers *c.* £65 per sample in 1994) is eliminated. There is therefore scope for introducing the traps into commercial practice as a wireworm detection system.

Unfortunately, it has not so far proved possible to develop a useable threshold using the traps. The difficulty of predicting the absolute level of wireworm damage to potato is probably

related to a combination of factors. These are likely to include harvest date of the crop and the availability of alternative food such as old turf material. The inherent behaviour of wireworms may also be important, as they are known to cease feeding for long periods (Evans & Gough, 1942). A threshold for wireworms above the limit of detectability may be inappropriate in any case. Potato growers need to produce high quality crops to command premium prices. They are therefore generally unprepared to take chances with unpredictable pests such as wireworms, and would be satisfied with a user-friendly presence/absence test for wireworms, a role which these traps could usefully fill.

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NEW WORLDWIDE EXPERT INTERVIEWING SYSTEM FOR TARGET PEST SELECTION

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ABSTRACT

The collection of information about infestation and damage in relation to weeds, insects and diseases has in the main been on an ad hoc basis, and few resources have been devoted to any systematic tracking of these phenomena over time. As a result some pest problems have been largely overlooked, while in other cases pesticides have been developed for problems that in reality are much less important than had been thought.

A new method has been developed over the last eight years to allow monitoring on a regular basis of the development of pest problems in all major agricultural and horticultural crops in over 60 countries.

INTRODUCTION

If there has been any 'responsibility' for collecting and making available information on the infestation and damage attributable to weeds, insects and diseases in either the industrialised or developing worlds, it has been with the Extension Services in most countries, supplemented with inputs from crop research institutes and universities. In practice little empirical survey work has been undertaken, certainly not on a regular or systematic basis for all the important crops and pests, and as a result there is not much information available. Limited public sector resources are part of the explanation, but the agrochemical industry has commissioned pest survey work on only a modest scale over the last 50 years, most of it on ad hoc surveys rather than as part of an ongoing monitoring process. Hence the dynamics of the changing importance of pests within a crop are difficult to detect. The logic of a commitment to such long term work is accepted but there have been greater priorities for available resources, and sometimes a sense that enough was known anecdotally to be able to make the judgements about one pest problem or another.

Of the limited survey work done, it is naturally the higher value crops that have attracted the most attention. For example, cereal weeds have been subject to quite regular surveys by the ITCF in France, the BBA in Germany and ADAS in the UK. Often these are broad agronomic surveys where the weed situation is only one of the parameters being investigated. In the USA the Sugar Growers' Association and the Cotton Growers' Association have produced regular annual bulletins on infestation and damage in their crops. In Japan several crops are monitored for pests at the level of the prefecture by the Ministry of Agriculture.

Of the attempts to look at pests in all important crops on a sustained basis every year, that undertaken by APHIS in the USA is the best known (Animal and Plant Health Inspection Service of the USDA).

Unfortunately this has yielded little usable information.

For the agrochemical companies the upshot has been that until recently they were unable to access a standard body of information on infestation and damage to crops that was comprehensive in its coverage of countries and updated to reflect changes in pest situations.

THE GAP - WHY PEST SURVEY WORK MAKES A DIFFERENCE

Today the agrochemical industry faces increased competition from seed companies and others looking for novel methods of controlling pests. Conventional chemicals still account for over 95% of all pest control world-wide, but major investments have been made in plant breeding, biopesticides and other new pest control activities at a technical level and as commercial ventures. Public policy across the globe is trying to switch pest control away from agrochemicals. How this is to be achieved is still being debated and governments have committed modest resources so far. As Lisansky (1993) showed, only a handful of commercial investments in this area have as yet made a satisfactory return. Several strains of the bacterium Bacillus thuringiensis have been successful as insecticides on some Lepidoptera, Diptera and Coleoptera. Certain nematodes and viruses also hold promise as insecticides. But these are the exceptions.

In this context the contribution that pest survey work can make becomes more important. Consider the way many new compounds are assessed for their suitability as pesticides. From first screening, some activity against one or more pests is discerned. The discovering company will need to know much about both the current and future potential markets before committing further resources to its development. For around two thirds of the current world market, data is available, but this is gathered mainly for marketing purposes and does not provide the additional information needed for the large R&D investment decision, such as:

- what crops does each pest infest, what proportion is being treated ?
- what losses can be attributed to it ?
- how well do current products control the pest ?
- is the pest problem growing or diminishing ?
- is any infested crop not treated; if so, why ?
- are there pest problems with which it overlaps or forms complexes ?

This is the gap that the pest data has to fill to allow more informed decisions by plant breeders and agrochemical companies alike.

A NEW APPROACH TO PEST TARGETING

The needs of the pest control companies had crystallized sufficiently by the mid 1980's to allow objectives to be set for the survey work.

1. To get a quantitative view of the extent to which crops are infested and damaged - over and above what is already being treated with pesticides. **This reveals emerging pest problems - often missed from farmer-generated surveys where the focus is on old or existing pests.**

2. To know what damage is being caused by pests on both treated and untreated crops as this **provides an indication of the cost of treatment/yield response parameters for any new pesticide, seed variety or other pest control measure to be introduced.**

3. To obtain more disaggregated information on pests as they occur in the field at the level of species, genus or complex to **assist in the long term development of pest management on a more individualistic basis.**

Coverage

To be an effective R&D tool it was clear that the coverage had to be wide in terms of countries, crops and pests. Information is often most needed at or just beyond the limit of current activities.

Nearly all new products these days are introduced with the intention of ultimately addressing the market world-wide, not least because of the extremely high costs of development and of meeting regulatory requirements. For this reason there is a core group for the survey work of 14 countries which are of the greatest strategic importance for most companies (Australia, Brazil, Canada, France, Germany, Italy, Japan, Mexico, Netherlands, S. Africa, S. Korea, Spain, UK, USA). Beyond that a further 49, where at least some crops are of global significance, are also included but with less intensive coverage.

In total over 60 crops need to be covered including some that might at first appear surprising, such as ornamentals, and crops grown under glass or plastic. A handful of crops (corn [maize], soybeans, wheat, barley, rice, cotton, sugar beet, potatoes and grapes) at present account for the bulk of pesticide sales world-wide. Partly because of this concentration, many of the most severe pest problems are to be found in other crops, so in time the list will grow.

Updating

Today's minor pest may become tomorrow's major one, depending on climate, crop rotation, crop selection, varietal development, pesticide use, cultivation practice and other factors. It is essential to track a wide range of pests to be able to spot the new and emerging problems, as well as those that are stable or in decline.

Two 40-crop pest surveys that were conducted state by state in the USA in 1988 and again in 1992 illustrate this. Aggregating all the crops together, the area infested with some insects increased significantly, whilst that with others remained stable or declined. Understanding these developments can provide a key to predicting their likely evolution over the next few years.

The lead time from discovering a new chemical entity to first commercial sale may be 10-12 years. The pest survey has to be updated sufficiently often to be able to judge any likely change in status of a pest. Weeds in general seem to remain more stable than insects or diseases. An update frequency of about four years has been followed.

TABLE 1. Changes in levels of insect infestation in the USA in 40 crops aggregated together

	Area Infested 1988	1992
	----x 1,000 ha----	
Aphis spp.	3,500	2,000
Diabrotica spp.	10,300	9,300
Eriophyes spp.	3,200	3,800
Heliothis spp.	4,600	2,000
Mayetiola spp.	300	300
Ostrinia spp.	4,600	3,100
Spodoptera spp.	800	2,200

METHODOLOGY

The principal source of information on pests has been the Extension Service in most countries since one of their usual responsibilities is monitoring pest infestation. Other sources include crop research institutes, university plant protection departments, agrochemical manufacturers and distributors, independent crop consultants and crop associations. In addition where available, empirical pest survey work has been incorporated. A key to the success of this methodology is identifying the appropriate specialists at a local level and persuading them to participate. All providers of information are offered a copy of the results of the survey as well as access to the rest of the data base world-wide.

Information exchange

After identification of the significant pests of a particular crop, the specialists are asked to provide an estimate for each pest of the area infested, area treatable, area treated, attributable economic loss, to specify the main active ingredients in use, the ease of control achieved by the active ingredients, any problems experienced by farmers with those actives, and any reasons for non-treatment of infested crop.

The emphasis in collecting the information is on describing the real situation as it exists in the field. Notwithstanding crop rotation, varietal selection, cultivation, use of pesticides and any other operations carried out by farmers, what is the infestation of such and such a pest, and how much yield or quality reduction can be attributed to it ? For some plantation crops information can be obtained from the growers directly. Apart from this, farmers generally do not have the information required.

There is a certain amount of feedback on the information collected, and where possible a consenus view is reached, but otherwise it is collated and entered into the data base without modification.

This is an example of what emerges.

TABLE 2. N. Dakota, USA: 1992 Sugar Beet Diseases (Area Grown: 78,000 ha)

	Area Infested ---x 1,000 ha---	Area Treated	Economic Loss $ m	Actives In Use	Ease of Control %	Active Problems
Aphanomyes cochlioides	32	0	7.00	None	-	*
Cercospora beticola	64	60	4.00	Fentin hyd.	90	**
Pythium spp.	78	78	0.70	Metalaxyl	90	
				Oxadixyl	90	***
Rhyzoctonia solani	8	0	2.00	None		

* Reason for non-treatment: resistant varieties in use
** Fungus showing resistance
*** Carry-over

CONCLUSIONS

The main purpose of collecting this information is to assist companies with their pest targeting for which the detail and accuracy of the information collected by this method is appropriate.

An example will illustrate this. Suppose that a new molecule has been shown from first screening to provide selective control of the diseases caused by several species of the genus Colletotrichum in various crops. The data base is searched for all crops and all countries of interest and the results aggregated. This indicates that the component species are present on 8,805,000 ha, are treated on 4,408,000 ha and cause loss worth $229.19 million, a substantial apparent deficiency in the current control methods.

TABLE 3. Infestation and damage due to Colletotrichum

Crop	Colletotrichum Species	Area Infested ----x 1,000 ha----	Area Treated	Economic Loss $ m
Alfalfa	C. trifolii	923	0	16.40
Cucurbits	C. lagenarium	19	0	3.00
Lentils	C. truncatum	5	0	0.20
Maize	C. graminicola	5,449	3,100	117.84
Soybeans	C. dematium	2,300	1,250	85.50
	C. truncatum	32	0	0.25
Sugar cane	C. falcatum	16	0	0.00
Tobacco	C. destructivum	61	58	6.00
Total		8,805	4,408	229.19

Other analyses can show the spread between countries.

TABLE 4. Infestation and damage due to Colletotrichum

Country	Area Infested x 000 ha	Area Treated x 000 ha	Economic Loss $ m
Australia	5	1	1.00
Japan	10	25	18.50
S. Korea	35	27	10.50
USA	8,755	4,355	199.19
Total	8,805	4,408	229.19

Taken together, these analyses help to describe where Colletotrichum occurs and where it is a problem. Maize suffers the greatest total damage, although losses per hectare are higher on lentils. The disease causes its greatest total loss in maize and soybeans in the USA; and of these maize suffers the higher loss and higher area infested.

Time series data would reveal the dynamic picture of the disease's development over several years. The resulting profile would suggest where further research may be necessary, subject in particular to the relative performance of the new molecule against the various species of Colletotrichum. Thus it is possible to gain a comprehensive view of a pest problem across all the important countries of the world, and to aggregate the information so that it can be compared and contrasted with other pest problems in an objective way. This in turn allows a fuller evaluation of the strengths and weaknesses of the new molecule.

The experience of companies which have used pest information in this way shows that R&D resources can be more efficiently deployed as a result. In particular they have found that a much higher success rate can be achieved in predicting the future development of pests in terms of infestation, crops affected, and scale of losses. Investment decisions are made with more confidence as to the future importance of the target pest by the time the first commercial product may appear on the market. The focus can be on developing fewer but more effective products since the likelihood of sales falling below expectations is reduced. This in turn allows a greater concentration of effort in R&D and marketing on those parts of the market where a company has particular strengths.

In the longer term it will assist in the development of more individualistic methods of pest control.

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CONTROL OF PEA APHID ON COMBINING PEAS AND IMPROVED MANAGEMENT STRATEGIES

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ABSTRACT

Previous work has indicated that a premature population crash can occur in pea aphids infesting combining peas which may result in the current management strategy wrongly indicating that treatment is economically justified. The results of a series of field experiments investigating the yield responses obtained from pea aphid control following four different pesticide application regimes are reported. They show that the timing of sprays resulting from the current management strategy protect the crop during the growth stages that are susceptible to pea aphid damage, but do not always identify fields where spraying may not be needed.

INTRODUCTION

In recent years damaging populations of pea aphids (*Acyrthosiphon pisum*) have been widespread on combining peas, and this has resulted in an increase in routine applications of insecticide to control aphids. A series of damage assessment trials was conducted between 1985 and 1989 which indicated that an economic yield response was likely to be obtained by spraying with insecticide when 20% or more plants became infested with pea aphids and populations were increasing, at any time between flowering and pods fully formed on the fourth truss growth stages (Lane and Walters, 1991). However, results from these experiments also indicated that at the growth stages investigated, the estimated action thresholds obtained from different analytical techniques varied widely, partly because aphid population development and decline was highly variable between sites. In particular, populations at some sites which were increasing exponentially suddenly declined to zero within a few days, while those at other similar sites continued to increase for several weeks. The data suggested that this resulted in recommendations for unnecessary insecticide applications on over 30% of occasions.

Work carried out on pea aphids in North America (eg Via, 1989) and some parts of continental Europe (eg Sandström, 1994), has shown that their biology varies markedly in different geographical regions and the species may be a complex of races and subspecies (Blackman and Eastop, 1984). Thus, extrapolation of the results to UK conditions is difficult. Much of the experimental work in Britain has studied pea aphids on plants other than peas, although a few investigations of aphid population dynamics in pea crops have been undertaken (Dunn & Wright, 1955). However, as the results of such studies could not explain the early population decline in the recent trial series (Lane and Walters, 1991), or the apparent inaccuracy of the current action threshold, a further series of field experiments was undertaken between 1991 and 1993 to investigate the success rate of using the action threshold in commercial fields and to identify relevant aspects of pea aphid biology which could be used to improve the current management strategy. This paper reports yield responses obtained from pea aphid control following four pesticide application strategies.

MATERIALS AND METHODS

A similar experimental design was implemented at two ADAS Research Centres, in each of three years, 1991, 1992 and 1993. Details of the location, soil type and cultivar grown at each site are given in Table 1. Two large blocks of combining peas were drilled at each site on different dates, with the first sowing made as early as possible in March, and the second about three weeks later (a minimum of two weeks, but not after mid-April). As pea aphids were mostly found on or very near the growing tip of the plant, population estimates were expressed as percentage shoots infested (a shoot defined as stems and leaves within 10 cm of the growing tip). The growth stage (GS) of each sowing was assessed at each visit to the field using the growth stage key of Knott (1987).

Randomised block designs consisting of four blocks of five treatments, with a minimum plot size of 25 m x 4 m, were established in each site/sowing date. Treatments consisted of an untreated control (T1), and single foliar sprays of the aphid-specific insecticide pirimicarb (140 g AI/ha) at the first assessment when aphid levels exceeded 10% shoots infested (before GS203; T2), when 20% shoots were infested between GS203 and GS209 (T3), and when 50% shoots were infested between GS203 and GS209 (T5). In addition there was a multiple spray programme in which plots were treated on each occasion when 20% or more shoots were infested between GS203 and GS209 (T4).

TABLE 1. Details of sites used to investigate the effects of pea aphids on yield of peas.

Site	Year	Location	Soil type	Cultivar	Sowing date	
					A	B
1	1991	Mepal, Cambs	Peaty loam	Solara	13 Mar	5 Apr
2	1991	Preston Wynne, Herefordshire	Silty clay loam	Solara	27 Mar	16 Apr
3	1992	Mepal, Cambridgeshire	Peaty loam	Solara	10 Mar	3 Apr
4	1992	Preston Wynne, Herefordshire	Silty clay loam	Solara	11 Mar	8 Apr
5	1993	Mepal, Cambridgeshire	Peaty loam	Solara	8 Mar	8 Apr
6	1993	Preston Wynne, Herefordshire	Silty clay loam	Solara	17 Mar	14 Apr

Immediately before sprays were applied, aphid populations were assessed on 50 shoots per plot, and at weekly intervals thereafter on 25 shoots per plot. A 3 metre wide strip down the length of each plot was harvested using a plot combine harvester and the weight of seed produced recorded. Dry weight (85% d.m.) and thousand seed weight were determined from a representative subsample of seed from each plot.

RESULTS

Development of aphid populations

Irrespective of the growth stage at the start of colonisation, a similar pattern of population development was observed (Table 2), with a gradual increase in aphid numbers until June. The differential drilling dates resulted in aphid colonisation occurring at a range of crop growth stages both within and between sites but usually before the green bud stage (GS202). A phase of exponential increase followed. At five of the sites this phase of rapid increase started between first flower (GS 203) and flat pod on the first truss (GS 205), but slightly earlier at site 6 (visible buds, GS 201). A significant relationship was found between the growth stage at which the phase of rapid increase started and that at colonisation (Spearman Rank Correlation Coefficient: $r_s = 0.64$, $t = 2.63$, $df = 10$, $P < 0.05$).

Peak populations were recorded between GS 203 and GS 207 (Table 2). Within sites population decline started on similar dates in both early and late drilled plots (with a maximum of 5-day difference in timing of population peaks). Within sites, the crop growth stages at which peak population were recorded were usually slightly more advanced in the early drilled plots, and between sites was significantly correlated with the growth stage at which the rapid increase phase started ($r_s = 0.55$, $t = 2.07$, $df = 10$, $P<0.05$). A weak relationship was also found between growth stage at the population peak and the growth stage at colonisation

TABLE 2. Development of pea aphid infestations in untreated plots (% shoots infested).

Site	Crop growth stage				
	201	203	205	207	209
1A	1.0	3.5	32.0	82.0*	0.0
1B	2.5	10.5	85.5*	14.0	0.0
2A	5.0	26.5	56.5	88.0*	1.0
2B	9.5	23.8	81.0*	8.0	2.0
3A	12.5	61.0	100.0*	98.0	10.0
3B	41.0	85.0	100.0*	5.0	7.0
4A	9.0	18.5	35.5	81.0*	0.0
4B	25.5	47.0	94.5*	87.5	0.0
5A	4.5	37.5	87.5	99.5*	5.0
5B	59.5	73.5	99.5*	32.0	1.0
6A	71.5	88.5*	76.5	21.5	28.0
6b	59.5	86.0*	85.0	11.5	39.5

* denotes the point on the growth stage scale nearest to the peak of aphid infestation

TABLE 3. Effect of insecticide treatment on yield of combining peas (t/ha at 85% DM).

Site	Treatment						
	T1	T2	T3	T4	T5	SED	CV%
1A	5.02	5.26	5.30	5.36	5.52	-	5.5
1B	5.69	5.46	5.59	5.75	5.55	-	6.2
2A	6.38	6.39	6.59	5.88	6.82	0.48	10.7
2B	6.13	6.63*	7.05*	6.97*	6.59	0.22	4.6
3A	3.97	5.02*	5.23*	5.28*	5.19*	0.16	4.5
3B	4.30	4.91*	5.01*	5.37*	5.22*	0.24	6.8
4A	3.08	3.36	3.43	3.44	3.45	0.22	9.5
4B	2.22	2.35	2.16	2.45	2.21	0.33	21.1
5A	4.82	5.50	5.37	5.11	5.25	0.64	11.6
5B	4.10	4.67	4.06	5.25	4.97	0.64	19.7
6A	5.30	6.00	5.60	6.00	5.80	0.27	6.6
6B	4.90	5.50*	5.30	5.60*	5.10	0.13	3.4

*denotes that yield is significantly different from the untreated control ($P < 0.05$)

($r_s = 0.53$, $t = 1.98$, $df = 10$, $P < 0.05$). Peak aphid populations ranged from 81% to 100% shoots infested and were followed at most sites by a rapid decline in numbers to zero, apart from site 6 where a very early population decline was followed by a partial recovery of aphid numbers (Table 2).

Effect of insecticide treatment on yield

During the phase of exponential increase, populations often rose from 10% to more than 60% shoots infested within a week and in treatments 3 and 4 the infestation levels were sometimes well over the 20% shoots infested threshold when sprays were applied. As this would probably reflect the situation encountered on a commercial farm due to delays in treatment application after assessment, no attempt was made to correct the data for this paper.

Effective control of pea aphid infestations was achieved by all treatments applied. The multiple aphicide regime (T4) resulted in a mean yield increase of 12.1%, a positive yield increase in 11 of the 12 sites and statistically significant increases at four sites (Table 3). Positive yield increases were also obtained from all single spray regimes at most sites. Mean yield increases of 9.6% were recorded from treatment T2, 8.9% from T3 and 10.7% from T5. Statistically significant increases were recorded for all single spray regimes at two sites, from all except treatment T5 at site 2B and for treatment T2 at site 6B.

Statistically significant differences in thousand seed weight were recorded at all except sites 2A and 2B (Table 4). Mean seed weight increases of 2.8% were obtained from treatment T2, 6.1% from T3 and 5.2% from T5. The multiple spray regime resulted in an 8.0% increase. Of the ten sites where significant responses to treatment were recorded, four showed significant increases in seed weight resulting from treatment T2, eight from T3, nine from T5 and all ten from the multiple spray regime.

TABLE 4. Effect of insecticide treatment on 1000 seed weight (g).

Site	Treatment						
	T1	T2	T3	T4	T5	SED	CV%
1A	305	313	322*	329*	320*	5.40	2.4
1B	308	318	327*	336*	329*	5.20	2.3
2A	651	641	672	643	673	17.48	3.8
2B	649	651	672	664	651	16.94	3.6
3A	271	279	294*	304*	286*	4.20	2.1
3B	257	261	263	288*	272*	5.20	2.7
4A	223	239*	240*	251*	245*	6.39	3.8
4B	216	221	233	248*	247*	10.65	6.6
5A	284	310*	319*	337*	303*	6.20	3.4
5B	266	289*	293*	306*	300*	6.20	3.0
6A	342	360*	361*	372*	358*	6.90	2.7
6B	319	322	343*	340*	321	7.66	3.3

*denotes that seed weight is significantly different from the untreated control ($P < 0.05$)

DISCUSSION

The range of growth stages over which the rapid decline in aphid numbers occurred (GS203-207) was similar to those found in earlier work, GS203-209 (Lane and Walters, 1991). As current action thresholds assume a continued population increase after assessment if treatment is not applied, an accurate forecast of the timing of this natural decline may be needed if the current management strategy is to be improved, particularly in situations where it occurs early in the crop growth cycle. At all sites aphids colonised crops before flowering but, although both the time of colonisation and the time at which rapid population increase starts influenced the time of peak aphid population, the relationships between these factors are not strong and could not be used to form the basis of a forecasting system on their own. However, work is underway to integrate these relationships with other factors that influence the pattern of population growth (Dunn and Wright, 1955), to simulate the timing of population decline (Aegerter & Walters, in prep).

The period of crop growth between flowering and pod formation has been shown to be most sensitive to aphid damage (Maiteki and Lamb, 1985) and coincided with the period of rapid population increase and thus application of control measures in this study. Larger aphid populations were recorded in the current work than in the earlier study (Lane and Walters, 1991), and consequently, bigger yield responses to insecticide applications were obtained. However, the large variation in yield between plots situated in different areas of a commercial field which has been highlighted before (Lane and Walters, 1991) were demonstrated again, and resulted in only four sites showing statistically significant yield increases. The variability in yields may be due to a variety of factors including aphid and crop biology, soil conditions (Lane and Walters, 1991) and harvesting difficulties. However, positive yield responses were recorded from all treatment regimes applied. The multiple spray regime resulted in either two or three sprays being applied at all sites, but in no case gave a significant yield improvement over a well-timed single spray.

Errors resulting from the variability in yield between small plots did not have such a great effect on the assessment of thousand seed weight, and statistically significant responses were obtained from 10 of the 12 sites. These results confirmed earlier work (Lane & Walters, 1991) which showed that increased yields after aphicide application were largely due to increased seed weight. Significant increases in seed weight were recorded from the multiple treatment plots, all but two of those treated with single sprays at 20% shoots infested, and all but one of those treated with a single spray at 50% shoots infested. However, only four sites showed a significant increase in seed weight after applying an insecticide at 10% shoots infested.

Where the action threshold currently advised by ADAS (20% or more plants infested and populations increasing), was exceeded, positive yield responses to insecticide application were obtained at all sites in this study. Thus, the timing of the sprays triggered by this threshold to control aphid population development do protect the crop during the growth stages susceptible to damage. However, significant improvements in yield were not always recorded, illustrating the difficulty in obtaining repeatable results showing the effect of management strategies from small scale experimental population manipulations. This is partly due to the complex biology of the pea aphid, and further work is underway to investigate the effect of a variety of factors on the phenology and scale of pea aphid population dynamics, and to refine and improve the action threshold.

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TEFLUTHRIN - A CEREAL SEED TREATMENT FOR THE CONTROL OF WHEAT BULB FLY (*DELIA COARCTATA*)

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ABSTRACT

Tefluthrin is a novel pyrethroid insecticide with physical and chemical properties which make it ideal for use as a soil insecticide. This paper describes replicated field trials in which tefluthrin, formulated for use as a cereal seed treatment, was tested against wheat bulb fly (*Delia coarctata*). Tefluthrin had no phytotoxic effects at rates up to 60g AI/100kg of seed. Application rates as low as 20g AI/100kg seed had a high level of activity against the pest.

INTRODUCTION

Delia coarctata (Diptera; Anthomyiidae) is the most damaging soil pest of cereals in eastern England and parts of Scotland and can occur as far west as Gloucestershire and Wiltshire. All varieties of wheat, barley and rye can be attacked. Attacks are more likely on heavy land following a fallow and on lighter soils after root crops, potatoes or peas.

D. coarctata has one generation per year. Adults lay eggs on bare or open soils during July and August. Eggs hatch between December and March and the larvae bore into the base of cereal plants where they feed. Damage to the crop can be seen between February and April. Generally, the central leaf withers and dies, producing a characteristic 'dead-heart'. Attacks on late drilled crops can be particularly devastating. Plants that have not produced tillers can be killed outright and germinating seeds can be killed before emergence.

Tefluthrin is a novel pyrethroid insecticide effective against a wide range of soil pests (Jutsum et al, 1986; Marrs & Gordon, 1987). Tefluthrin has little effect on non-target organisms (Dewar et al, 1988) and is not persistent in the soil. To protect cereals from *D. coarctata*, tefluthrin was specially formulated in a slow release micro-capsule suspension for use as a seed treatment. This paper describes a representative selection of field trials conducted to test this formulation against *D. coarctata*.

MATERIALS AND METHODS

Over thirty small plot field trials, testing efficacy and crop safety, were conducted in wheat and barley in the UK between 1988 and 1994. The trials covered a wide range of soil types, drilling dates, crop varieties and pest populations. All seed was treated with fungicide seed treatments to control seed and soil borne diseases. All seed treatments were applied using Mini Rotostat or commercial seed treatment machinery. The trial plots were sown using small plot drills and harvested by Claas Compact or Hege plot combine harvesters.

Crop safety was determined by monitoring crop emergence, establishment and yield in the absence of pests. Activity against *D. coarctata* was determined by monitoring crop establishment, the appearance of 'dead-hearts' and crop yield under a range of pest pressures. Site details for the trials presented in this paper are shown in the tables below.

TABLE 1. Site details for crop safety trials.

	Site 1	Site 2	Site 3
Crop	Wheat	Wheat	Wheat
Cultivar	Beaver	Mercia	Beaver
Drilling date	14.12.92	25.10.93	27.10.93
Soil type	Clay loam	Peat	Sandy loam
Location	Yorkshire	Lincolnshire	Yorkshire

TABLE 2. Site details for pest control trials.

	Site 4	Site 5	Site 6	Site 7
Crop	Wheat	Wheat	Wheat	Wheat
Cultivar	Beaver	Mercia	Beaver	Beaver
Drilling date	10.11.92	18.1.93	6.12.93	21.1.94
Soil type	Sandy loam	Clay loam	Sandy loam	Peat
Location	Lincolnshire	N.Humberside	Yorkshire	Cambridgeshire
Egg Count (million/ha)	11.0	11.2	6.6	6.8
Previous crop	Bare fallow	Vining peas	Potatoes	Sugar beet

All the trials were of a randomised block design with four to ten replications. Assessment data were analysed using an analysis of variance and, in cases where the effect of treatment was found to be statistically significant (at the 5% probability level), an LSD test was used to differentiate between individual treatment means. For all the assessment data described in this paper, treatment means with no letter in common are significantly different at the 5% probability level.

RESULTS AND DISCUSSION

Crop Safety

Tefluthrin seed treatment was tested in crop safety trials with a range of standard fungicide seed treatments. Tefluthrin treated seed showed no symptoms of phytotoxicity in any trial. Typical crop safety effects are shown in Table 3 where tefluthrin, applied at 40 and 60g AI/100kg seed, had no adverse effect on crop emergence and establishment.

TABLE 3. Site 1: Effects of tefluthrin on emergence and establishment of wheat (plants per m^2) in the absence of pests when applied with two different fungicide seed treatments.

Seed treatment	Rate g AI per 100kg seed	thiabendazole + carboxin		triadimenol + fuberidazole	
		Emergence	3 leaves	Emergence	3 leaves
No insecticide	-	328	432	256	448
Tefluthrin	40	344	432	240	432
Tefluthrin	60	328	432	264	456

Tefluthrin compared favourably with standard insecticide seed treatments which often caused delayed emergence and occasionally resulted in reduced plant populations when applied at recommended rates. Examples from two trials are shown in Table 4.

TABLE 4. Effect of insecticide seed treatment on emergence and establishment of wheat (plants/m^2).

Seed treatment	Rate g AI per 100kg seed	Site 2		Site 3	
		Emergence	3 leaves	Emergence	3 leaves
No insecticide	-	171 a	414 a	215 a	294 a
Tefluthrin	20	160 a	422 a	206 a	307 a
Fonofos	108	60 b	409 a	82 b	287 a
Chlorfenvinphos	97	6 c	318 b	37 c	254 b

A satisfactory level of crop safety is an essential characteristic for a seed treatment intended for the control of *D. coarctata*. High risk fields are often drilled into cold, rough seedbeds after late-harvested crops such as sugar beet. Delays in emergence at this time of year can have an adverse effect on plant establishment and can give rise to crops that are less well developed and so more susceptible to attack by *D. coarctata*.

Pest control

To determine the activity of tefluthrin against *D. coarctata* field trials were conducted with a wide range of drilling dates, soil types and pest populations. In every one of these trials tefluthrin seed treatments performed at least as well as the standard insecticide seed treatments and were sometimes more effective. Typical examples are shown in Tables 5-8.

TABLE 5. Site 4: Effect of seed treatments on crop establishment, number of 'dead-hearts' and yield in a November drilled crop of wheat: Lincolnshire, 1992.

Seed treatment	Rate g AI per 100kg seed	Healthy tillers per m^2 after attack	Ears per m^2	Crop yield t/ha
No insecticide	-	29 a	22 b	1.0 b
Tefluthrin	20	322 b	245 a	6.1 a
Fonofos	108	235 b	240 a	6.0 a

In this trial cold conditions following drilling reduced the rate of plant growth and resulted in a crop that had produced few tillers by the time the larvae of *D. coarctata* started to invade. A fine tilth allowed the larvae to move freely through the soil and resulted in a very high proportion of tillers becoming infested in plots with no insecticide seed treatment. Both tefluthrin and fonofos seed treatments reduced infestation significantly and helped produce a satisfactory plant stand (Table 5). Crop yield was increased by 5 t/ha as a result of controlling the pest.

TABLE 6. Site 5: Effect of seed treatments on crop establishment, number of 'dead-hearts' and yield in a January drilled crop of wheat: North Humberside, 1993.

Seed treatment	Rate g AI per 100kg seed	Healthy tillers per m^2 after attack	Ears per m^2	Crop yield t/ha
No insecticide	-	54 a	132 c	3.7 c
Tefluthrin	20	392 b	373 a	7.9 a
Fonofos	108	316 b	305 b	6.9 b

This trial was drilled after the start of egg hatch allowing larvae to attack during the early stages of plant growth. Significant plant losses had occurred before the plants had produced tillers and continued plant invasion resulted in the death of more tillers. By the time the attack was completed the plant population in untreated plots was only 14% of that in the tefluthrin-treated plots. Good soil conditions allowed compensatory tiller development but crop yield was reduced by over 4 t/ha. Tefluthrin was more effective than fonofos seed treatment.

A comparison of these two trials shows that the damage potential of *D. coarctata* is not a simple relationship between pest population and crop growth stage at the time of egg hatch. Soil conditions affect the ability of the larvae to locate host plants and also affect the ability of the crop to compensate for plant losses.

TABLE 7. Site 6: Effect of seed treatments on establishment, number of 'dead-hearts' and yield in a December drilled crop of wheat: Yorkshire, 1993.

Seed treatment	Rate g AI per 100kg seed	Healthy tillers per m^2 after attack	Ears per m^2	Crop yield t/ha
No insecticide	-	102 c	98 b	5.7 b
Tefluthrin	20	434 a	257 a	10.4 a
Chlorfenvinphos	97	285 b	257 a	10.1 a

TABLE 8. Site 7: Effect of seed treatments on establishment, number of 'dead-hearts' and yield in a January drilled crop of wheat: Cambridgeshire, 1994.

Seed treatment	Rate g AI per 100kg seed	Healthy tillers per m^2 after attack	Ears per m^2	Crop yield t/ha
No insecticide	-	52 b	77 c	2.0 b
Tefluthrin	20	190 a	275 a	5.6 a
Chlorfenvinphos	97	58 b	158 b	3.8 b

CONCLUSIONS

Tefluthrin seed treatment has been tested for crop safety, at application rates up to 60g AI/100kg seed, with a number of fungicide seed treatments. Trials have been conducted with a wide range of drilling dates and soil types. No symptoms of phytotoxicity have been seen in any trial.

The activity of tefluthrin against *D. coarctata* has also been tested at a wide range of drilling dates and soil types and under extremes of pest population. Tefluthrin, applied at 20g AI/100kg seed, has shown consistently high levels of control and has proved to be at least as effective as any of the products currently approved for this use.

ACKNOWLEDGEMENTS

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SUPPRESSION OF THE SPREAD OF POTATO LEAFROLL VIRUS AND POTATO VIRUS Y BY APHICIDE SPRAYS

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ABSTRACT

The efficacy of pirimicarb and a mixture of deltamethrin and heptenophos was assessed against potato-infesting aphids and the subsequent spread of Potato Leafroll Virus (PLRV) and Potato Virus Y (PVY^N). Both spray products reduced aphid numbers. The spread of PLRV was reduced by both types of aphicidal spray, the least effective treatment being a single, late application. Spread of PVY^N to adjacent plants was controlled by use of the mixture of deltamethrin and heptenophos, but not by pirimicarb.

INTRODUCTION

The ability of insecticides to suppress the spread of potato leafroll virus (PLRV), is well known (Woodford *et al.*, 1983). PLRV is transmitted by aphids, principally by the peach-potato aphid, *Myzus persicae*. There is, however, little published about the efficacy of modern insecticide products against the various strains of potato virus Y (PVY), and none within Scotland, where there is a need to protect the quality of seedstocks by use of aphicidal sprays. PVY is spread in the non-persistent manner, with a very brief acquisition period and immediate ability to transmit the virus by the vector. Again, *M. persicae* is an efficient vector of PVY, but aphids that do not colonise potatoes are also able to transmit this virus by non-feeding probes during host plant selection. PVY has several strains causing "severe mosaic" symptoms in potatoes, and one, tobacco veinal necrosis (PVY^N), which produces only a slight mottle (Beemster & Rozendaal, 1972). Synthetic pyrethroids can reduce the spread of some aphid-borne, non-persistent viruses. Pyrethroids have repellent and antifeedant properties which may contribute to control, but it is likely that intoxication of viruliferous aphids by extremely low doses is largely responsible for their effect (Gibson & Campbell, 1986). Mixtures of non-fumigant, non-systemic pyrethroids with systemic and fumigant organophosphorus insecticides give good control of both aphids and persistent virus spread, also being claimed to provide better control than when either type of insecticide is used alone.

The present trial was designed to compare the efficacy of several aphicide spray products, this report being concerned with those products currently approved for use in the UK. The unusual abundance of peach-potato aphids early in the season provided an excellent opportunity to assess efficacy and to reveal the difference in performance between a mixture and a carbamate insecticide used alone.

MATERIALS AND METHODS

Crop details and trial design

Potatoes (cv. Maris Piper, grade Super Elite 2) were planted into a sandy loam field at Auchincruive on 15 May 1992 at 71 cm row spacing. Brestan 60 (WP containing 540 g AI/kg fentin acetate and 160 g AI/kg maneb) was applied three times for the prevention of late blight (*Phytophthora infestans*).

The experimental area was divided into four blocks, 20 rows by 84 m. Each block was divided into six plots 14 m square, within each of which six infector units were planted. The latter comprised four PLRV-infected tubers cv. Romano, in rows 5 and 15, five metres from the end of each row, and two PVY^N-infected tubers cv. King Edward, planted similarly in row 10. Six treatments were randomly assigned within each block, the four presented here being: (1) untreated controls, sprayed with water twice; (2) pirimicarb at 140 g AI/ha (Aphox) sprayed twice; (3) deltamethrin at 7.5 g AI/ha + heptenophos at 120 g AI/ha (Decisquick) sprayed once; (4) the deltamethrin + heptenophos mixture sprayed twice. The treatments were applied in 200 litres water/ha using a hand-held boom fitted with four F11003 Tee-Jet nozzles, pressurised to 200 kPa with a compressed air Azo Trial Plot Sprayer. The measured output was 3.9 litres per plot. Pesticides were applied at minimal wind speed to avoid drift between plots. The treatment dates were 27 June and 9 July, treatment 3 being applied on the second occasion.

Aphid sampling

Aphid populations were estimated on 9 June and 1 July in the field by beating plants onto white boards. Subsequent counts were based on samples of upper, middle and lower leaves (C_u, C_m, C_l) on each of a series of randomly chosen haulms in each plot. Thirty per cent of upper leaf samples included the associated flower head. The number of haulms per planting position (H), and the numbers of upper, middle and lower leaves per haulm (U, M, L) were estimated so as to provide a weighted estimate of the number of aphids per planting position, e.g. with 10 leaves of each type sampled per plot:

$$\text{Aphids per planting position} = \frac{H(UC_u + MC_m + LC_l)}{10}$$

Samples of *M. persicae*, for the purpose of resistance-typing, were taken on 7 July from control plots, and on 11 August from the control plots and those treated twice either with deltamethrin + heptenophos or with pirimicarb. Plants were searched in a transect across plots, taking young nymphs, which were reared to 4th instars or adults on prior to testing by an esterase immunoassay (Devonshire *et al.*, 1992).

Infector unit sampling and assessment

Six tubers were harvested from each of the "inner", "outer" and "neighbouring row" positions comprising an infector unit (**i**, **o** and **n** in Figure 1). These were stored and planted in 1993. The incidence of PLRV was assessed in July by inspection of each plant for characteristic, leaf-roll symptoms. Field assessment of PVY^N was difficult because of poor symptom expression, with too many borderline assessments. Estimates of PVY^N infection were therefore based on an antibody-trapped antigen form of enzyme-linked immunosorbent assay (ATA-ELISA), using the procedures described by Barker et al. (1993). One middle leaf was collected on 12 July from each of the six plants grown-on from inner positions, this procedure being repeated on 4 August for plants grown-on from the outer and neighbouring row positions. PVY^N assessments were made on one pooled sample per planting position, consisting of a slice, weighing c. 1 g, cut through a "sandwich" of the six terminal leaflets removed from the collected leaves. Leaf sap was extracted in 10 ml buffer using a Pollahne leaf press. Two replicate wells were used for each test sample.

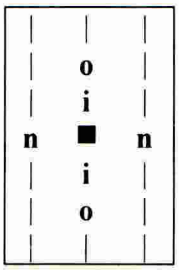

FIGURE 1. Plan of the harvesting of the each infector unit. Each symbol represents a planting position, ■ being position of the infected tuber, **i**, **o** and **n** indicating the "inner", "outer" and "neighbouring row" positions.

Analysis of results for PVY^N was confined to infection of each planting position around an infector unit, whereas spread to a sample of daughter tubers from planting positions around the PLRV infector units was also possible.

RESULTS

Aphids

Migrants of the main potato-infesting species were unusually numerous in 1992, with, for example, 75 *M. persicae* alatae recorded on 200 untreated plants on 1 July. The peak numbers of *M. persicae* (147 per planting position in control plots on 13 July) occurred earlier than those of the most abundant species, the potato aphid, *Macrosiphum euphorbiae*, with a peak of 6,951 aphids per planting position in control plots on 27 July (Figure 2). The numbers of all species were reduced by a minimum of 76% of control values by all treatments, without evidence either of differences between species or of population resurgences. The numbers of *M. persicae* that could be found and tested successfully for resistance type were low because of these high levels of population reduction and also because of infection with fungi (*Entomophthora* spp). The most resistant form, "R3" (ffrench-Constant & Devonshire, 1986), was detected in samples from the pesticide-treated areas, but not in the samples from untreated areas, which were dominated by R1 and R2 forms, with occasional S forms.

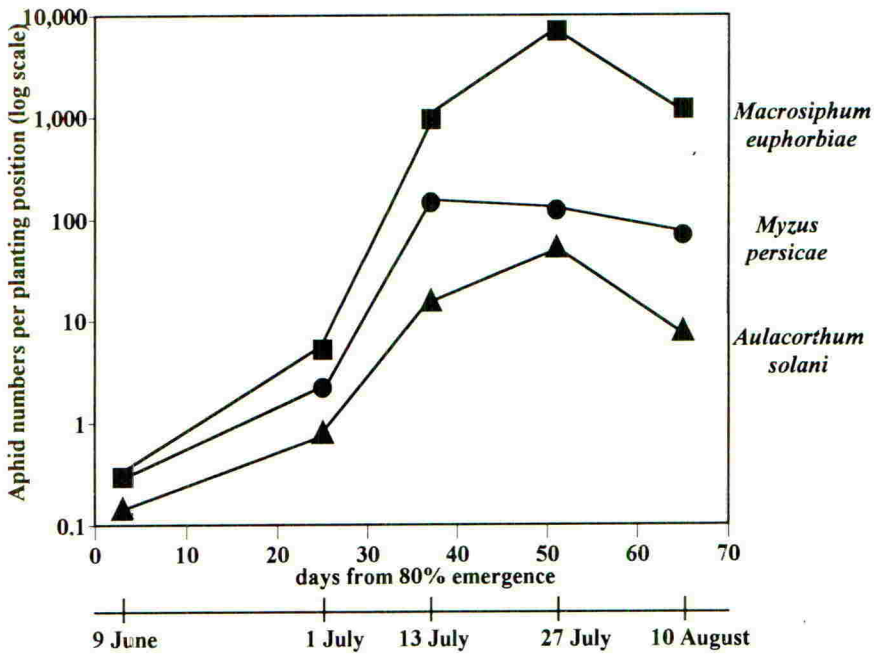

FIGURE 2. Aphid numbers in untreated plots of potatoes in 1992.

Viruses

One block had no PLRV spread and it was excluded from the analysis in Table 1. Block-by-block inspection of the data did not reveal any trends to explain the patchiness of PLRV infection in terms of differing levels of aphid attack. All treatments suppressed the spread of PLRV, the least effective being the single, late application of deltamethrin + heptenophos (Tables 1 and 2). With the exception of pirimicarb, the incidence of infection with PLRV was highest in the plants next to the infectors within the same row and least in other planting positions tested, confirming that background spread was negligible. Pooled control values for daughter tuber infection (Table 1) and for planting position infection (Table 2) were significantly different from any treatment as assessed by calculation of exact probabilities from contingency tables. Valid confidence limits could not be calculated for the figures in Table 2, each being based on 16 observations, as opposed to the larger number (> 139) of observations on daughter tubers in Table 1.

Out of 96 samples tested for PVY^N from the inner plant positions, 28 were clearly infected (substrate absorbance at 405 nm more than twice that of virus-free samples), and four were on the borderline and regarded as infected. Outer and neighbouring row positions yielded far fewer samples infected with PVY^N and no borderline cases. The results for PVY^N infection of planting positions (Table 2) were more variable than those for PLRV. Differences between the proportions of samples infected from the inner plants were significant for the control plots and for pirimicarb versus the use of deltamethrin + heptenophos (exact probability tests, $P<0.05$). There were no significant differences based on pooled results for inner, outer and neighbouring rows.

TABLE 1. Percentages of daughter tubers infected with PLRV in stock grown-on from infector units exposed to a range of insecticidal treatments in 1992. Figures in parentheses are the 95 per cent confidence limits based on the binomial distribution.

	Inners	Outers	Neighbouring rows	Overall
PLRV				
control	35 (±8)	20 (±7)	11 (±5)	18
pirimicarb twice	2 (±2)	4 (±3)	2 (±2)	2
deltamethrin + heptenophos once	18 (±6)	5 (±4)	0	7
deltamethrin + heptenophos twice	6 (±4)	6 (±4)	0	4

TABLE 2. Percentages of planting positions infected with aphid-borne viruses in stock grown-on from infector units exposed to a range of insecticidal treatments in 1992.

	Inners	Outers	Neighbouring rows	Overall
PLRV				
control	38	31	25	31
pirimicarb twice	6	13	13	10
deltamethrin + heptenophos once	19	19	0	13
deltamethrin + heptenophos twice	19	6	0	8
PVY^N				
control	44	0	6	17
pirimicarb twice	56	0	6	21
deltamethrin + heptenophos once	25	19	0	15
deltamethrin + heptenophos twice	31	0	19	17

DISCUSSION

The results for PVY^N, which were based on half as many infector plants as PLRV and on bulked ELISA assessment of leaflet samples from each planting position, were not as consistent as for PLRV. Nevertheless, the results clearly indicated that the mixtures reduced PVY^N spread whereas pirimicarb failed to give control. This is in contrast to the result for the spread of PLRV, of which both products gave good control when applied twice.

The results demonstrate that a pyrethroid/organophosphorus insecticide mixture can suppress spread of both of the major aphid-borne viruses of potatoes whereas pirimicarb controls only PLRV. It is likely that a similar picture would emerge when comparing mixtures with other cholinesterase-inhibiting insecticides. Both products appeared to select for R3 aphids.

On the basis of infection of the plants next to infectors within the rows, two applications of deltamethrin + heptenophos resulted in 50% suppression of PLRV and 30% suppression of PVY^N in a year with an unusually high number of *Myzus persicae*. Such treatments should not be claimed to be a substitute for roguing, which would reduce infection levels yet further; however, roguing cannot take place until symptoms are detectable and is in any case difficult for PVY^N. Virus transmission is more likely early in the season when the plants are more

susceptible to infection; spraying therefore provides vital protection before roguing can take place.

The danger of delaying the first application was evident from PLRV infection. PLRV spread to inner plants was the same for one and two applications of deltamethrin + heptenophos (19%), but the proportion of infected daughter tubers was reduced by two-thirds by the inclusion of an earlier application. This did not occur with PVY^N, differences between a single, late application and two applications being non-significant, though each was significantly less than spread in the untreated plots.

The advisory policy of SAC is to recommend the use of insecticide mixtures for virus suppression, despite the reputed environmental advantages of the use of pirimicarb alone. The SAC policy also precludes giving advice about aphid control *per se*, since it is suppression of virus that is all-important, this being not necessarily associated with perfect aphid control. The industry must understand that trials demonstrating aphid control in potatoes have no value if they fail to demonstrate suppression of the spread of aphid-borne viruses.

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EFFECTS OF MINERAL OIL APPLICATIONS ON APHID BEHAVIOUR AND TRANSMISSION OF POTATO VIRUS Y

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ABSTRACT

Aphids (adult apterous *Myzus persicae*) were allowed a single, video-monitored stylet penetration of a potato virus Y (PVY)-infected tobacco leaf disc which had been painted or sprayed with 1% mineral oil. The oil was applied either as an ethanolic solution or an aqueous suspension. Following natural withdrawal of the stylets, aphids were immediately transferred to individual tobacco test plants (2-3 leaf stage), where they remained overnight for inoculation access. All applications of oil to PVY-infected leaves significantly delayed the initiation of stylet penetration by aphids and also reduced their ability to transmit PVY to test plants, compared with aphids on the solvent-treated control leaves. When aphids were given an opportunity to acquire the virus and then allowed a single electrically-recorded penetration of a test plant, inoculation of this plant was not reduced when oil was present on the leaf surface, but the oil treatment reduced subsequent virus transmission to untreated test plants.

INTRODUCTION

Applying mineral oil to plants has been shown to reduce non-persistent transmission of plant viruses by aphids in laboratory and field studies (Bradley *et al.*, 1962; 1966; Vandervecken, 1977; Simons & Zitter, 1980). A reduction in virus transmission is achieved if oil is brought into contact with aphid mouthparts before acquisition of virus, or between virus acquisition and inoculation, and even a single brief labial contact with an oil-covered surface can be effective (Bradley, 1963; Simons *et al.*, 1977; Powell, 1992). Protection therefore depends on obtaining an even cover of oil over plants, to ensure that aphids have a good chance of encountering oil before stylet insertion (during labial exploration of the leaf surface) or during penetration of the leaf. Oil is usually sprayed onto plants in aqueous suspension, and, because of the hydrophobic properties of cuticular waxes, forms discrete droplets on the surface. Gibson *et al.* (1988) suggested that aphids probing virus-infected plants through oil droplets do not transmit virus, but those probing between droplets were able to acquire and subsequently transmit. Plants are usually left for several hours after spraying before testing for the effects of oil the laboratory, as the droplets eventually form a continuous film (Gibson *et al.*, 1988). Aqueous oil has also been painted onto leaves in laboratory tests, but ethanolic treatments of plant surfaces often give a better cover of leaves (e.g. for insect antifeedant applications: Gibson *et al.*, 1982; Powell *et al.*, 1994). The aim of these laboratory experiments was to compare the effects of spraying and painting oil onto plants, in aqueous suspension and ethanolic solution, on

the behaviour of the aphid *Myzus persicae* and non-persistent transmission of potato virus Y (PVY).

MATERIALS AND METHODS

Culture of aphids, plants and virus

A clone of *M. persicae* was reared on Chinese cabbage (*Brassica Pekinensis* cv. 'Tip Top') in a controlled environment room at 15°C and 16 h:8 h light:dark photoperiod. Apterous adult aphids were collected using a camel hair brush and starved for 1-2h in plastic Petri dishes before use. Tobacco (*Nicotiana tabaccum* cv. 'White Burley') was used as a virus host; test plants were inoculated at the 2-3 leaf stage and used as sources of virus 13-20 d later. The tobacco veinal necrosis strain of PVY was maintained in tobacco by aphid transmission.

Mineral oil applications

Sunoco 7E mineral oil, containing its own emulsifier at 1.2%, was prepared at 1% in water or ethanol. The oil was mixed by shaking, and aqueous oil formed a cloudy suspension, whereas the ethanolic oil was a clear solution. Each formulation of oil was sprayed onto upper leaf surfaces using an aerosol spray (Sigma product no. S 8399) held above the plants at a distance of 30 cm. This method deposited approximately 15 μl of ethanolic treaments and 20 μl of aqueous treatments per cm^2 leaf surface. Sprayed plants were left >24 h before use. The oil treatments were also painted onto upper leaf surfaces, at approximately 2.5 (ethanolic) or 2.0 (aqueous) $\mu l/cm^2$. Painted ethanolic applications dried rapidly, and were used after 2 min, but aqueous oil was used after 15 min.

Oil treatment of virus source plants

Close-up video recording (Hardie *et al.*, 1992) was used to investigate the effects of oil treatments on aphid behaviour. Leaf discs 16 mm diameter were cut from an inter-veinal area of the oil- or solvent-treated PVY-infected 'source' plants and floated on water in a transparent dish (Powell *et al.*, 1994), positioned below a video camera with macro lens. A time-base enabled display of a clock accurate to 0.1 s on the monitor. Using a hand-held microaspirator, aphids were placed onto the discs and their behaviour video recorded until antennal movements (Hardie *et al.*, 1992; Powell *et al.*, 1993) indicated that a single stylet penetration had been completed. Aphids were then immediately transferred to an untreated test plant and confined overnight for 15-20 h inoculation access. Video tapes were analysed for behavioural parameters; stylet penetration times and pre-penetration times were normalised by $\log_{10}$ transformations (Hardie *et al.*, 1992) and analysed using Student's t tests.

Oil treatment of virus test plants

Aphid stylet activities and consequent PVY transmission efficiencies were investigated as described previously (Powell, 1991), by electrically recording (Tjallingii, 1988) the acquisition and inoculation processes. Aphids were attached to a fine gold wire using

conductive silver paint, and allowed a single penetration of an untreated virus source leaf. Following electrical recording of this penetration, each aphid was immediately transferred to the first leaf of a 'test' plant (test 1), which had been painted with aqueous or ethanolic oil, or with the solvent treatments, and a further single penetration was recorded. The insects were then removed from the wire and placed on a second, untreated test plant (test 2) overnight. Electrical signals were recorded on a chart recorder (Graphtec WR7200) at a paper speed of 2.5 mm/s, for measurement of stylet penetration times.

Assessment of virus transmission efficiencies

Following inoculation access periods, all test plants were transferred to a glasshouse kept free of aphids by regular nicotine fumigation, and symptoms of systemic PVY infection scored after 10-14 d. PVY transmission efficiencies were calculated as the proportions of aphids which transmitted the virus to test plants, and compared using the chi-square (χ^2) test.

RESULTS AND DISCUSSION

Oil treatment of virus source plants

Video recording aphid behaviour on leaf discs (Tables 1 & 2) showed that oil caused a small but significant delay in the initiation of stylet penetration, as reported previously (Wyman, 1971; Simons *et al.*, 1977; Powell, 1992). Aphids on leaf surfaces sprayed with ethanolic oil made stylet penetrations of a shorter duration than those on control-treated leaves, but none of the other oil treatments affected this parameter.

TABLE 1. The effects of painted applications of mineral oil on video-recorded stylet penetration parameters and PVY transmission efficiencies by *M. persicae*. n=30; ***P<0.001; **P<0.01; *P<0.05; ns = no significant difference, Student's t test (penetration parameters) or χ^2 test (transmission efficiences), cf. solvent controls.

	penetration parameters		
Treatment of leaf disc	Mean±SE log pre-penetration time (s)	Mean±SE log penetration duration (s)	PVY transmission efficiency (%)
water	0.96±0.05	1.11±0.03	56.7
	*	ns	***
aqueous oil	1.15±0.04	0.98±0.04	3.3
ethanol	0.99±0.03	1.21±0.03	60.0
	*	ns	***
ethanolic oil	1.18±0.05	1.15±0.05	6.7

All applications of oil to source leaves significantly reduced virus transmission, compared with the corresponding solvent control treatments. The oil-induced reduction in virus transmission cannot be accounted for by its behavioúral effects on aphids (Powell, 1992); oil probably acts by disrupting the interactions between non-persistent viruses and their aphid retention sites (Qui & Pirone, 1989), or by interfering with the initiation of virus infection following delivery of virus into plant cells by aphids (Powell, 1991; Powell *et al.*, 1992). Similar reductions of virus transmission occurred when the oil was applied in aqueous suspension, or in ethanolic solution. The results therefore suggest that cover of the leaf surface was not limiting in these experiments. Remarkably, although spraying deposited approximately ten times more oil on leaf surfaces than the painted applications, the two application methods had very similar effects on PVY transmission by aphids (Tables 1 & 2).

TABLE 2. The effects of sprayed applications of mineral oil on video-recorded stylet penetration parameters and PVY transmission efficiencies by *M. persicae*. n=30; ***P<0.001; **P<0.01; *P<0.05; ns = no significant difference, Student's t test (penetration parameters) or χ^2 test (transmission efficiences), cf. solvent controls.

	penetration parameters		
Treatment of leaf disc	Mean±SE log pre-penetration time (s)	Mean±SE log penetration duration (s)	PVY transmission efficiency (%)
water	0.98±0.07	1.20±0.03	50.0
	***		**
aqueous oil	1.44±0.09	1.21±0.07	10.0
ethanol	0.95±0.05	1.15±0.04	66.7
	***	*	***
ethanolic oil	1.39±0.07	1.03±0.06	3.3

Oil treatment of virus test plants

When stylet penetration of test plants was electrically recorded, aphids tested on plants treated with aqueous oil penetrated for longer than those on control, water-treated plants, but the behaviour of insects following application of ethanolic oil was similar to the solvent controls (Table 3). Oil-treatment of the first test plant, on which a single stylet penetration was electrically recorded, did not significantly reduce PVY inoculation of that plant by aphids, compared with the solvent treatments. Subsequent virus transmission to the second, untreated test plant, on which aphids were confined overnight, occurred more frequently than transmission to solvent-treated first test plants, presumably because aphids made multiple stylet penetrations of the second plant. However, virus transmission to the second test plant was significantly reduced by both oil treatments of the first test plant.

The reason that oil did not reduce inoculation of the first plant is not clear. When virus transmission to test 1 and/or test 2 were considered together, oil treatment reduced the numbers of aphids which transmitted PVY, but the reduction was only significant following the aqueous treatment.

TABLE 3. The effects of painted applications of mineral oil on stylet penetration durations and PVY transmission to a test plant (test 1) during single electrically-recorded stylet penetrations, or to a second test plant (test 2) during subsequent 15-20 h inoculation access. n=20; ***P<0.001; **P<0.01; *P<0.05; ns = no significant difference, Student's t test (penetration durations) or χ^2 test (transmission efficiences), cf. solvent controls.

		PVY transmission efficiency (%) to:		
Treatment of test 1	Mean±SE log duration of penetration of test 1 (s)	test 1	test 2	test 1 and/or test 2
water	1.17±0.04	25.0	65.0	70.0
	*	ns	**	*
aqueous oil	1.33±0.05	15.0	10.0	25.0
ethanol	1.30±0.04	35.0	65.0	70.0
	ns	ns	***	ns
ethanolic oil	1.29±0.05	35.0	5.0	40.0

In conclusion, it seems likely that during stylet penetration of oil-treated, PVY-infected source plants, oil contamination of aphid mouthparts occurred and reduced subsequent transmission to test plants. Moreover, although oil-treatment of a healthy test plant did not reduce PVY-infection of that plant during single stylet penetrations, sufficient oil was carried over on aphid mouthparts to inhibit inoculation during subsequent penetrations of a second test plant. Perhaps effective contamination of aphid mouthparts occurs less frequently before or during stylet penetration of an oil-covered leaf than during stylet withdrawal, so that oil treatment of test plants offers less protection during an infective aphid's first penetration than during subsequent penetrations of the same or other plants.

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A SEMI-FIELD METHOD TO ASSESS THE EFFICACY OF INSECTICIDES FOR THE CONTROL OF THE LENTIL SEED BEETLE (*BRUCHUS LENTIS*)

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ABSTRACT

A semi-field method to assess the efficacy of insecticides against the lentil seed beetle, *Bruchus lentis*, is described. Experimental plots were covered by walk-in cages and a fixed number of beetles were released inside before the products were applied. A previous trial had established that there is a good correlation between the number of beetles released and the infestation level of the collected seeds. The first pesticide bioassay showed that this pest was controlled by γ-cyhalothrin or endosulfan sprays during the flowering of the crop. Treatments with carbaryl or malathion were less effective.

INTRODUCTION

The lentil seed beetle is one of the most serious pests of the lentil crop (*Lens culinaris* Medikus) in Spain. In Castilla - La Mancha, the principal Spanish producer region, *Bruchus lentis* Fröehlich (Coleoptera: Bruchidae) is the most important species by far (Mozos Pascual, 1992a). The larval stages develop inside the seeds and when the attack is heavy, much of the crop cannot be sold. This species have a monovoltine cycle and infest the seeds exclusively in the field, never in warehouses. The control measures normally employed in the region consist of the fumigation of seeds immediately following the harvest. This treatment prevents the emergence of the new generation, but not the infestation of the seeds, which occurs previously in the field. In the short-term, the most feasible alternative measure to control this pest is the application of insecticides against the adults in the field, in order to prevent oviposition on the pods. In this work we describe a method, with controlled infestation levels, to evaluate reliably the efficacy of pesticides against *B. lentis* in semi-field conditions.

METHODOLOGY

During 1992 a preliminary trial was planned to establish if artificial infestation of the crop in cages was possible, and to determine the number of individuals which would need to be released to obtain an adequate level of infestation. In 1993 the first trial to evaluate insecticides with controlled infestation levels was developed. Both trials were established on the farm of the C.I.A. Albaladejito near the city of Cuenca (Central Spain). The crops were sown in February (6/2/1992 and 22/2/1993 respectively) using lentil seeds cv. "Magda" at a rate of 100 kg/ha. The trial area in 1992 had been previously fertilized with the compound N:P:K = 8:36:16 at a rate of 200 kg/ha. In both experiments the plots were 10 m^2 (5x2 m), with 10 crop rows each (20 cm between rows). In all cases the plots were weeded by hand.

1992 trial

For this experiment 16 plots were delimited in the trial area. At the beginning of May, just before the bruchids appeared in the crop, 12 plots were covered with walk-in cages made of polypropylene fibre netting (spun-bonded) supported by an iron structure. Four plots had no cages (external plots) to allow monitoring of crop development and natural infestation levels in the area. When the plants inside the cages were at flowering (100% of plants with flowers, 8.6 flowers per plant, n=120), adults of *B. lentis* were released (26/5/1992). At that moment some plants had a few young pods (30%, 3.5 pods per plant, n=120). The quantities of beetles released per cage were 20, 40, 80, 160 and 320, with two replicates for each treatment. The sex-ratio was 1:1, which is the pattern of the species in natural conditions. Two cages were not infested to detect possible interference from the external bruchid population. The beetles had been maintained in a fridge at 3-5 °C during the autumn and winter; 5-7 days before release they were brought out and fed with fresh flowers of *Hypecoum* sp. (Papaveraceae) which are frequently used as a food source by *B. lentis* in the field before the lentil flowering period (Mozos Pascual, 1992c). In the first days of June, a great number of aphids (*Acyrthosiphon pisum* and *Aphis* sp.) were detected inside some cages. To control the aphid proliferation, 100 adults of *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) were released in each cage. After harvesting, yield data of external and covered plots were compared using the *t*-test. The infestation levels were calculated on a sample of 400 seeds per plot. The average number of larval penetration holes per seed was recorded from 100 infested seeds per plot. A Pearson correlation test was made on the data to know if variables were correlated.

Evaluation trial: 1993

The cages for this experiment were made of nylon netting, which was more robust than the material used in the preliminary trial. They were installed at the begining of May. The insects were again released during flowering of the plants inside the cages (100% of plants with flowers, 7.6 flowers per plant, n=30), but no pods were detected on the plants this time (1/6/1993). Taking into account the results of the preliminary trial, 200 beetles per cage (sex-ratio 1:1) were released to obtain an infestation level of around 30% of the seeds. To prevent aphid proliferation in the cages, two different control measures were utilized. A preventive treatment with pirimicarb (Z-Z Aphox 50% G) at 250 g AI/ha was applied the day before the installation of the cages. The application of this pesticide (specific for aphids) did not affect the infesting bruchids, which were released one month later. Complementary to that treatment, 100 adults of *C. septempunctata* were released per cage, 50 immediately after installation of the cages and 50 some days after the application of the test pesticides. Four pesticides and an untreated control were replicated three times in a randomized complete-block design. The following chemicals and doses were tested: carbaryl (Suvamil 48% F) at 1320 g AI/ha, γ-cyhalothrin (Karate 2.5% EC) at 15 g AI/ha, endosulfan (Arasulfan 35% EC) at 613 g AI/ha and malathion (Malafin 50% EC) at 1250 g AI/ha. The selected doses were the mean between the maximum and minimum recommended by the respective commercial firms. Out of the trial, three external plots were delimited to compare the development of the crop and the natural infestation levels with those inside the cages (untreated plots). The pesticides were applied in 1 litre of water using a continuous pressure sprayer (Matabi Super 16) supplied with a front quadruple nozzle (hydraulic cone type). The operating pressure was 3 x 10^5 Pa and the flow rate 630 ml/min per nozzle. The same

quantity of water alone was applied to the untreated plots. The pesticides were applied during the midday, three days after insect release. Air temperature 20 cm above the ground (among the plants) oscillated between 27.5 and 31.5 °C inside the cages during the pesticide application, but was higher (28.5 - 35.5 °C) outside the cages for the same period.

To know the effect of the cages on the plants, several parameters (plant height, viable pods per plant, 100 seeds weight and yield) were measured in external and untreated plots, and the results were compared using the *t*-test. The infestation levels were determined on a sample of 400 seeds per plot. The efficacy of treatments was calculated using Abbott's formula (Abbott, 1925). An analysis of variance on efficacy data (previously transformed by arcsin $\sqrt{p}$) was made to detect any significant variation between all insecticides tested, and means were separated with Duncan's multiple range test.

RESULTS

1992 trial

The beetles released into the cages copulated, females oviposited on lentil pods, and larvae penetrated the seeds as if they were in natural conditions. The average level of seed infestation was clearly correlated with the number of beetles released in the respective cages ($r=0.93$, $p<0.05$), and the average number of larval penetration holes was also highly correlated with the average level of seed infestation ($r=0.95$, $p<0.05$). Increasing the population level of the pest inside the cages resulted not only in a greater percentage of infested seed, but in an increase of seed infested by several larvae. In the four external plots the infestation levels were very similar (12.94 % on average), and the number of penetration holes per seed was lower than in caged plots with the same level of infestation (Table 1).

Two different methodological problems became apparent during the experiment. At first, the material used to make the cages deteriorated after some weeks, and it was necessary to make periodical repairs to the cages. It is possible that some insects could have escaped from the cages during the final period of the trial, which might have influenced the infestation results, especially in plots with low pest populations. However, in the control cages (with no bruchids) no infested seeds were detected, which implies that there was no interference by the external bruchid population during the installation and later manipulation of the cages. Another problem was the aphid proliferation inside some cages. The adults of *C. septempunctata* did not manage to control the aphids, perhaps because the aphid colonies were already well established when the predators were released into the cages. The important differences of grain yield between cages were undoubtely due to aphid attack (mean: 898; standard deviation: 318; range: 1416-368 = 1048). However, such differences did not appear to have a great influence on the global results of the trial. Severe aphid attack provoked the abortion of many young pods, both infested and uninfested by bruchids, and perhaps for this reason the relative proportion of infested pods and seeds would stay the same. In the four external plots, the grain yield was consistently higher and more homogeneous (mean: 1877; standard deviation: 194; range: 2103-1656 = 447), and the differences with caged plots was highly significant ($p<0.001$).

TABLE 1. The effect of the number of released beetles on seed infestation and average number of holes per seed. At the bottom of the table are the same data for external plots.

N° of released beetles	Infestation (%)	Holes per seed
0	0	0
20	3.25	1.115
40	6.88	1.130
80	18.75	1.165
160	32.50	1.265
320	38.75	1.395
External plots	12.94	1.063

TABLE 2. The effect of insecticides on yield and infestation of lentil seeds by *B. lentis* at 1993. Means of efficacy followed by the same letter are not significantly different ($p < 0.05$; Duncan's multiple range test).

Active ingredient	Rate (g AI/ha)	Yield (g/plot)	Infestation (%)	Abbott's Index (%)	
γ-Cyhalothrin	15	1324	0.25	99.24	a
Endosulfan	613	1209	0.50	98.55	a
Malathion	1250	1170	4.33	87.31	b
Carbaryl	1320	1241	14.18	58.95	c
Untreated		1159	34.42		
External plots		531	6.50		

Evaluation trial

The methodological problems detected in the 1992 trial were successfully solved in the evaluation trial. The stronger nylon netting remained undamaged throughout the trial period. The aphid populations were effectively controlled and the yield in the caged plots was very homogeneous (Table 2). There were significative differences ($p < 0.05$) in average yield between untreated control plots and external plots, which indicates a beneficial effect of the cages on the crop. Not only yield, but plant height, number of viable pods per plant and seed weight were significantly higher ($p < 0.001$; $p < 0.05$ and $p < 0.01$ respectively) in untreated controls (58.3 cm, 19.9 pods per plant and 8.33 g / 100 seeds) than in external plots (42.3

cm, 13.8 pods per plant and 7.31 g / 100 seeds). The remarkable differences in grain yield between 1992 and 1993 external plots can be explained by the fertilizer application in the first year. The infestation levels of untreated plots was very homogeneous and up to 30% on average, as was expected. The analysis of variance showed highly significant differences ($p < 0.01$) between treatments and the residual variance of the trial was very low (coefficient of variation = 9.74%). The pyrethroid γ-cyhalothrin was the most effective (99.24% control on average), but it did not differ statistically ($p < 0.05$) from the organochlorine endosulfan. Treatments with malathion and carbaryl had lower efficacy levels, particularly the last chemical, which did not reach 60% control on average (Table 2). The average yield in all treatments was higher than in untreated plots, probably due to the control of secondary phytophagous insects inside the cages, however such differences were not statistically significant.

DISCUSSION

Several insecticides are proposed in the literature for controlling bruchids on lentil crops, including organochlorines (Dörtbudak, 1975; Moreau, 1978; Tahhan & Weigand, 1988), organophosphates (Hoffman *et al*, 1962; Moreau, 1978; Zeren & Yabas, 1986; Domínguez García-Tejero, 1989) and pyrethroids (Mansilla Martínez *et al.*, 1987). Most authors consider that the most appropiate time to spray is during the flowering period of the crop. However, it is remarkable that different authors testing the same products have obtained different results (Zeren & Yabas, 1986; Mansilla Martínez *et al.*, 1987). Even the results of trials with the same pesticides on different years have been different (Mozos Pascual, 1992b). Usually the residual variance of data is unacceptably high, which reduces the precision and reliability of the results. The infestation level of the seeds harvested from different points of the field under natural conditions may be very different, even in an apparently homogeneous area (Mozos Pascual, 1992c). The patchy distribution of the seed infestation is a serious obstacle for experiments in field conditions which cannot be easily solved. Scattering the blocks or breaking up block designs are proposed as a solution when the heterogeneity in the trial area is known (E.P.P.O., 1990). Nevertheless, the final level of bruchid infestation in the lentil crops may be influenced by several factors. Of course, differences in pest intensity, which could probably be detected by previous sampling, must play an important role; however other factors such as the rate of parasitism of bruchid eggs by trichogrammatid wasps or the productive differences through the field should be considered. That impredictability of the infestation levels across the trial area does not allow the choice of an appropriate lay-out of the plots to improve the results. Once the whole process is under control (cage materials, number of infesting bruchids, operations and appropiate date to do them) the semi-field methodology followed in this work unites the reliability of laboratory bioassays with the real conditions of field trials. The residual variance of the data in the first evaluation trial is very low and the results can be considered as quite acceptable. In addition, these results can be easily compared with those obtained in other trials which are planned for the future.

In the 1993 trial, the pest was well controlled by two products. The treatment with γ-cyhalothrin was the most effective, however it is much more expensive than the treatment with endosulfan. In the other hand, endosulfan is less advisable because of its higher global toxicity to animals, including humans. Anyway, insecticide application will be profitable only

in those areas where the infestation levels are usually high. In this way, complementary studies to establish an approximate economic threshold based on the relation between intensity of bruchid populations in the field and post-harvest infestation levels would be very useful.

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POPULATION DYNAMICS OF THE POTATO TUBER MOTH, *PHTHORIMAEA OPERCULELLA*, IN YEMEN AND ITS EFFECTS ON YIELD

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ABSTRACT

80-90% of the Yemeni potato farmers cultivate their own seed potatoes which they store for months until planting. Those are often highly infested by potato tuber moth (30-50% of the tubers). Predominantly, the build-up of the potato tuber moth population results from the farmers' practice of using infested seed potatoes. In an elaborate experimental set-up, since all plots had to be screened off to prevent moths flying in from other fields, the first potato tuber moth adults were observed only a week after infested seed was planted. The adults were able to work their way up through the soil, despite heavy rainfall and furrow irrigation at intervals of 10-12 days. The first symptoms of potato tuber moth larvae were apparent on the leaves three weeks after planting. The infested seed affected two important yield parameters of the potato: the number of plants per unit area and the number of shoot axes per plant. The result of these changes was an appreciable reduction in yield.

INTRODUCTION

The potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera, Gelechiidae), is present in almost all tropical and subtropical regions of the world, and is considered to be the most serious insect pest of potato in Yemen (Kroschel, 1993). The larvae mine in all the vegetative parts of the crop. The foliage may be completely destroyed, which, according to Broodryk (1979), can result in a substantial loss of yield, especially after early tuber infestation in the field, generally where the adult moths have laid eggs through cracks in the soil (Richardson and Rose, 1967). The pest is transferred to the potato stores with the harvested tubers, where it can reproduce and infest other tubers. Infestation of stored tubers can result in anything from slight damage to total destruction (Broodryk, 1979). In Kenya, losses of up to 90% have been reported in potato stores (Raman, 1987).

The preconditions for potato production are favourable in the Yemeni highlands (between 2100 to 2400m above sea level). Production can take place under irrigation from February to the beginning of November. From November to February the mean temperature ranges between 12.5 and 15°C and soil frost can occur in the morning hours (Alex, 1985). After the interval of cultivation, the potato tuber moth population has to re-establish itself in the fields in spring. Since 80-90% of the Yemeni potato farmers cultivate their own seed potatoes which are often highly infested by the potato tuber moth (30-50% of the tubers), the hibernation of the potato tuber moth in the potato store may have a key impact on their population dynamics. Preliminary field studies in farmers' fields already indicated very obviously that fields cultivated with farmers' own seeds are earlier and more heavily infested than fields planted with non-infested seeds (Kroschel, 1994). The aim of this experiment was twofold. Firstly, it was intended to show whether the potato tuber moth develops from infested seed after planting, and whether it is then possible for the resulting adults to emerge from the soil to establish a population in the field. Secondly, it was designed to investigate the effects of infested seed on the development of the potato plant and on tuber yield.

MATERIALS AND METHODS

The experiment was prepared in January and set up in a field in April on the Shoub-farm in Sana'a in 1989. Preparation consisted of subjecting the infested and uninfested seed to the same pregerminating conditions. Uninfested seed potatoes of the variety Baraca were distributed among six boxes (100 x 70 x 50cm) sealed with nylon gauze, and stored at an average room temperature of 22°C. Potato tuber moth was mass-reared in the laboratory as described by Kroschel (1993). Approximately 150 potato tuber moth adults were released into three of the boxes to infest the seed, which was individually classified into one of three classes of intensity of infestation before planting: Infestation Class 1: slight infestation, 1-4 boreholes with an average of 2 infested eyes per seed potato; Infestation Class 2: moderate infestation, 5-8 boreholes with an average of 4 infested eyes per seed potato; Infestation Class 3: severe infestation, 9-12 boreholes with an average of 6 infested eyes per seed potato. The uninfested seed potatoes had an average of nine eyes per tuber.

Experimental set-up

The plot size was 4.30 x 6m; potatoes were planted at an inter-row distance of 0.7m and at 0.35m distance between each row at a depth of 10cm. The minimum distance between plots was 1.50m. The experiment was set up in a two-factor block arrangement (split plot) in two blocks. Factor 1 (the small plot factor) was infested and uninfested seed; factor 2 (the large plot factor) was with and without pheromone trap. Each block of infested seed contained two rows of each infestation class, each row containing 16 potatoes planted randomly. In the control plot, six rows of potatoes were planted in each block. The potatoes were watered as necessary, generally every 10-12 days, using the standard furrow irrigation technique of flooding the plots between the potato ridges. The sexual pheromones of the female moth were used to help trap male specimens, with the aim of monitoring the population growth of the potato tuber moth. The substance used was a 1:1.5 mixture of the synthetic components trans-4, cis-7-tridecadien-1-ol acetate and trans-4-cis-7, cis-10-tridecatrien-1-ol acetate supplied by the CIP (Centro International de Papas, Peru). Rubber capsules were impregnated with the pheromone and were kept deep-frozen to preserve their effectiveness until required. Plastic water bottles (1.5 l) served as traps and were prepared as described by Kroschel (1993). The traps were set 80cm above the ground in the middle of four plots. The individual plots were covered after planting by large walk-in cages, 6.8 x 5 x 1.3m, covered in 1mm mesh netting to prevent immigration and emigration of adult moths. Screening reduced the light intensity inside the plots, when the sun was at its height, to around 12,000 lux below normal field conditions of between 60,000 and 65,000 lux. However, this did not adversely affect plant growth. Air temperature and relative humidity were measured inside one of the plots, using a thermohygrograph set up 0.6 m above the ground and shielded from direct sunlight by a small wooden roof.

Test parameters

The pheromone traps were used firstly, to detect the emergence of moths from infested seed, and secondly to reduce the population and thereby control infestation. To this end, the traps were checked weekly and a record of catches was kept. Beginning with the growth stage 10-15 (emergence) of the potato, the growth and yield parameters of the potatoes were measured weekly and the infestation of leaves by the potato tuber moth (mines per plant) was assessed from 18 plants in each plot. For all the plants up to growth stage 40 (end of extension growth), a record was kept of shoots which failed to emerge and the number of shoots formed on each stem. The effect of various intensities of infestation of the seed by the potato tuber moth on growth was recorded in terms of the maximum sprout height, taking three randomly selected plants from each row up to growth stage 70 (formation of berries). After more than four months, the plots were harvested and the yield from each row was measured. Definitions of the growth stages were used according to Bätz et al. (1980).

RESULTS AND DISCUSSION

Population dynamics from infested seed

In the control plots planted with uninfested seed, no potato tuber moth adults were caught in the traps, and no signs of damage were found on the foliage. This indicated that no moths penetrated the plots through the nets, and that the infested seed was therefore the sole source of the moths inside the plots. The trial confirmed that the use of infested seed stock contributes to the development of a new potato tuber moth population in the field. The first potato tuber moth adults were observed only a week after infested seed was planted, and males were subsequently caught in the pheromone traps (Fig. 1). The adults were able to emerge from the upper soil layer, despite heavy rainfall and furrow irrigation at interval of 10-12 days. They were probably also aided in their emergence by the holes the shoots made as they broke through the soil. The emerging adults began laying eggs on the leaves, and the first generation of potato tuber moths developed in the field. The first symptoms were apparent on the leaves three weeks after planting. On the basis of the climatological data and the regression analysis of an additional experiment on the duration of the individual growth stages of the potato tuber moth in different seasons (Kroschel, 1993), the development of the first generation was calculated to be 40 days. Since infested seed could contain developmental stages ranging from egg to pupa, the moths trapped up to the seventh week after planting could have come only from the seed potatoes.

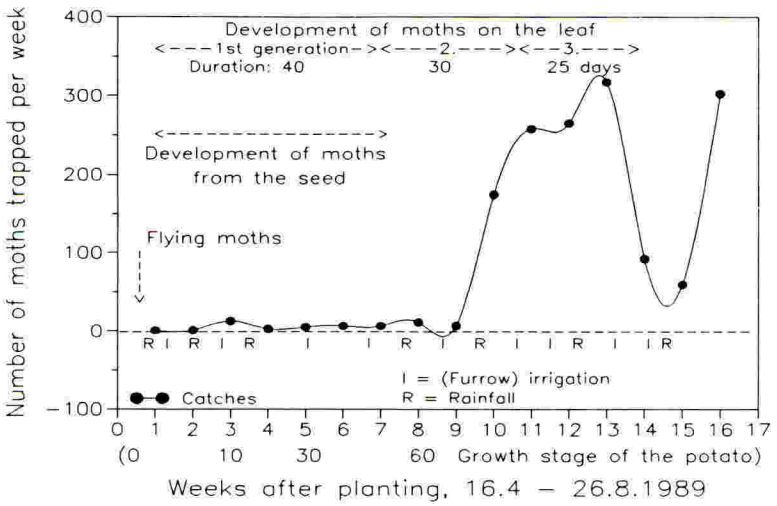

FIGURE 1. The population dynamics of the potato tuber moth emerging from infested seed, as shown by pheromone trap catches

It can be concluded that the males caught from the eighth week after planting had all developed on the leaves. Assuming a sex ratio of 1:1 and that all the males were caught (which is probably not the case), an average of 68 adults developed from the seed in the two blocks in which pheromone traps were set. This figure is low, considering that 96 potatoes were planted in each block with an average of six infestation points each, in addition to an unknown number of eggs and L1 larvae. Although each infestation point cannot be assumed to represent a seperate individual, the facts nonetheless suggest that there is some mortality of the potato tuber moth in the soil. However, the small initial population of about 34 females developed from the seed in each block was quite sufficient to trigger mass propagation, with rising mean daily temperatures in May/June speeding up the developmental stages. The reproduction for

each female could therefore be calculated at 97 individuals by the end of the experimental period, or a total of 3,298 per block. The explosion in the potato tuber moth population brought about a steadily increasing infestation of the plants, which became so great that many of them were found to have more than 150 infestation points. Use of the pheromone traps reduced infestation by over 50% of all growth stages of the potato, so, by harvest, tuber infestation stood at only 14% in plots with traps, as against 47% in plots without traps.

Yield losses due to the use of infested seed

Infested eyes on the tubers are destroyed through feeding by the potato tuber moth larvae, and germination vigour is reduced mainly through secondary infestation by fungal and bacterial pathogens (Winning, 1941). This affects two important yield parameters of the potato: the number of plants per unit area and the number of shoot axes per plant (Geissler, 1983) (Table 1). The more severe the infestation of individual seed potatoes, the larger were the gaps in the stand, i.e. the larger the reduction in number of plants per unit area. Plants grown from infested seed (class I) produced a higher number of shoot axes, although the shoots themselves developed less vigorously and the plants did not grow as high (Table 2).

TABLE 1. The effect of potato tuber moth on percent emergence and number of shoot axes at different growth stages (128 potatoes per batch)

Seed batch	Growth stages				
	15		**30**	**40**	
	% emerge	shoots/plant	shoots/plant	% emerge	shoots/plant
Uninfested	100	2,9	3,0	100	3,0
Infestation Class 1*:	97.5	3,4	3,6	99.0	3,6
Infestation Class 2*:	90.5	3,0	3,4	93.5	3,4
Infestation Class 3*:	77.5	2,8	3,1	79.5	3,2

* see text for details

As early as growth stage 15, two to five small mines were counted on the individual pinnate leaflets and at the growing points of each plant, so from this growth stage onwards leaf infestation also had some effect on the development and, consequently, the height of the potato plant. The clear delay and inhibition of development up to growth stage 30 (extension growth), however, must be primarily attributed to the reduced germination vigour of the infested potatoes. The net result of these changes in the yield parameters due to the use of infested seed was an appreciable reduction in yield - determined, however, not only by the degree of infestation of the seed, but also by that of the foliage (Table 3).

The use of pheromone traps, as already mentioned, reduced infestation by over 50%. Loss in yield from the plots where traps were set was accordingly less severe, owing to the lower degree of leaf infestation. The experiment was not designed to determine the loss in yield caused by the seed infestation alone, as this would have involved excluding the factor of leaf infestation. With or without the pheromone traps, however, it is evident that yield is inversely proportionate to the degree of infestation in the seed potatoes, and the use of infested seed therefore results in clear losses in yield.

The infested seed stock replanted by the farmers play a key role in the potato tuber moth's population development. The pest overwinters and breeds in the infested seed during storage and thus is transferred back to the fields at planting time. This form of hibernation does not appear to be of much importance in other countries. Only Verma (1967, quoted in Lal, 1987) reports from India of a renewal of population development of this kind, and Broodryk (1971) mentions that more attention should be paid to infested seed as a carrier in South Africa.

TABLE 2. Height of potato plants grown from infested seed, average values from two replications (mean of 12 plants): % reduction from uninfested control

Seed batch	Growth stages					
	15	**30**	**40**	**50**	**60**	**65**
Uninfested:	13,1	28,6	49,9	58,1	75,0	83,0
Infestation Class 1*:	10,9	25,1	42,2	52,9	63,5	69,3
% reduction	**16,8**	**12,2**	**15,4**	**9,0**	**15,3**	**16,5**
Infestation Class 2*:	8,8	19,9	36,3	45,3	56,9	65,4
% reduction	**32,8**	**30,4**	**27,3**	**22,0**	**24,1**	**21,2**
Infestation Class 3*:	7,0	17,8	34,0	38,4	53,0	63,7
% reduction	**46,6**	**37,8**	**31,9**	**33,9**	**29,3**	**23,3**
Infestation Class 1**:	10,6	21,3	38,2	42,4	52,9	58,9
% reduction	**19,1**	**25,5**	**23,4**	**27,0**	**29,5**	**29,0**
Infestation Class 2**:	10,8	21,3	37,2	44,3	50,9	55,2
% reduction	**17,6**	**25,5**	**25,5**	**23,8**	**32,1**	**33,5**
Infestation Class 3**:	6,9	15,6	31,3	36,4	48,0	57,5
% reduction	**47,3**	**45,5**	**37,3**	**37,3**	**36,0**	**30,7**

* with pheromone trap; ** without pheromone trap

TABLE 3. Losses in yield through the use of seed infested by the potato tuber moth with subsequent leaf infestation

Seed batch	yield/row (kg)	% reduction
Uninfested	18.5 ± 1.1	-
Infestation Class 1*	12.5 ± 1.8	32.6
Infestation Class 2*	10.7 ± 1.9	42.1
Infestation Class 3*	9.7 ± 4.3	47.4
Infestation Class 1**	8.6 ± 2.3	53.4
Infestation Class 2**	7.4 ± 1.5	60.0
Infestation Class 3**	4.8 ± 0.8	74.3

* with pheromone trap; ** without pheromone trap

According to Kroschel (1993, 1994) overwintering on secondary host plants plays a minor role in Yemen. Extremely low temperatures and the threat of frost make the cultivation of primary hosts (potato, tomato or aubergine plants) impossible in the highland valleys from November to February. Secondary host plants, such as *Solanum nigrum* L. and *Datura stramonium* L., are also killed by frost, and do not serve as food for the larvae at this time of year.

Hibernation in the field entirely inside unharvested tubers is possible, as Lal (1987) observed in India and Broodryk (1971) in South Africa. Yet the Yemeni farmers harvest very thoroughly, leaving few tubers in the field. However during field inspections in spring 1990, potato tubers were found containing live potato tuber moth larvae, but it should be mentioned that the preceding winter had been particularly mild, with little frost.

In this presented field trial, the development of just a few adults was sufficient to provoke a population explosion. Assuming only 10% infestation of the seed stock, with a normal plant density of 60,000 potato plants/ha 6,000 infested seed potatoes would have been planted out. Even if an imago develops in only 50% of those, taking the male/female ratio to be 1:1 (as do Al-Ali and Talhouk, 1970), then the initial population will be 1,500 males and females per hectare. Considering that the reproduction per female is influenced by temperature, at average February temperatures of 15°C, according to Broodryk (1971), approx. 20 eggs will be laid per female, so population growth start in spring with a maximum of 30,000 eggs per hectare. And the rate of infestation is not 10%, but according to farmers' estimates backed up by our own research, between 20% and 50%. Therefore, the use of healthy seed potatoes would be one of the main improvements in Yemen potato production to reduce the infestation by the potato tuber moth and to increase yields.

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EFFECTS OF SEED COATING FORMULATION No.17 ON THE HISTOPATHOLOGY AND ULTRASTRUCTURE OF *PUCCINIA STRIIFORMIS* IN WHEAT

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ABSTRACT

The seedlings from wheat seeds coated with seed coating formulation (SCF) No. 17 were inoculated with a virulent race of *Puccinia striiformis* and the histopathological and ultrastructural changes in the fungus and host were studied. Results from fluorescence staining, whole leaf clearing technique and electron microscopy showed that SCF No. 17 caused a series of changes on both fungal and host cells. The lipid bodies and vacuoles increased in the cytoplasm of the hyphae and primary haustoria. There were no septa formed at the bases of branches and the septal walls could not extend regularly. Some of the haustorial mother cells produced malformed penetration pegs which could not penetrate through the host cell walls. The degeneration of hyphae induced the leaking of some materials from the hyphae into the host intercellular space and eventually caused the necrosis of the host cells. The infection was totally inhibited in the treated seedlings.

INTRODUCTION

Seed coating treatment has been proved to be an effective way of controlling seedborne and soilborne diseases and insects at the seedling stage. A series of seed coating formulations (SCF) for different crops have been developed at Beijing Agricultural University and most of them have been manufactured and used in different parts of China. The seed coating treatment offers an integrated pest control in one operation before planting, improves the seed sowing quality and enhances the production yield substantially. The total extension acreage in China was about 8.7 million ha for the period of 1984 to 1993, increasing the total production yield of grain, cotton and oil crops about 4.4 million tons with estimated profit of 640 million US dollars. (Li *et al* 1994, 1993).

SCF No. 17 is an effective formulation against wheat yellow rust at the seedling stage and is widely used in the north part of China. The objective of this research was to study the histopathological and ultrastructural changes of the fungus and the host cells in the SCF No.17 treated wheat seedlings.

MATERIALS AND METHODS

SCF No. 17 was developed and provided by Beijing Agricultural University. The active ingredients included fungicides (e.g. triadimenol and carbendazim), insecticide (carbofuran), minor elements, adhesives and other chemicals (Li and Liu, 1986). The susceptible wheat cultivar HiuXianHong and a virulent race No. 29 of *Puccinia striiformis* were used in this study.

The wheat seeds were treated with SCF No. 17 at 3.75 (Treat. I) and 5.36 (Treat. II) mg AI of triadimenol/kg seed. The wheat seedlings were cultivated in a growth chamber at 13-14°C for 10 days. When fully extended, the first leaf was inoculated by brushing the leaf with uredospores of *P. striiformis*. The leaf samples were taken at 2, 4, and 6 days after the inoculation and treated according to the conventional method (Dahmen *et al* 1988; Buchenauer, 1987; Heller *et al* 1990; Kang *et al* 1993a, 1993b; Smolka and Wolf, 1986) for histopathological and electron microscopic observation.

RESULTS AND DISCUSSION

Effect on the extension of hyphal colony

Results from fluorescence staining observation and whole leaf clearing technique showed that SCF No. 17 had no influence on uredospore germination or germ-tube penetration on the treated leaves, but the hyphal growth and colony extension were strongly inhibited (Table 1). The diameter of the hyphal colony on the treated leaves was considerably smaller than that on the untreated leaves. The extension of the colony ceased 120 h after inoculation in treatment II and the diameter of the colony on the treatment II leaves was much smaller than that on the treatment I leaves.

These observations clearly indicated that SCF No. 17 treatment could effectively inhibit the hyphal growth and the colony extension of *P. striiformis* on treated wheat leaves (Table 1).

TABLE 1. Effect of SCF No. 17 on colony extension

Treat.	Diameter (um) of colony after inoculation				
	24h	48h	72h	96h	120
CK	53.3a*	59.4a	104.9a	211.2a	448.9a
I	50.8a	54.7a	68.8b	103.6b	163.6b
II	50.7a	54.6a	62.3b	87.3b	8.4c

* numbers followed by the same letter in the same column are not significantly different at P=0.01 level.

Effect on hyphal branching and haustorial mother cell formation

Hyphal branching and haustorial mother cell formation were inhibited 96 h after inoculation, resulting in much lower numbers of branches and haustorial mother cells in the treated leaves than in the untreated leaves (Table 2).

TABLE 2. Effect of SCF No. 17 on hyphal branching (HB) and haustorial mother cell (HMC) formation

Treat.	Number of HB or HMC after inoculation							
	24h		48h		72h		96h	
	HB	HMC	HB	HMC	HB	HMC	HB	HMC
CK	1.7a*	1.6a	3.1a	2.3a	9.8a	8.6a	31.5a	28.7a
I	1.9a	1.9a	3.3a	2.3a	6.2a	5.4a	11.6b	9.6b
II	1.8a	1.9a	2.9a	2.5a	6.4a	6.0a	10.5b	8.3b

* numbers followed by the same letter in the same column are not significantly different at P=0.01 level.

Effect on haustorium formation

primary and secondary haustoria were formed inside each infection site of both untreated and treated leaves 24 h after the inoculation. However the number of haustoria was considerably decreased in treated leaves 72 h after the inoculation. There was an average of less than 2 haustoria formed in each site in the treated leaves compared to 6.5 in untreated leaves (Table 3).

TABLE 3. Effect of SCF No.17 on haustorium formation

Treatment	Number of haustoria after inoculation			
	24 h	48 h	72 h	96 h
CK	1.1a*	1.7a	6.5a	18.7a
I	1.3a	1.4a	1.7b	1.6b
II	1.2a	1.5a	1.4b	1.5b

* Numbers followed by the same letter in the same column are not significantly different at $P=0.01$ level.

Ultrastructural changes in the fungus and host cells

The results from electron microscopic observation showed that SCF No. 17 caused a series of changes in the fungal and host cells. The lipid bodies and vacuoles increased in the cytoplasm of the hyphal and primary haustoria. Electron-dense vesicles were accumulated in between the plasmalemma and the cell walls of both hyphae and haustoria resulting in a irregular thickening of their cell walls. The thickening of the cell walls at the tips of hyphae was most obvious. There was no septum formed at the base of the hyphal branch. The septal walls could not expand to the center of the hyphal cells due to the accumulation of the electron-dense materials at their extending points. Some haustorial mother cells were found to produce malformed penetration pegs which could not penetrate through the host cell walls.

The extrahaustorial matrix of primary haustoria became enlarged and contained some electron-dense materials. The secondary haustoria could form several branches, but could not generally extend at all. The degeneration of hyphae induced the leaking of some materials from the hyphae into the intercellular space of the host cells and eventually caused the necrosis of host cells. Most infected host cells secreted a large amount of collar materials which usually extended beyond the joint part between the neck and body of the haustorium. Sometimes, the materials secreted by infected host cells could enclose a whole haustorium. These results indicated that SCF No. 17 not only directly acted on the fungus, but also indirectly affected the fungus by influencing its host.

Effect on the host cells and their interaction with the fungus

Although the penetration of the spores into the epidermal cells occurred in the same way on both untreated and treated leaves, the interactions between the host and the pathogen

were greatly changed in the treated leaves. The necrosis of the host cells due to the accumulation of electron-dense materials secreted by both fungus and host cells prevented the fungus from further extending to the healthy areas of the leaves. The histopathological characteristics mentioned above were similar to the hypersensitive necrotic reaction of resistant wheat cultivars infected by the fungus.

In summary, seed coating treatment is an economical and effective measure for plant protection (Scott, 1989). Dahmen (1988), Buchenauer (1987, 1983) and Heller *et al* (1990) reported that triazole fungicides were sterol biosynthesis inhibitors. Triadimanol was used as one of the principal active ingredients in SCF No. 17. The combination of triadimanol and carbendazim in SCF No. 17 had greatly enhanced its spectrum and bioactivity against rust, powdery mildew and bunt, smut, and root rot of winter wheat. Our research results showed that SCF No. 17 treatment provided integrated control of both seed- and soilborne and airborne diseases at the seedling stage (Li *et al*, 1991) and the mode of action on *P. striiformis* in winter wheat was similar to that of the single compound triadimenol (Buchenauer, 1987; Heller *et al*, 1990).

Results showed that SCF No.17 treatment caused a series of changes in fungal and host cells: (1) hyphal growth, branching and haustorium formation were inhibited and decreased; (2) the lipid bodies and vacuoles increased in the cytoplasm of the hyphae and haustoria, the cell walls of the hyphae and haustoria were thickened irregularly; (3) some haustorial mother cells produced malformed penetration pegs which could not penetrate through host cell walls, several branches from the secondary haustoria could be formed but not be expanded; (4) some electron-dense materials leaked from the hyphae into the host intercellular space, which caused necrosis of the host cells proventing further expansion of the fungus; (5) the histopathological characteristics mentioned above were similar to the hypersensitive necrotic reaction of resistant wheat cultivars infected by *P. striiformis*.

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HISTOLOGICAL STUDIES ON THE FUNGICIDAL ACTIVITY OF THE STROBILURIN BAS 490 F

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ABSTRACT

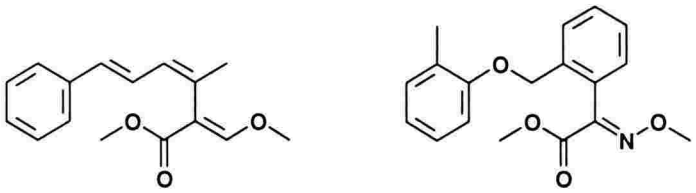
BAS 490 F is a highly active, broad-spectrum fungicide belonging to a new class of fungicides, the strobilurins. It exhibits excellent protective, curative and eradicative activity and provides long-lasting residual disease control. Microscopy was used to identify the specific sensitive developmental stages of three important target fungi, *Venturia inaequalis*, *Erysiphe graminis*, and *Puccinia recondita* inhibited by BAS 490 F. Pre-infectional treatments effectively inhibited spore germination of all three pathogens, but complete inhibition of mycelial growth by post-infectional applications was confined to *E. graminis*, whose mycelia and spores develop exclusively on the leaf surface. These results together with its unique biochemical mode of action are important to the future practical use of BAS 490 F in the field.

INTRODUCTION

One of the greatest challenges to the crop protection industry is the actual *discovery* of highly effective and safe substances that protect plants from pests and pathogens and reduce competition by weeds. In an effort to meet this challenge, research at BASF has focussed since 1983 on the synthesis, biological activity and market development of an extraordinary new class of fungicides, the strobilurins (Sauter *et al.*, 1994). Two years ago at the Brighton Conference we announced the development of the broad-spectrum strobilurin BAS 490 F and described its physico-chemical properties, toxicology, formulation and biological activity (Ammermann *et al.*, 1992). In this paper we describe additional details on the biological activity of BAS 490 F (proposed common name: kresoxim-methyl).

BAS 490 F (methyl-(E)-methoximino[α-(o-tolyloxy)-o-tolyl]acetate, Fig. 1) is very active against a broad spectrum of economically important plant pathogenic fungi. It has excellent protective, curative and eradicative activity and provides long-lasting residual disease control (Ammermann *et al.*, 1992).

FIGURE 1. Structure of strobilurin A (left) and the active ingredient in BAS 490 F (right).



BAS 490 F is a fungicide belonging to the strobilurins. These fungicides, which are synthetic analogs of strobilurin A (Fig. 1) originally isolated from the fungus *Strobilurus tenacellus* (Pers. ex Fries) Singer, have a novel biochemical mode of action (Anke *et al.*, 1977; Beautement *et al.*, 1991; Clough, 1993; Sauter *et al.*, 1994). They strongly inhibit mitochondrial respiration by blocking electron transfer at the cytochrome bc_1 complex (Becker *et al.*, 1981; Brandt & von Jagow, 1991). Their unique mode of action will be useful in spray management programs aimed at maximizing biological activity and minimizing the danger of fungicide resistance developing to any given active ingredient (AI) in the treatment schedule.

MATERIALS AND METHODS

The biological activity of BAS 490 F was investigated in laboratory, greenhouse and field experiments using three target fungi, *Venturia inaequalis* (Cooke) Winter (apple scab), *Erysiphe graminis* DC. ex Merat f. sp. *tritici* Em. Marchal (wheat powdery mildew) and *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* (wheat leaf rust) following foliar treatments. Spores of all three fungal pathogens were produced in vivo in the greenhouse on leaves of their respective host plants.

Plant materials

Wheat (cv. Kanzler) and apple (cv. Golden Delicious) were cultivated from seeds in the greenhouse at 20-24° C and 60-80% r.h. Additional HQI-light was provided for up to 16 h d^{-1} when the natural light intensity fell below 7000 lx.

In vitro germination

Spores were either incubated on collodion membranes (*P. recondita*) or on dialysis tubing (*V. inaequalis*) on water agar at 20°C in the dark. Percent germination was evaluated at 6 or 24 h after spores of *P. recondita* and *V. inaequalis* were transferred to their respective test surface.

Histological studies

Fungicide applications were made either in a spraying cabinet (run-off) or with a hand-held glass sprayer (up to run-off) in both the pre-infectional and post-infectional experiments. Light microscopy (after Gessler & Stumm, 1984), fluorescence microscopy (Kuck *et al.*, 1981) and low temperature scanning electron microscopy (Guggenheim *et al.*, 1990) were used to determine the growth stages inhibited by the fungicide.

RESULTS AND DISCUSSION

The effect of BAS 490 F on spore germination and germ tube growth was first evaluated using in vitro methods. The results showed that spore germination is particularly sensitive to the AI, as evidenced by *V. inaequalis*, whose germ tubes formed only at concentrations below 0.1 mg AI l^{-1} (Table 1).

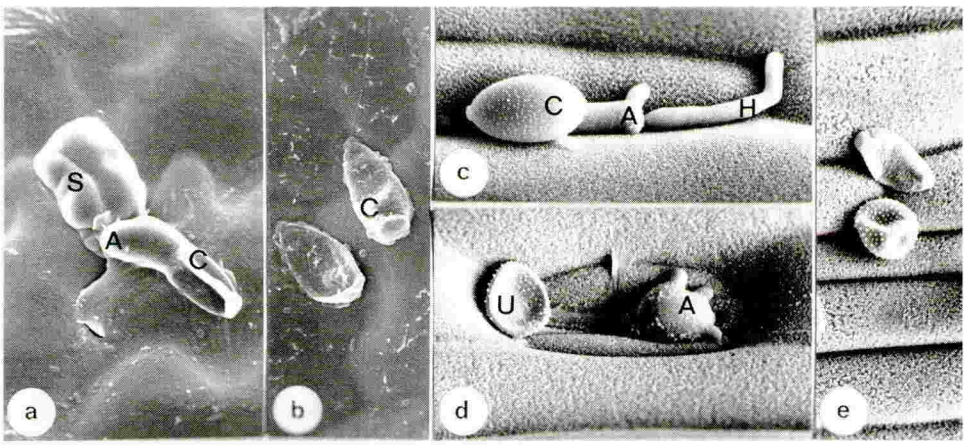
TABLE 1. Effect of BAS 490 F on spore germination in vitro

Concentration (mg AI l^{-1})	% Germination	
	V. inaequalis (10)[a]	*P. recondita* (4)[a]
1	0	0
0.1	0	17.0
0.01	12.4	-
Control	89.4	89.5

[a] Number of replications per treatment

These observations were confirmed by greenhouse studies with *V. inaequalis*, *E. graminis* and *P. recondita* spores on their respective host leaf surfaces (Fig. 2).

FIGURE 2. Protective activity of BAS 490 F against plant parasitic fungi in the greenhouse



a, Germling of *V. inaequalis* on apple leaf, untreated control. Conidium (C) with germ tube and appressorium (A). Following cuticular penetration below the appressorium, an irregularly swollen hypha (=stroma, S) developed subcuticularly. **b**, Ungerminated conidia (C) of *V. inaequalis* on a leaf pre-treated with BAS 490 F (2 mg AI l^{-1}). **c**, Germling of *E. graminis* on wheat leaf, untreated control. Conidium (C) with appressorial germ tube (A) and secondary hypha (H). **d**, Germling of *P. recondita* on wheat leaf, untreated control. Urediniospore (U) with germ tube and prominent appressorium (A) over a stoma. **e**, Ungerminated spores of *E. graminis* (top) and *P. recondita* on a wheat leaf treated with BAS 490 F (16 mg AI l^{-1}). All scanning electron microscopic images were obtained 1-2 d after inoculation.

Both pre-infectional and post-infectional treatments are very effective in arresting the growth and sporulation of *E. graminis* in the greenhouse (Table 2).

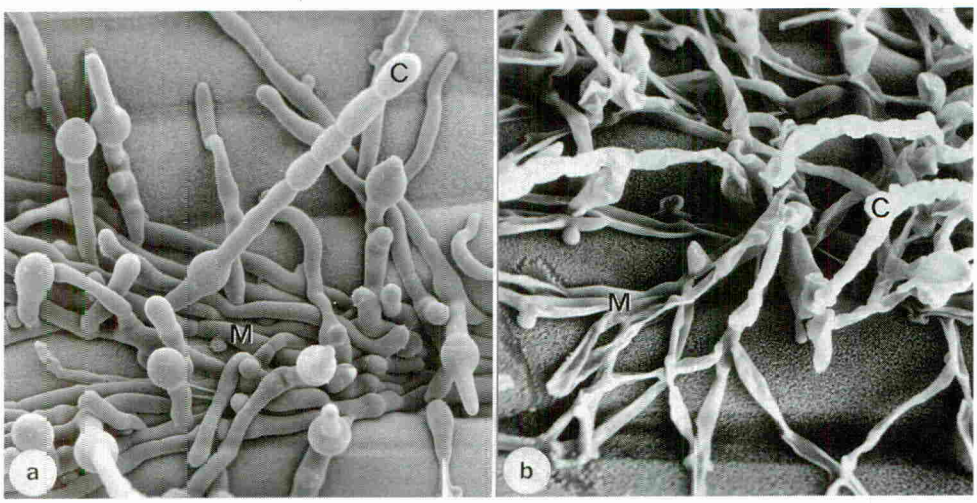
TABLE 2. Control of *Erysiphe graminis* by BAS 490 F in the greenhouse

Concentration (mg AI l^{-1})	% Leaf area infected[a]	
	Pre-infectional treatment[b] (n=15)[d]	Post-infectional treatment[c] (n=17)[d]
63	0	0.3
16	0.2	2.3
4	2.5	18.2
1	31.3	57.2
Control	66.2	71.2

[a] Plants were evaluated 7-10 d after inoculation
[b] Treatment 1 d before inoculation
[c] Treatment 3-4 d after inoculation
[d] Number of experiments

Scanning electron microscopy revealed that the mycelia and spore chains of *E. graminis* on the leaf surface collapse within 48 h of treatment (Fig. 3).

FIGURE 3. Eradicative activity of BAS 490 F against *E. graminis* in the greenhouse



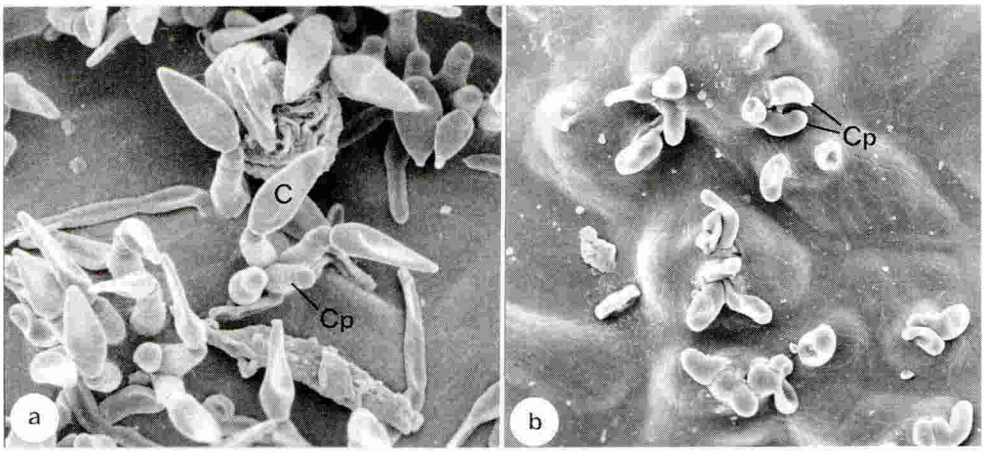
a, 7 d after inoculation a dense layer of mycelia (M) and chains of conidia (C) developed on the surface of untreated leaves. **b**, The eradicative activity of BAS 490 F is shown after treatment with 16 mg AI l^{-1}. Both mycelia (M) and conidial spore chains (C) are completely collapsed. Plants were treated 4 d after inoculation.

During the same time period, the epidermal haustoria became encapsulated with callose. This was determined by fluorescence microscopy following staining with aniline blue. A causal relationship between fungal death and encapsulation is, however, controversial (Heath,

1988; Smolka & Wolf, 1986) and the precise role of BAS 490 F in these events has yet to be established.

Light microscopical studies showed that a single post-infectional application of BAS 490 F does not completely stop the growth of the subcuticular mycelium (=stroma) of *V. inaequalis*. However, sporulation of the fungus is strongly inhibited (Fig. 4). A mean reduction in sporulation of 98% was achieved with a treatment of 67 mg AI l^{-1} applied 2 or 4 d after inoculation in the greenhouse.

FIGURE 4. Inhibition of sporulation of *V. inaequalis* in the field by BAS 490 F



a, Numerous conidia (C) have formed on the untreated leaf. Several conidia are seen detached from their conidiophores (Cp) and are dispersed on the leaf surface. **b**, 28 d after eradicative treatment with 100 g AI ha^{-1} BAS 490 F (67 mg AI l^{-1}) sporulation of the fungus is still inhibited. A few rudimentary conidiophores (Cp) have formed on the leaf surface; however, conidia have not developed.

The curative and eradicant activity observed against *V. inaequalis* and *E. graminis* was not found in parallel greenhouse experiments with *P. recondita*. Postinfectional treatments with BAS 490 F up to 100 mg AI l^{-1} had no effect on radial growth of mycelial infection sites of the brown rust fungus as determined by fluorescence microscopy. This is apparently due to insufficient uptake of the AI into the leaf mesophyll (Köhle *et al.*, 1994), where the growth of rust mycelia occurs, and to the insensitivity of the mycelium to the AI relative to spore germination. Furthermore, an accumulation of the AI in the leaf is limited by enzymatic cleavage of its methylester group to give the corresponding carboxylic acid, which is biologically inactive (Röhl & Sauter, 1994). Adding an adjuvant to BAS 490 F can, however, significantly increase the uptake of the AI and lead to complete inhibition of mycelial growth within host tissues and to inhibition of sporulation.

In summary, BAS 490 F is characterized by its long-lasting protective, curative and eradicative fungicidal activity. Together with its unique biochemical mode of action and favorable toxicological and environmental characteristics, it will be a valuable new tool for integrated pest management programs in the future.

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ICIA5504 : EFFECTS ON DEVELOPMENT OF CEREAL PATHOGENS

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ABSTRACT

ICIA5504 (methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate) is a novel, broad spectrum systemic β-methoxyacrylate fungicide which inhibits mitochondrial respiration. The dominant effect of preventative applications of ICIA5504 against *Puccinia recondita, Septoria tritici* and *S. nodorum* was inhibition of spore germination, but this was less important against *Erysiphe graminis* f. sp. *hordei*. However, in all instances post-germination effects were also seen including germinated uredospores with short germ tubes that stained intensely in Evans blue (*P. recondita*) and restriction of fungal development after appressorium formation (*S. nodorum*).

ICIA5504, in marked contrast to epoxiconazole, clearly has major effects on the early stages of fungal development. This reflects the different biochemical targets of β-methoxyacrylates and the triazole family of fungicides.

The effects of ICIA5504 against *P. recondita* were the same whether the active ingredient was applied directly or reached the target site via systemic movement. Eradicant treatment with ICIA5504 caused mycelial collapse of *P. recondita*.

INTRODUCTION

ICIA5504 is a novel, broad spectrum, systemic β-methoxyacrylate fungicide for use on a wide range of crops (Godwin *et al.*, 1992). ICIA5504 has the same biochemical mode of action as the naturally occurring strobilurins, namely inhibition of mitochondrial respiration by blocking electron transfer between cytochrome b and cytochrome c_1 (Wiggins & Jager, 1994), a specific mode of action different from all compounds currently sold as agricultural fungicides.

Glasshouse tests involving microscopy studies were undertaken to determine how ICIA5504 affects the development of cereal pathogens. Chemical applications were made prior to inoculation and disease development was assessed in parallel tests in order to identify the most influential effect of ICIA5504 on a population of spores as they progressed from germination to pustule or pycnidium formation. Epoxiconazole was tested alongside ICIA5504 to allow direct comparison with the triazole family of fungicides. Additional experiments examined the systemic and eradicant effects of ICIA5504 on fungal development.

MATERIALS AND METHODS

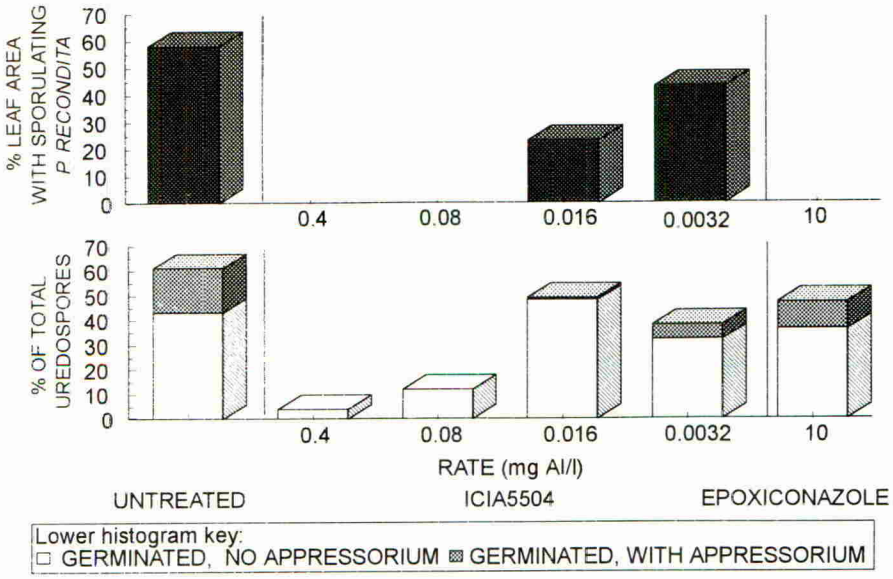
Wheat and barley seedling cultivation, chemical treatment and pathogen inoculation were as previously described by Waller *et al.* (1990). Treatments were applied 1h prior to inoculation unless otherwise stated., Inoculated plant material was fixed and cleared for light microscopy studies using the method of Carver *et al.* (1992) to ensure no displacement of spores from the leaf surface. Fungal structures were stained by placing leaf pieces for 24h on filter paper soaked in Evans blue (0.1% in 2:1 lactoglycerol : ethanol). Fluorescence microscopy was carried out by the method of Godwin (1985). In all quantitative microscopy studies, 3-4 replicates of 25 or 50 spores were assessed 48h after inoculation unless otherwise stated. Standard techniques were used for the cryofracture scanning electron microscopy studies.

RESULTS

Puccinia recondita

ICIA5504 had a major effect on uredospore germination, particularly at 0.4mg AI/l (Figure 1).

FIGURE 1. *Puccinia recondita* : disease development, uredospore germination and appressorium formation on wheat (parallel tests).

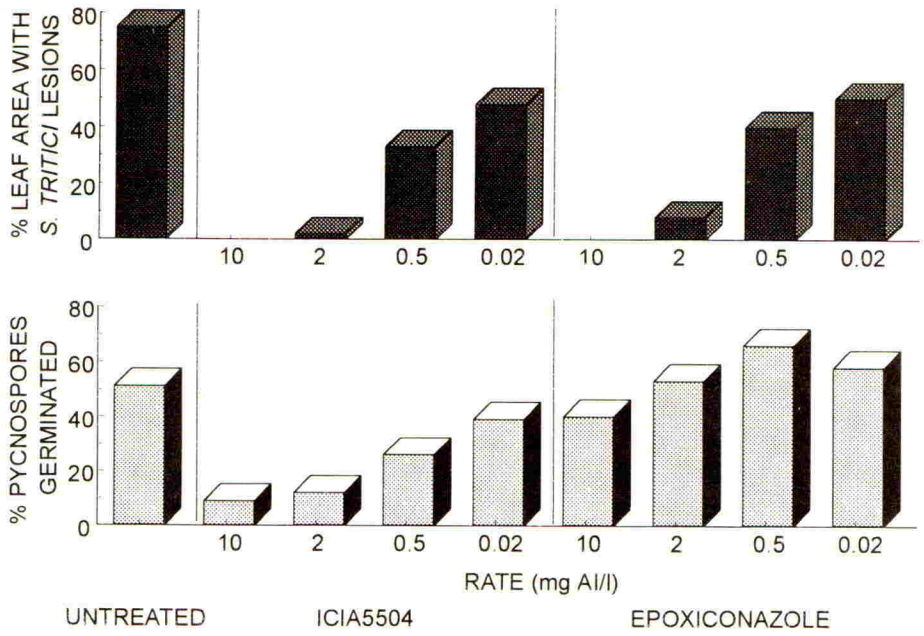


In addition, uredospores with short germ tubes that stained intensely in Evans blue were recorded on plants treated with ICIA5504 at 0.4, 0.08 or 0.016 mg AI/l (17%, 83% or 61% of germinated uredospores respectively). Epoxiconazole, applied at 10mg AI/l, had no significant effect on uredospore germination and did not cause germinated uredospores to develop short, intensely stained germ tubes.

Septoria tritici

Inhibition of pycnospore germination was clearly the major effect of ICIA5504 against *S. tritici* (Figure 2). Interestingly, although 9% of pycnospores sampled had germinated on plants treated with 10mg ICIA5504/l, no disease developed. This indicates that ICIA5504 was also exerting a post-germination effect. Epoxiconazole had no significant effect on pycnospore germination at the rates tested.

FIGURE 2. *Septoria tritici* : disease development and pycnospore germination on wheat (parallel tests).

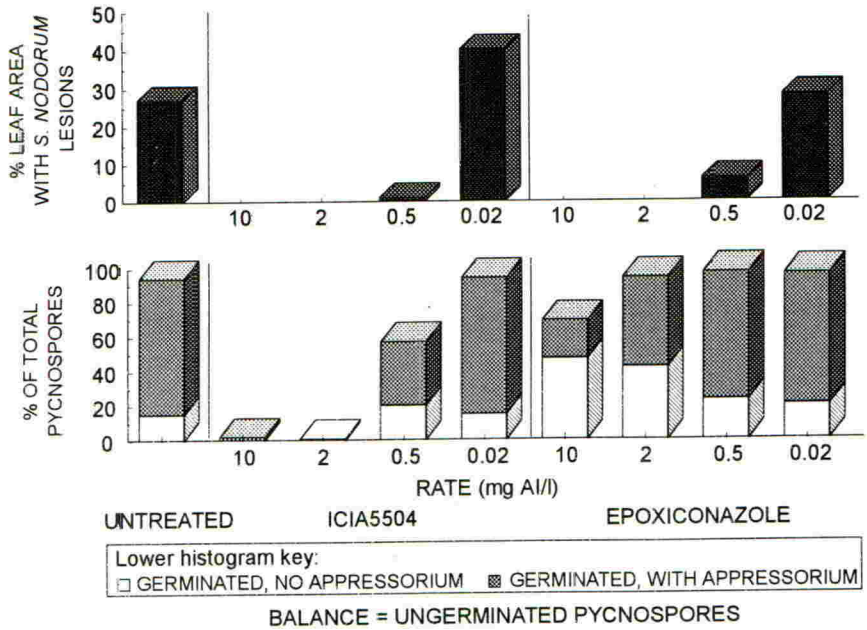


Septoria nodorum

Treatment with ICIA5504 at 10 and 2 mg AI/l provided almost complete inhibition of pycnospore germination (Figure 3). In addition, although 57% of pycnospores sampled at 0.5 mg AI/l had germinated and most had also formed an

appressorium, the disease level was low in the parallel efficacy test. This suggests that ICIA5504 is inhibiting not only spore germination but also fungal development after appressorium formation. Epoxiconazole had no significant effect on pycnospore germination at the rates tested.

FIGURE 3. *Septoria nodorum* : disease development, pycnospore germination and appressorium formation on wheat (parallel tests).



Erysiphe graminis f.sp. *hordei*

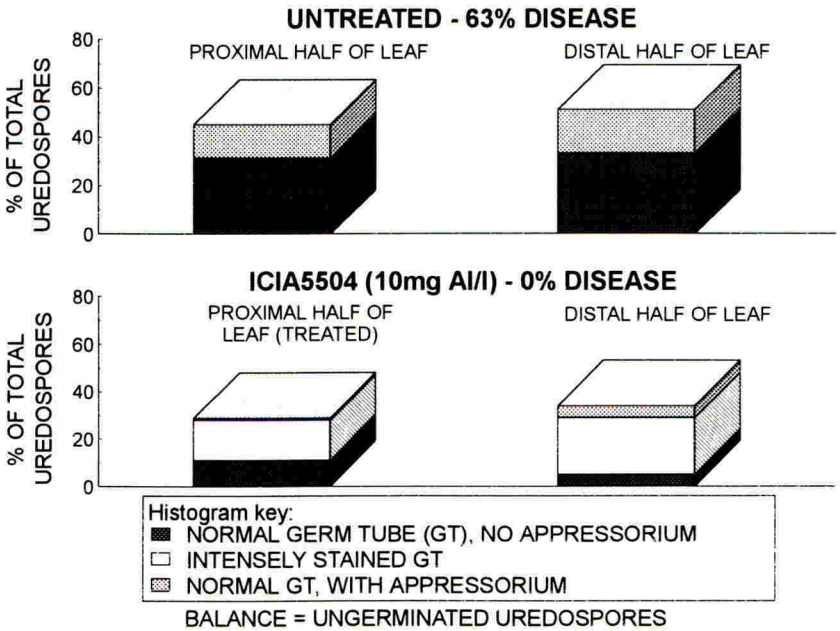
ICIA5504 inhibited the germination of conidia of *E. graminis* f.sp. *hordei* on barley when sampled 7h after inoculation. However, this effect was less marked than with *P. recondita* and *Septoria* spp. on wheat. For example, ICIA5504 applied to barley at 30mg AI/l gave only 55% inhibition of germination of conidia of *E. graminis* f.sp. *hordei*, despite complete disease control at the same rate.

71% of germinated conidia on untreated plants had developed a primary hypha. This compared with 5-10% on plants treated with 3mg ICIA5504/l. In addition, fluorescence microscopy showed that 53% of conidia having formed an appressorium had also developed a seemingly normal haustorium on untreated plants. This compared with 15% on plants treated with 3mg ICIA5504/l. These data, coupled with complete disease control at the same rate, indicate that ICIA5504 slows post-germination fungal development and/or prevents the successful establishment of haustoria.

Systemic effects

ICIA5504 applied to only the proximal half of wheat leaves inhibited the germination of *P. recondita* uredospores and caused most of those uredospores which did germinate to develop short germ tubes that stained intensely in Evans blue. These effects were seen equally in the proximal and distal halves of treated leaves (Figure 4). ICIA5504, being inactive in the vapour phase, could only have caused such effects in the distal half of treated leaves through systemic movement.

FIGURE 4. Systemic effects : *P. recondita* disease development, uredospore germination, germ tube elongation and appressorium formation on wheat (parallel tests; proximal half of leaf treated 24h prior to inoculation).



Eradicant effects

ICIA5504 (2mg AI/l) applied to wheat seedlings 5 d after inoculation with *P. recondita* was seen to cause mycelial collapse when cryofractured material was viewed by scanning electron microscopy 24h after treatment. Although no mycelial collapse was apparent with epoxiconazole at the same rate and sampling time this effect was clearly evident at the second sampling time, 7d after application.

DISCUSSION AND CONCLUSIONS

The dominant effect of preventative applications of ICIA5504 against *P. recondita, S. tritici* and *S. nodorum* was inhibition of spore germination, but this was less important against *E.graminis* f.sp. *hordei*. However, in all instances post-

germination effects were also observed, including germinated uredospores with short germ tubes which stained intensely in Evans blue (*P. recondita*) and restriction of fungal development after appressorium formation (*S. nodorum*). Further work is necessary to understand why only *P. recondita* uredospores on ICIA5504 treated plants formed germ tubes which stained intensely in Evans blue, a specific stain for dead material (Fischer *et al*, 1985), given that all inoculated plant material studied was fixed prior to staining.

Inhibition of mitochondrial respiration by ICIA5504 clearly has major effects on the early stages of fungal development. In contrast, the triazole family of fungicides inhibits the 14-demethylation step in the ergosterol biosynthesis pathway. Therefore, triazoles do not impair the early stages of fungal colonisation because during this time the pathogen obtains a supply of ergosterol or its precursors from the spore (Hänssler & Kuck, 1987).

The effects of a protective application of ICIA5504 against *P. recondita* were the same whether the active ingredient was applied directly or reached the target site via systemic movement. Eradicant treatment with ICIA5504 caused mycelial collapse of *P. recondita* within 24h of application.

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FORECASTING LIGHT LEAF SPOT (*PYRENOPEZIZA BRASSICAE*) ON WINTER OILSEED RAPE

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ABSTRACT

The incidence of light leaf spot (% plants affected) in ADAS surveys of commercial crops in Eastern England from 1977 to 1993 ranged from 0 to 43% for disease on leaves at early stem extension and from 0 to 75% for stem disease or pod disease in July. Regression analyses indicated that the % plants with disease on leaves related well to the % plants with pod disease the previous season but not well to rainfall for any month. The % plants with stem or pod disease related well to the % plants with disease on leaves and % plants with pod disease slightly to May and October rainfall. In 1990 the first appearance (27 November) of light leaf spot on plants in inoculated plots at Bristol could be associated with the occurrence of weather which fulfilled infection criteria of $\geq$17h wetness (27 October), followed by a period with sufficient accumulated temperature to complete the latent period of light leaf spot. In a second successive oilseed rape crop at Rothamsted in 1990 light leaf spot was first observed on plants sampled from the crop on 16 November and first developed on healthy bait plants exposed in the crop from 3-5 October. Symptomless plants infected with light leaf spot sampled from a winter oilseed rape crop in 1994 or from pots of artificially inoculated plants had developed more light leaf spot after 6 days at 10 or 20°C than at 4 or 15°C.

INTRODUCTION

Light leaf spot (*Pyrenopeziza brassicae*) can cause damaging epidemics on winter oilseed rape in the UK (Wale *et al.*, 1990; Hardwick *et al.*, 1991). The development of severe epidemics on leaves, stems and pods in spring and summer appears to be associated with the widespread infection of crops in the autumn, since the greatest yield responses to control of light leaf spot are often achieved by spray programmes including fungicide applications in autumn (Rawlinson & Cayley, 1984; Jeffery *et al.*, 1994). However, since *P. brassicae* may have a long latent period on oilseed rape crops in the winter in the UK (Figueroa *et al.*, 1994), widespread autumn infection may not be observed until the symptoms appear in late winter or early spring. There is a need to estimate the frequency of severe light leaf spot epidemics and to identify the factors which cause them, both nationally and in individual crops. This paper investigates the relationship between the incidence of light leaf spot on commercial oilseed rape crops from 1977 to 1993 and previous inoculum concentrations or weather (monthly rainfall) and considers methods to study the early development of light leaf spot in winter oilseed rape in order to forecast the subsequent severity of epidemics.

MATERIALS AND METHODS

As part of a national ADAS disease survey, samples of 25-100 plants were taken from 9-44 commercial winter oilseed rape crops in Eastern England in 1977-1993 at both early stem extension (GS 2.1-2.5) in late February to early April and at maturity (GS 6.4) in July. The incidence (%) of plants with light leaf spot symptoms on leaves (first sample), stems or pods (July sample) was assessed. Step-wise regression techniques were used to investigate the relationships between the % of plants with light leaf spot on leaves, stems or pods and both monthly rainfall and previous inoculum concentrations (the incidence of light leaf spot on previous samples). At ADAS Bristol, plots of three oilseed rape cultivars, Jet Neuf, Ariana and Falcon, were inoculated with infected stem debris immediately after sowing (30 August 1990). Twenty-five plants per plot were marked and assessed *in situ* for symptoms weekly in order to calculate the cumulative incidence of light leaf spot (% plants affected). Samples of ten plants per plot were also taken weekly and incubated in polyethylene bags at 20°C for 1 week before assessment. Maximum and minimum temperatures, rainfall and estimated rainfall duration were recorded hourly at a meteorological station 4km from the site. Accumulated temperatures above 0°C were calculated using the mean of the maximum plus minimum temperatures as the mean temperature.

At Rothamsted, the incidence (% plants affected) of light leaf spot was assessed on winter oilseed rape (cv. Cobra) sown after oilseed rape on four samples of 20-50 plants taken between 1 and 19 November and then on monthly samples of ten plants per plot from 24 November 1990 to June 1991. Before assessment, plants were incubated in polyethylene bags at 3°C for 7-10 days. Plots were sprayed with prochloraz (500g a.i. ha^{-1}) in autumn, spring or summer or were unsprayed. Healthy oilseed rape plants (cv. Cobra, GS 1.5), grown in plastic pots, were exposed in unsprayed plots of this experiment twice each week from 1 September to 20 December 1990 for periods of one week (four pots with three plants per pot) and then returned to a glasshouse for incubation before assessment of light leaf spot development. Samples (cv. Envol, GS 1.9) were taken on 2 March 1994 from unsprayed plots of another winter oilseed rape experiment inoculated with infected debris. Plants, which showed no symptoms of light leaf spot, were placed in polyethylene bags at 4, 10, 15 or 20°C (ten plants per temperature). Glasshouse-grown oilseed rape (cv. Envol, GS 1.5) was inoculated with a spore suspension of *P. brassicae* ($3x10^5$ spores ml^{-1}) on 24 March 1994 and kept for 7 days at 100% r.h. and 15°C. Twelve days after inoculation, plants were sampled and placed in polyethylene bags at 4,10, 15 or 20°C (ten plants per temperature). In both experiments, plants were assessed daily for light leaf spot (% leaf area affected) after 48h from sampling.

RESULTS

The average incidence of light leaf spot (% plants affected) in ADAS surveys of crops in Eastern England ranged from 0 (1992) to 43% (1988) for disease on leaves, from 2 (1991) to 75% (1983) for stem disease and from 0 (1978, 1980) to 75% (1983) for pod disease (Fig. 1). Regression analyses suggested that the % plants with light leaf spot on leaves in spring was related better to the % plants with pod disease the previous July (43% variance accounted for) than to the % plants with stem disease (25%) or disease on leaves the previous spring (17%) or to monthly rainfall (maximum 20% previous October). The % plants with stem (59%) or pod (44%) disease in July both related well to the % plants with

disease on leaves in spring and the % plants with pod disease was also related to May and October rainfall (46%). In 1990 at Bristol, periods with rainfall wetness duration $\geq$17h, when infection could have occurred (Figueroa, 1993), were recorded on 29 September, 5, 18-19, 27 October and 9-12 November (30, 36, 49-50, 58 and 71-74 days after sowing) (Fig. 2). No symptoms were observed on leaves of marked plants on 20 November and the first symptoms were observed on 27 November (89 days after sowing) on all three cultivars. The estimated accumulated temperature during the latent period was therefore 263 day-degrees if infection occurred on 27 October. However, symptoms were first observed on incubated plants sampled on 31 October.

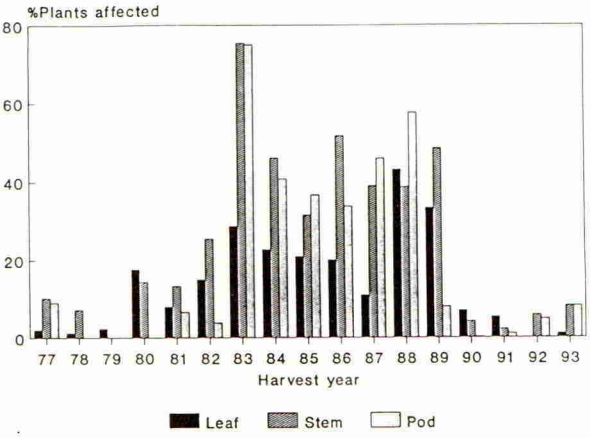

FIGURE 1. Incidence (%) of plants with light leaf spot on leaves at early stem extension or stems or pods in July, assessed in ADAS surveys of crops in Eastern England in 1977-1993. Crops were not sampled in July 1979.

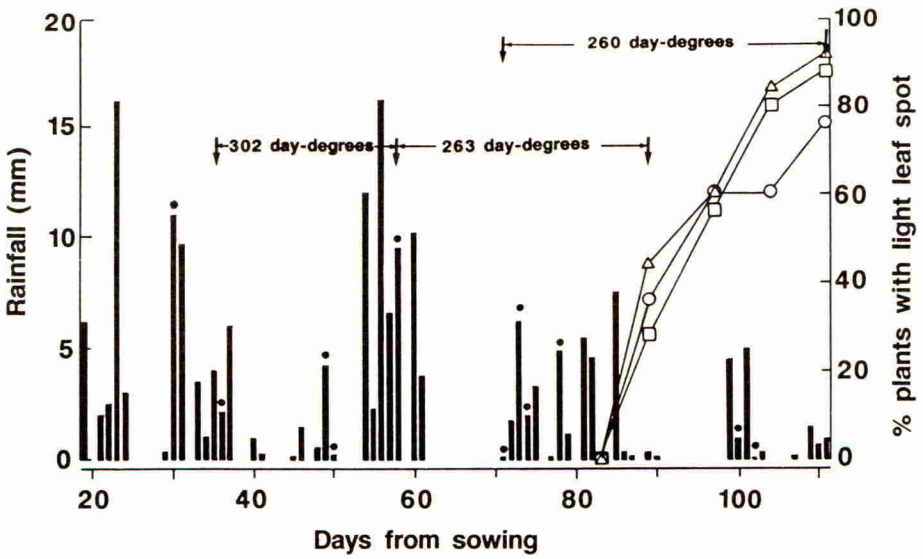

FIGURE 2. Development of light leaf spot on winter oilseed rape cvs. Jet Neuf (Δ), Ariana (○) and Falcon(□) (% plants affected) in 1990/91 in relation to rainfall (columns) and occurrence of infection periods with wetness duration $\geq$17h (●)

At Rothamsted in 1990, light leaf spot was observed on plants sampled from the crop on 16 (4%) and 19 November (12%) but not on 7 November and was observed on nearby volunteer plants on 1 November (5%). Subsequently the incidence of light leaf spot steadily increased in incubated samples from unsprayed plots between November and March (Fig. 3). Bait plants exposed in the crop first became infected between 3-5 October at a low incidence (2 from 24 plants infected) and from late October to December 8-50% of plants in all batches of bait plants developed symptoms. Autumn fungicide sprays decreased the incidence of light leaf spot for several months but spring or summer sprays had little effect. Symptomless plants sampled from a winter oilseed rape crop at Rothamsted in 1994 or from pots of artificially inoculated plants had developed more light leaf spot (% area affected) after 6 days at 10 or 20°C than at 4 or 15°C (Fig. 4). Plants kept at 15 or 20°C then rapidly lost their leaves whilst those at 4 or 10°C developed more lesions.

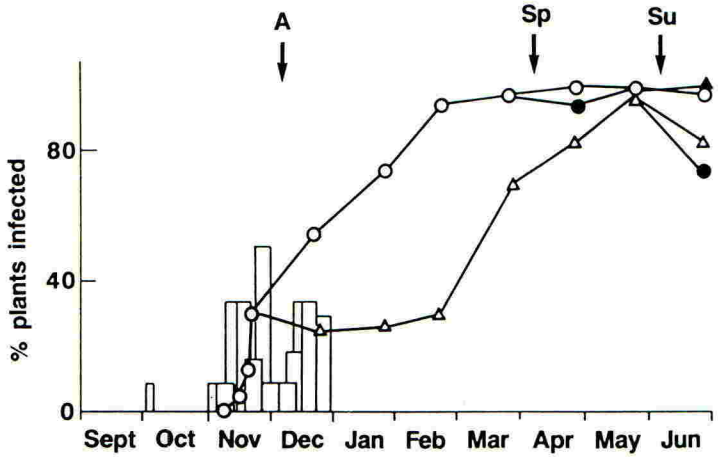

FIGURE 3. Incidence of light leaf spot (% plants infected) in a winter oilseed rape crop (cv. Cobra) sown after rape, assessed on samples from unsprayed plots (○) or plots sprayed with prochloraz in autumn (A, △), spring (Sp, ●) or summer (Su, ▲) and on healthy bait plants (cv. Cobra) exposed in the crop twice weekly for periods of 1 week before incubation in a glasshouse (columns). Arrows indicate spray dates.

DISCUSSION

These results suggest that the forecasting of light leaf spot on oilseed rape can be considered in three stages:

1. Identify seasons with risk

In October, ADAS national survey data can be used to identify seasons with a high risk of severe light leaf spot, when there is both inoculum available and weather favourable for inoculum dispersal and infection of new winter oilseed rape crops. The regression analyses suggest that an indication of the inoculum available in autumn can be obtained from the incidence of light leaf spot on pods in July. Such infected pods are likely to provide a

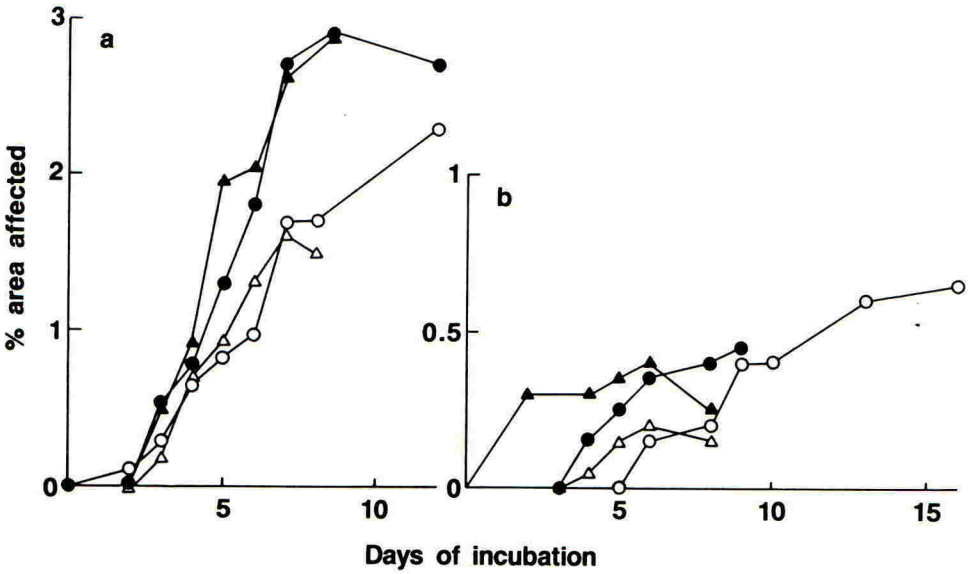

FIGURE 4. Development of light leaf spot (% area affected) on leaves of oilseed rape plants (cv. Envol) sampled from either (a) a winter oilseed rape crop or (b) pots of plants inoculated with spores of *Pyrenopeziza brassicae* and incubated at 4 (○), 10 (●), 15 (△) or 20°C (▲).

means by which the pathogen can survive between seasons, along with infected stem debris and volunteer plants, whereas infected leaves would decay too quickly. The relationship may be improved slightly by inclusion of July rainfall (favouring inoculum production) and October rainfall, favouring dispersal of inoculum as ascospores or conidia and providing sufficient wetness periods for infection (Rawlinson *et al.*, 1978). It may be possible to present predictions of seasons with a high risk on a regional basis since both regional survey data and regional weather data are available. However, in some areas (e.g. the north of Scotland) there is a high risk of severe light leaf spot in most seasons (Wale *et al.*, 1990) and it may not be necessary to proceed further before deciding to spray.

2. Identify crops at risk

In high risk seasons, individual growers or advisers can then identify crops at risk, using information about cultivar susceptibility, proximity to previous infected crops and sowing date (early-sown crops are likely to be at greater risk). This information can then be complemented by using local weather information to identify when infection periods with leaf wetness duration $\geq$ 17h (Figueroa, 1993) have occurred. From subsequent accumulated temperature records it should be possible to establish when symptoms resulting from these infection periods are likely to appear in the crops and the grower or adviser can choose a sampling date if they wish to determine the incidence of the disease.

3. Confirm autumn disease incidence and make spray decision

These results suggest that samples can be taken before symptoms appear; however, they should not be taken when the crop is frosted or wet. Plants sampled can be examined immediately to assess the incidence of light leaf spot but should also be incubated in polyethylene bags for *c.* 6 days to allow for additional symptom development, either at 20°C (in the farmhouse) or preferably at 10°C (in the barn). In future, the accuracy of disease assessments could be improved by developing precise serological or molecular diagnostic techniques. However, there is still a need to determine the threshold values on given dates for incidence of light leaf spot to determine when application of spray treatments is justified. These values will also determine the size of samples needed (e.g. a sample size of *c.* 60 plants is needed to detect a 5% incidence of disease (Figueroa, 1993)) and the period of time over which sampling needs to be continued. Furthermore, a forecasting scheme for light leaf spot also needs to take into account the time period over which fungicide sprays can be effectively applied and to be amalgamated with a scheme for forecasting stem canker (*Leptosphaeria maculans*), which may need to be controlled at the same stage in the growing season of winter oilseed rape.

ACKNOWLEDGEMENTS

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OCCURRENCE, DEVELOPMENT AND CONTROL BY FUNGICIDES OF *SEPTORIA NODORUM*, *S. TRITICI*, AND *PSEUDOCERCOSPORELLA HERPOTRICHOIDES* ON WHEAT IN POLAND IN 1993

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ABSTRACT

The occurrence, development and control by fungicides of *Septoria nodorum*, *S. tritici*, and *Pseudocercosporella herpotrichoides* on wheat in Poland in 1993 were investigated using DuPont diagnostic immunoassays. At 25 locations throughout Poland, all pathogens were present at GS 25-31 and attained high levels by GS 75. *S. nodorum* and *S. tritici* occurred in lower foliage at GS 25 and spread to upper leaf levels and heads by GS 61-75 while *P. herpotrichoides* infection increased significantly between GS 61 and GS 75. In a field trial in southeastern Poland, flusilazole combinations with carbendazim or tridemorph were effective in controlling these pathogens and exhibited excellent residual activity. These results demonstrate the importance and development of *S. nodorum*, *S. tritici*, and *P. herpotrichoides* in Poland as well as the utility of fungicide applications for control of these cereal pathogens in Eastern Central Europe.

INTRODUCTION

Septoria nodorum, *S. tritici*, and *Pseudocercosporella herpotrichoides* are important pathogens of wheat in Poland and can cause significant yield losses (Malinski, 1992; Pokacka, 1985). Their occurrence in this country has been previously described using traditional visible disease assessment and fungal isolation techniques. With the recent availability of diagnostic immunoassays for *S. nodorum*, *S. tritici* and *P. herpotrichoides* from DuPont, a highly quantitative, sensitive, and specific measure of the incidence of these three pathogens is now possible (Joerger *et al.*, 1992; Smith *et al.*, 1990). This paper presents results on the occurrence, development and control by DuPont fungicides of *S. nodorum*, *S. tritici*, and *P. herpotrichoides* on wheat in Poland in 1993 as determined by diagnostic assays.

MATERIALS AND METHODS

To investigate disease occurrence and development, samples were collected in 1993 from 25 winter wheat fields, located throughout Poland, which had not been treated with fungicides. Each field was *c.* 1 ha in size and was fertilized according to local agronomic practice. Disease developed from naturally occurring inoculum. Thirty main shoots were collected per field using a uniform, randomized sampling pattern at GS 25-31 (late April to early May), GS 51-55 (mid to late May), and GS 75 (late June to early July).

To study further pathogen development and the effect of fungicides, a separate field trial was established at the Institute of Soil Science and Plant Cultivation, Pulawy in southeastern Poland. Winter wheat (cv. Rosa) was planted following wheat in fall, 1992 and fertilized according to general agronomic practice. A randomized, complete block design with four replicates and 11 x 2.3 m plot sizes was used. Treatments consisted of either an untreated control or a foliar fungicide spray program of 'Alert' 375 SC (flusilazole 125 g/l + carbendazim 250 g/l) applied at 1 l/ha at GS31 (8 May), followed by 'Cerelux' 510 EC (flusilazole 160 g/l + tridemorph 350 g/l) applied at 0.8 l/ha at GS 49 (23 May). Sprays were applied using a pressurized knapsack sprayer with flat fan nozzles, water volumes of 300 l/ha, and pressures of 250 kpa. Thirty main shoots were collected per plot at GS 25 (27 April), GS 31 (6 May), GS 49 (21 May), GS 61 (3 June), GS 75 (24 June), and GS 92 (29 July).

For all locations, samples for GS 25 and 31 were divided into lower (basal 5 cm) and upper leaf sections. At subsequent sampling dates, the 30 shoots from each field or plot were bulked by stem bases, leaf layers (with leaf 1 denoting the flag leaf), and heads.

Samples were homogenized in 150 ml buffer and tested following the protocols described for the DuPont 'Advisor' enzyme-linked immunosorbent assays (ELISA). Leaves and heads were assayed for *S. nodorum* and *S. tritici* whereas stems and lower plant sections were tested for *P. herpotrichoides*. ELISA results were expressed as the number of *S. nodorum*, *S. tritici*, or *P. herpotrichoides* antigen units/ml of homogenized plant tissue (AgU/ml). AgU/ml values for replicates were averaged.

RESULTS

Occurrence of *S. nodorum*, *S. tritici*, and *P. herpotrichoides* in Poland

Significant levels ($\geq$10 AgU/ml) of *S. nodorum*, *S. tritici*, and *P. herpotrichoides* antigens were present in all locations at GS 25-31. *S. nodorum* antigens increased during the season to attain high levels in all leaf layers and heads at all sites by GS 75 while *S. tritici* was also widely present in the upper canopy and heads by GS 75 (Fig. 1a,b). *P. herpotrichoides* antigens were detected at levels $\geq$40 AgU/ml in stems in all regions by GS 75.

Development of *S. nodorum*, *S. tritici*, and *P. herpotrichoides* in untreated wheat in Pulawy

Significant levels of *S. nodorum* and *S. tritici* antigens were present in both lower and upper leaf sections at GS 25 and GS 31 (Fig. 2a,b). Antigen concentrations increased in lower sections but decreased in upper sections during the period between the two growth stages. At GS 49, *S. nodorum* and *S. tritici* antigens continued to be found in lower leaves (leaf 4), but few or no antigen units were detected in upper leaf layers (leaves 1-3). By GS 61, *S. nodorum* antigens were present at significant levels in all foliar layers (leaves 1-4) as well as in heads. Infection increased in all leaves through GS 75 and in heads through GS 92. Although *S. tritici* antigens were detected only in leaf 4 at GS 61, significant levels were found in leaves 2-4 at GS 75. Infection in heads increased through GS 92. At each sampling date from GS 49-75, antigen units of both *S. nodorum* and *S. tritici* were highest on lower leaves and progressively decreased at higher leaf layers.

Antigens of *P. herpotrichoides* occurred at significant levels in lower pseudostem and stem sections at GS 25-61 (Fig. 3). Antigen levels greatly increased from GS 61 to GS 75 and attained very high levels by GS 92.

Control of *S. nodorum, S. tritici, and P. herpotrichoides* by fungicide treatments in Pulawy

Applications of flusilazole combinations with carbendazim or tridemorph resulted in significantly lower levels of *S. nodorum* antigens in leaves 1-3 at GS 61 and 75 compared to untreated controls (Fig. 4a). Reduced *S. tritici* antigen concentrations were present in leaves 2-3 at GS 75 in plants treated with fungicides relative to unsprayed controls (Fig. 4b). Significantly lower levels of *P. herpotrichoides* antigens occurred in fungicide-sprayed plants compared to untreated controls at GS 61 and GS 75 (Fig. 5).

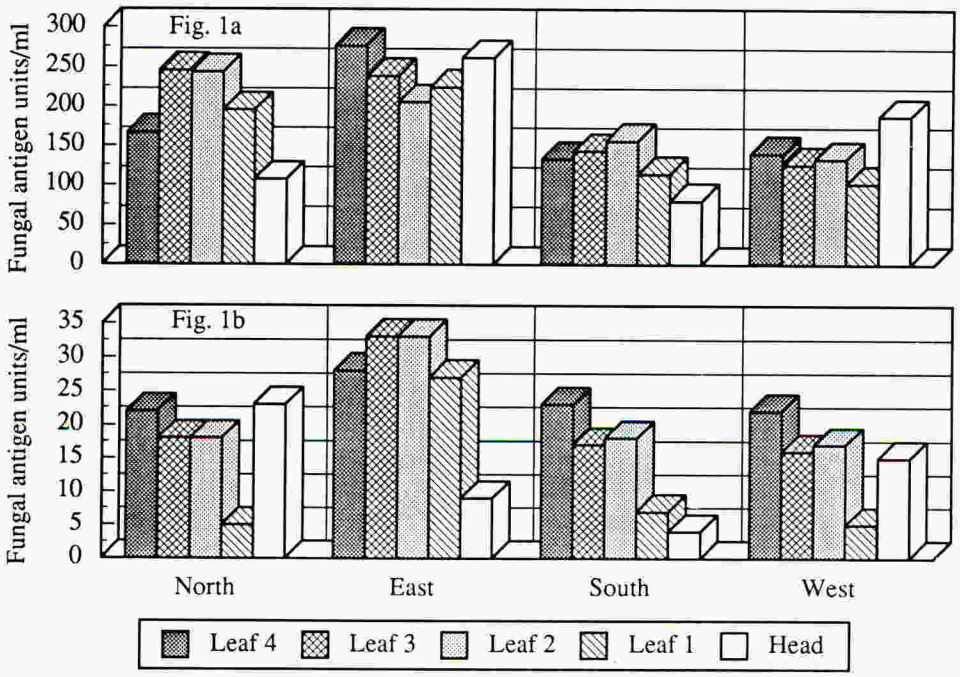
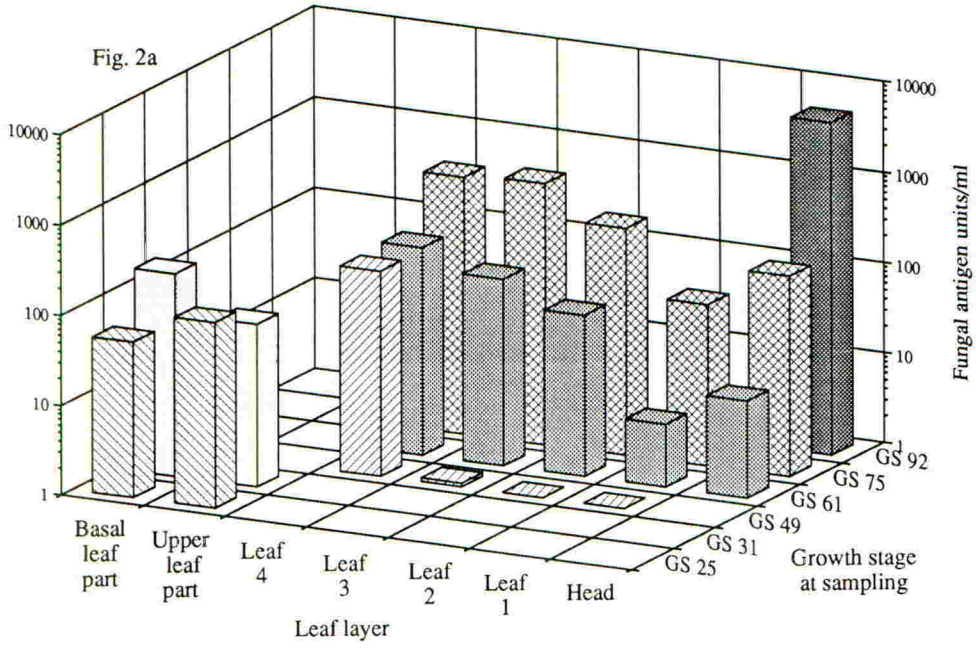


FIGURE 1. Levels of *Septoria nodorum* (a) and *S. tritici* (b), as expressed in fungal antigen units/ml, in untreated wheat at GS 75 in four geographic regions in Poland in 1993. Leaf 1 denotes flag leaf.

DISCUSSION

The use of the DuPont diagnostic assays has demonstrated that *S. nodorum*, *S. tritici*, and *P. herpotrichoides* are widely present in wheat throughout Poland and can attain high levels of infection during the growing season. The highly sensitive and quantitative nature of the three imunoassays insures that the fungi were correctly identified and infection levels were determined with significant precision. These results confirm earlier reports on the occurrence of these fungi in Poland (Malinski, 1992; Pokacka, 1985) and demonstrate the prevalence of these cereal pathogens from Western to Eastern Central Europe (Joerger *et al.*, 1992; Royle *et al.*, 1986; Smith *et al.*, 1990).



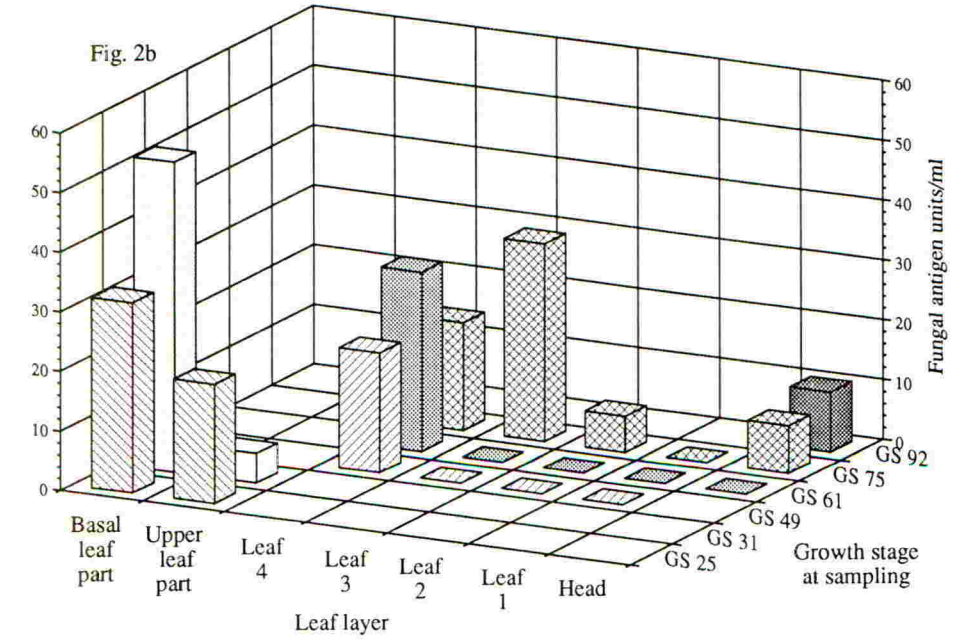


FIGURE 2. Development of *Septoria nodorum* (a) and *S. tritici* (b), as expressed in fungal antigen units/ml, in untreated wheat in Pulawy, Poland, in 1993. Leaf 1 denotes flag leaf.

Pathogen development in southeastern Poland was described using the diagnostic assays. *S. nodorum* and *S. tritici* were present in lower foliage at GS 25-49 to serve as inoculum sources. By GS 61-75, infection of both *Septoria* spp. had increased significantly and spread to upper leaf levels and heads. Initial *P. herpotrichoides* infection at GS 25 greatly increased between GS 61 and 75. This disease progress was typical of that found in other locations throughout Poland and is comparable to the disease development described in areas of Western Europe (Joerger *et al.*, 1992; Royle *et al.*, 1986; Smith *et al.*, 1990).

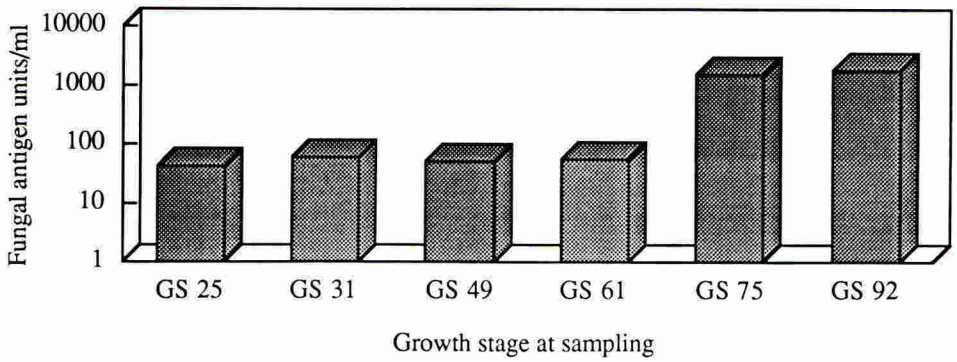


FIGURE 3. Development of *Pseudocercosporella herpotrichoides*, as expressed in fungal antigen units, in untreated wheat in Pulawy, Poland in 1993.

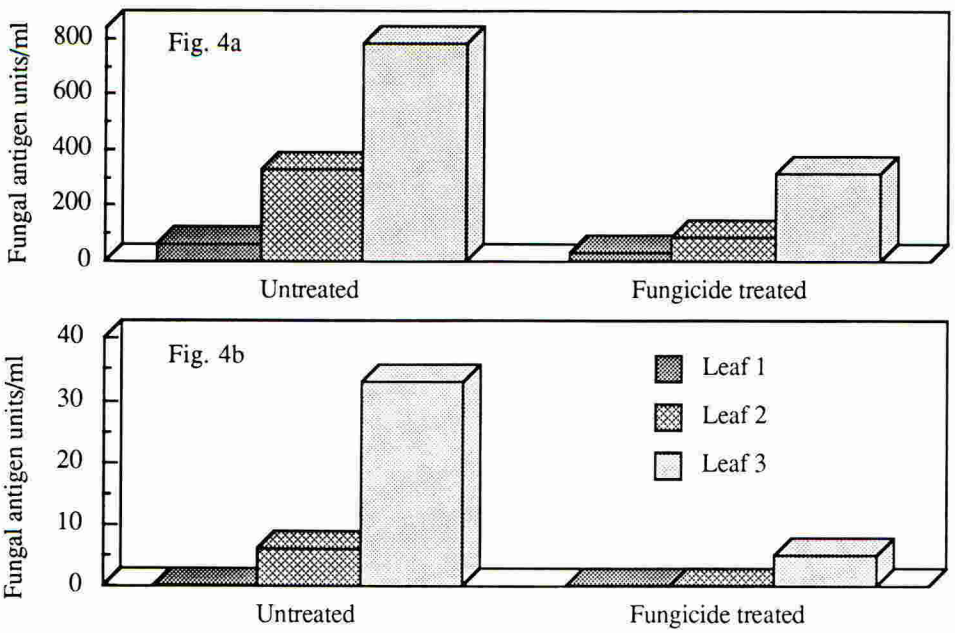


FIGURE 4. Control of *Septoria nodorum* (a) and *S. tritici* (b) at GS 75 in wheat, as expressed in fungal antigen units/ml, following applications of flusilazole plus carbendazim at GS 31 and flusilazole plus tridemorph at GS 49 in Pulawy, Poland in 1993. Leaf 1 denotes flag leaf.

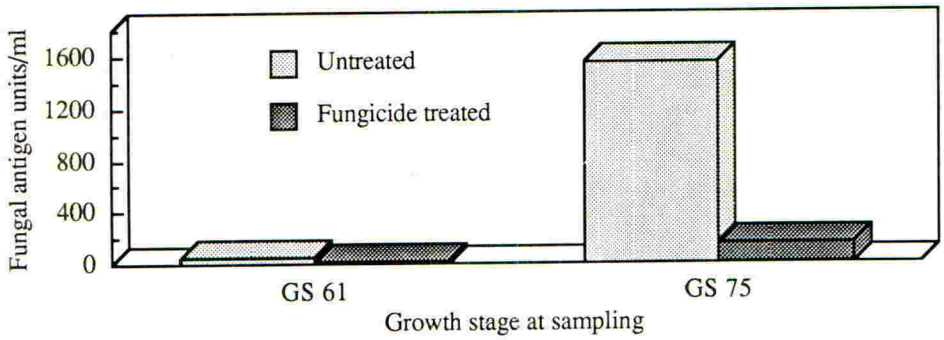


FIGURE 5. Control of *Pseudocercosporella herpotrichoides* at GS 61 and GS 75 in wheat, as expressed in fungal antigen units/ml, following applications of flusilazole plus carbendazim at GS 31 and flusilazole plus tridemorph at GS 49 in Pulawy, Poland in 1993.

Effective control of *S. nodorum*, *S. tritici*, and *P. herpotrichoides* by flusilazole products has been demonstrated in Western Europe (Austin, 1986). This excellent activity was confirmed in the Pulawy field test in which applications of flusilazole combinations with carbendazim or tridemorph significantly reduced infections of both *Septoria* species and *P. herpotrichoides*, compared to untreated controls. The fungicide mixtures exhibited strong residual efficacy, providing control for over one month after the last application.

The results presented in this paper demonstrate the importance and development of *S. nodorum, S. tritici,* and *P. herpotrichoides* in Poland as well as the utility of fungicide applications in controlling cereal diseases under conditions in Eastern Central Europe.

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APPLICATION OF DIAGNOSTICS AS A FORECASTING TOOL IN BRITISH AGRICULTURE

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ABSTRACT

Septoria diseases are of major importance in winter wheat crops leading to significant losses of yield and quality. Accurate diagnosis is important for effective control, and identification from visual symptoms is often difficult even for the experienced plant pathologist, whilst laboratory analysis is both time consuming and expensive.

Ciba Agriculture started work in 1988 screening for antibodies for *Septoria tritici* and *Septoria nodorum*. These were successfully isolated and used in a standard multiwell ELISA kit to provide a highly sensitive and accurate system for the identification and quantification of *Septoria* spp.. Ciba Agriculture used this immunoassay technology as a basis for a national *Septoria* spp. diagnostic survey called 'Septoria Watch'. This is the first system for *Septoria* spp. control to incorporate ELISA technology and apply it to British agriculture as a usable nationwide disease management system. Trials showed presymptomatic warning and good yield benefits in combination with possible reductions in fungicide usage.

'Septoria Watch' provides a large database for *Septoria* disease incidence and epidemics, which has the potential of being ultimately integrated into a *Septoria* spp. forecasting / ICM system.

INTRODUCTION

Septoria diseases occur throughout the world and affect numerous crops, causing mostly leaf spots and blights. *Septoria tritici* and *Septoria nodorum* are among the most important leaf spot pathogens of wheat causing considerable losses of yield and quality each year.

Current agricultural practice is to time fungicide applications on host phenology rather than disease progress. From an epidemiological point of view these growth stage oriented treatments are randomly timed and only randomly successful. Accurate disease diagnosis would allow the use of the most appropriate fungicide, whilst quantification of infection levels and pre-symptomatic detection would help to establish disease thresholds for spray timing, also allowing the possibility of fungicide dose reduction. Immunoassays are rapid, sensitive, specific and quantitative.

With R&D costs increasing, resulting in the likelihood of fewer new fungicides being introduced onto the market, many agrochemical companies are developing diagnostics to

support the use of their current products and to demonstrate an environmental awareness by helping to improve integrated crop management (ICM) strategies.

Ciba started work in 1988 screening for highly specific antibodies for *S. tritici* and *S. nodorum* (Miller *et al.*, 1988, Mittermeier *et al.*, 1990). This has since been developed into a commercially useful diagnostic tool for these diseases, the use of which is reported in this paper. Also presented are the results of field trials comparing disease management using the immunoassay diagnostic to routine programmes and a disease risk assessment model (Verreet & Hoffman, 1989)

The principle of immunoassays and ELISA

The basic tenet behind immunoassays is the binding of a specific antibody to its corresponding antigen in a stable and specific manner. Plant pathogens possess as part of their structure, specific antigenic determinants (proteins, polysaccharides or other moieties), and their distinct serology can be exploited through immunoassay techniques.

One of the most widely used techniques is using an enzyme-labelled antibody. These are stable, easily prepared, generally nonhazardous to health and inexpensive. Furthermore, assays can be performed quickly with relatively inexpensive and simple equipment. The technique is known as enzyme - linked immunosorbant assay (ELISA).

Several variations of ELISA are currently in use, and can be divided into those which are 'direct' where enzyme labelled specific antibody is reacted directly with antigen, and indirect where the antibody is unlabelled and is, itself, reacted with an enzyme-labelled antibody. The method used in this study, and preferentially used for many plant pathogens and by plant health monitoring agencies worldwide is the double antibody sandwich (DAS) ELISA. This uses antibody coated microtitre plates or membranes, and 'sandwiches' the antigen between a primary coating and secondary detector antibody.

To increase accuracy, the Ciba ELISA uses monoclonal antibodies as the capture system (highly specific) and polyclonal antibodies for the detection (highly sensitive).

'Septoria Watch'

During 1993 and 1994 a disease monitoring system has been in operation throughout the UK called 'Septoria Watch'. This involves sampling representative wheat crops throughout Britain at weekly intervals, during the main wheat growing period. During 1993 these crops were monitored by distributor technical advisors, agricultural colleges, ADAS (UK official advisory service) as well as Ciba personnel.

Samples from crops were sent by first class post to the plant pathology lab. at Ciba Agriculture headquarters at Whittlesford for processing through the DAS ELISA system for both *S. tritici* and *S. nodorum*.

The leaf samples were processed and the results sent to customers, usually by fax. within a 48h period of sample receipt. Participants in the system were therefore in possession of an up to date assessment of the Septoria status in their area. In addition to this rapid information dissemination to participants in the system, the data were made available to the whole of farming industry by means of a 'Septoria Map' which was published in a commercial farming magazine.

MATERIALS AND METHODS

Microtitre plates

The immunoassay described for the detection of *S. tritici* and *S. nodorum* are based on the principle of a DAS ELISA. Immunoassays were made from plant material i.e. leaves sampled from GS 21 (Zadoks *et al.*, 1974) onwards.

The microtitre plates were pre-coated with primary antibody. The filtered extract (100µl per well) was pipetted into two wells. All samples (maximum of 44 per plate) were added to the plate (approx. 5 min), and the plates incubated at room temperature for 10 min on a plate shaker. The plates were then automatically washed with wash solution five times. After washing, 100µl of antibody-peroxidase conjugate was added to each well. The incubation and wash steps were repeated. To each well 100µl of working substrate solution was then added, and incubation was repeated. Finally, 50µl of stop solution was added and the absorbance value of each assay well at 650 nm was recorded.

Positive and negative standards were included in each plate to determine variance between plates and to establish the background level, i.e. non-specific binding.

'Septoria Watch' Samples.

Registered fields were sampled at weekly intervals. Comprehensive instructions were issued at registration to enable the sampler to collect the samples correctly. A sample of 30 wheat plants/leaves were collected in a 'W' transect per maximum 10 ha area that was expected to achieve statistically significant comparisons.

Septoria Watch ran from late March to mid July, to incorporate the main growth stages for application timing. Traditionally fungicide applications are made at GS 31-33, GS 39 and GS 55. Samplers were instructed to send in whole wheat plants in order that the appropriate leaf levels were tested and after GS 39 only flag leaves were sampled. The leaf level tested was the most recently fully emerged leaf, giving the opportunity to evaluate the movement of Septoria disease through the leaf levels.

Field Trials

Two trials were initiated during 1993 in winter wheat crops, cv. Riband, in East Anglia. Plot sizes were 3x12m with a randomised complete block design and four replicates. The fungicide used was Tilt 250EC (propiconazole, 125 g AI / ha). Leaves were sampled at weekly intervals starting at GS32 to determine disease levels and subsequently base spray decisions (Table 1). This was either by evaluating the visual % leaf area infected (LAI) and using threshold criteria (Verreet & Hoffman, 1990), or using the multiwell diagnostic to monitor leaves from 1 leaf level above the indicator level in the Verreet/Hoffman system (MW1); or in the Septoria Watch programme by using the multiwell reading plus consideration of cultivar susceptibility and weather conditions. A diagnostic threshold value of 50 (absorbance reading at 650 nm x100) was used for both MW1 and Septoria Watch programmes. In this project, decisions for applications were made on the level of *S. tritici* detection since this is the more important *Septoria* species in the UK.

TABLE 1. Application details and spray plan in field trials.

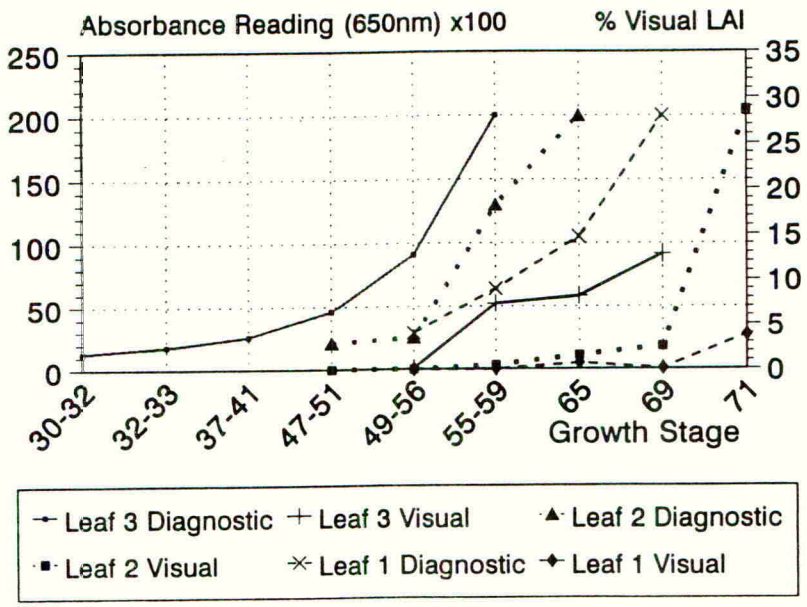
Treatment	Date				
	28/04/93	14/05/93	01/06/93	14/06/93	28/06/93
Untreated	-	-	-	-	-
3-spray programme	X	-	X	-	X
Verreet / Hoffman	-	-	X	-	-
MW 1	-	-	X	-	-
'Septoria Watch'	-	X	X	-	-
GS at Elmdon site	31	32-33	45-55	49-59	65-70
GS at Comberton site	31	32-34	45-55	49-59	65-70

RESULTS

Disease detection

The Ciba diagnostic gave elevated absorbance readings of *S. tritici* in the diagnostic monitoring programme assayed in 1992 (Fig. 1) at least 1 week before visual symptoms appeared and gave early warning of visual symptoms that could easily be missed by a crop advisor for 1-2 weeks. In the 1993 field trials, *S. tritici* and S. *nodorum* developed at both sites. The diagnostic MW1 triggered a fungicide application at the same time as the Verreet / Hoffman criteria.

Fig. 1. Diagnostic results and visual symptoms for *S. tritici*. Whittlesford 1992 - untreated



It became clear that *S. nodorum* was present at a high level in the trials (Table 2) indicating a revival of this pathogen as a major late season disease, as last seen in the early 1980s. 'Septoria Watch' surveys run in 1993 and 1994 have shown an increase in *S. nodorum* incidence at GS59-65 from 16% samples infected in 1993 to 57% in 1994. This confirms field observations as reported by Du Rieu *et al.* (1994).

TABLE 2. *S. nodorum* diagnostic results for the Comberton site, showing absorbance at 650nm x 100. "Threshold value" = 50.

Treatments	Diagnostic results for flag leaf			
	01/06/93	14/06/93	28/06/93	08/07/93
Untreated	14.3	87.9	192.3	200.0
3-spray programme	16.8	9.4	37.1	82.7
Verreet / Hoffman	14.3	16.1	39.8	200.0
MW1	14.3	24.2	84.1	77.7
'Septoria Watch'	16.8	8.5	35.4	55.5
GS	45-55	49-59	65-70	75-83

Disease control and yield in field trials

Good control was achieved by each spray programme on this susceptible cultivar under a high disease pressure (Table 3). 'Septoria Watch' consistently gave good disease control, equal to the standard 3-spray programme but with only two sprays applied. The Verreet / Hoffman and MW1 programmes, which triggered only one fungicide application, under the heavy disease pressure gave similar results to the other spray programmes.

In both trials, all treatments yielded significantly better than the untreated, and at Comberton both the three-spray and 'Septoria Watch' programmes yielded significantly better than the Verreet / Hoffman and MW1 programmes.

TABLE 3. Visual control of *Septoria* spp. on flag leaf and yield (t /ha).

Treatment	Elmdon		Comberton	
	% foliar disease	yield t / ha	% foliar disease	yield t / ha
Untreated	32.5	5.99	18.0	3.70
3-spray programme	1.3	7.16	2.5	6.35
Verreet / Hoffman	1.3	6.61	4.0	5.42
MW1	0.3	6.70	4.8	5.53
'Septoria Watch'	0.1	7.07	2.5	6.39
LSD ($P \leq 0.05$)	15.55	0.770	6.52	0.878
Date	08/07/93	31/08/93	08/07/93	20/08/93
GS	75-83	93	75-84	93

DISCUSSION

From several years work (Miller *et al.*, 1988, Mittermeier *et al.*, 1990, Petersen *et al.*, 1990) we know that the Ciba *Septoria* diagnosic system gives reliable and specific identification of *Septoria* diseases. The detection of the disease is very early in the epidemic, allowing more time to apply control measures and to optimise fungicide inputs.

The trials carried out in 1993 demonstrated that the use of the multiwell diagnostic system interpreted appropriately gave an equivalent yield response to a prophylactic three-spray programme; but by optimising fungicide application a saving of one spray was made. Use of the Ciba diagnostic (MW1) gave pre-symptomatic detection of Septoria disease, triggering sprays at the same time as Verreet / Hoffman; however, 'Septoria Watch' interpretation of the diagnostic produced significant disease control and greater yield benefits. Following the commercial success of the 1993 programme, 'Septoria Watch' has been repeated in 1994 in the UK and also in Germany.

'Septoria Watch' is an information service to advisors and is an important part of the disease control decision making process, but is not in itself a definitive spray advice system. It is potentially a valuable part of Decision Support Systems (DSS) which must be used in context by experienced advisors in combination with their knowledge of the crop cultivars, weather conditions, yield potential, agronomic conditions, etc. The system gives an important measure of the amount of disease in the crop even if the disease cannot be seen - this is a major advance over current risk assessment systems.

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USE OF FUNGICIDE-MANIPULATED EPIDEMICS TO DETERMINE CRITICAL PERIODS FOR DISEASE CONTROL AND YIELD RESPONSE IN WINTER BARLEY

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ABSTRACT

Critical stages for disease control and prevention of yield loss were investigated in two seasons at different sites using fungicide programmes in a systematic linear design. In 1988/89, control of mildew and brown rust by applying fungicide no later than GS31 was essential to avoid yield loss. There was no advantage in active mildew during the autumn and winter at this high-yielding site (10.91 t/ha with full fungicide programme) where yield losses were up to 39.9%. In 1991/92, net blotch and brown rust were severe by GS77 (60% and 23% area leaf 1 affected respectively) and yield losses were up to 40.9% (full fungicide treated yield 8.45 t/ha). Spray programmes initiated at GS30-31 and continued up to GS55 were essential to avoid yield loss. Significant yield-disease or green leaf models were identified from GS31 onwards. Each 1% green leaf area on the top two leaves at GS77 was estimated to contribute 0.59 - 0.84% to yield.

INTRODUCTION

Winter barley crops have consistently shown cost-effective yield responses to a broad-spectrum fungicide treatment in the spring at GS30-31 (Cook & Jenkins, 1988; Gladders & Hims, 1994). A second fungicide application during the period from flag leaf emergence to ear emergence (GS39-59) may also be cost effective if disease pressure is high (Gladders & Hims, 1994). Disease control during the autumn and winter has rarely been cost-effective except when mildew is active in crops on lighter soils (Stevens & Yarham, 1981). There are opportunities to reduce fungicide use and to improve timing of treatments. For example, a single fungicide treatment at GS33-37 gave comparable yields to a two spray programme when disease pressure was low at GS31 (Gladders & Hims, 1994).

This paper present the first results from two experiments from a series carried out by ADAS during 1988-1992 to investigate the effects of variably-timed disease epidemics on the yield of winter barley. These experiments also had the objectives of identifying critical periods for disease control, and the weather conditions which favoured epidemic development.

MATERIALS AND METHODS

A series of differently tired disease epidemics was induced by sequential application of fungicides in an unreplicated linear design similar to that used on wheat by Thomas *et al.*

(1989). In 1988/89, 28 plots (72m²) of winter barley cv. Plaisant sown at Croft, N. Yorkshire, on 10 September 1988 after two previous crops of winter wheat were used in an experiment in which fungicide programmes were started progressively later. Plots 1 and 2 received the first spray on 11 October and programmes started on successive plots at two week intervals up to the end of April, thereafter, new treatments were initiated weekly until 20 June. The first spray was fenpropidin at 0.75 kg AI/ ha (Patrol) and prochloraz + carbendazim 0.40 + 0.15 kg AI/ha (Sportak Alpha) applied by MDM pressurised knapsack sprayer in water at 220-250 l/ha. Subsequently all treated plots were sprayed with fenpropidin and prochloraz + carbendazim at half the initial rate until 20 June (GS 77). The half rate treatments were applied at monthly intervals until mid April and then at 2-3 week intervals. Plots 25 and 26 were untreated controls. Plot 28 received tridemorph 0.525 kg AI/ha (Ringer) on 9 November followed by the fenpropidin and prochloraz + carbendazim treatments at full rate on 16 May, plot 27 received tridemorph on 31 January and fenpropidin and prochloraz + carbendazim also on 17 May. A total of 23 different dates were used to initiate fungicide programmes as follows: 11 October (GS 12), 25 October (GS 21), 2 November (GS 21-22), 25 November (GS 22), 6 December (GS 23), 22 December (GS 24), 3 January (GS 25), 17 January (GS 25), 31 January (GS 26), 14 February (GS 25), 3 March (GS 30), 15 March (GS 30-31), 29 March (GS31-32), 12 April (GS 32), 26 April (GS 33), 2 May (GS 33), 10 May (GS 39-47), 16 May (GS 52-56), 23 May (GS 61-63), 1 June (GS 69), 8 June (GS 71), 15 June (GS 73), 20 June (GS 77).

Leaf diseases and green leaf area were assessed on all green leaves (10 shoots/plot) in previously treated plots and on a sample of 25 tillers representative of the remaining unsprayed areas at each spray date. Eyespot was assessed at GS 31 and GS 75. Plots were combine harvested on 31 July 1989 and grain yield corrected to 85% d.m.

In 1991/92 winter barley cv. Igri at Haverfordwest, Dyfed, sown on 25 September 1991 as a third successive winter barley crop was used to generate two series of disease epidemics in a linear design of 44 plots (33.3m²). Plots 1, 2, 22, 23, 43 and 44 were untreated controls; plots 3 to 21 were used for spray programmes starting successively later, whilst plots 24 to 42 received sprays on 25 November but subsequent sprays were all applied for successively longer periods. Adverse weather conditions during the winter prevented sprays being applied on all target dates and some treatments were repeated on additional plots, but the dates of application were as follows: 25 November (GS 22), 31 December (GS 24), 25 February (GS 24), 17 March (GS 25), 8 April (GS 30-31), 16 April (GS 31), 21 April (GS 32-33), 29 April (GS 37), 5 May (GS 39-41), 15 May (GS 45-49), 19 May (GS 55), 1 June (GS 69-71). The fungicides were used fenpropimorph at 0.375 kg AI/ha (Mistral) and prochloraz + carbendazim at 0.40 + 0.15 kg ha AI/ha (Sportak Alpha) were applied during the period November to mid April, propiconazole + tridemorph at 62.5 + 175g AI/ha (Tilt Turbo 475) was used from 21 April until 1 June. The interval between the first spray and successive treatments was 4 to 8 weeks during the winter but fortnightly from 8 April. All sprays were applied by pressurised knapsack sprayer in water at 250 l/ha. Disease assessments were carried out as described above for Croft. Plots were combine harvested on 22 July 1992 and grain yields adjusted to 85% d.m.

Regression analysis were carried out using MINITAB (Minitab Inc., USA) using yield as the dependent variable and foliar disease severity, eyespot index and green leaf area on individual leaf layers at each assessment date as the independent variables. In order to

simplify the data presented only significant ($P \leq 0.001$) single variable models identified from GS 31 onwards are presented together with the coefficient of determination (r^2) adjusted for degrees of freedom. Yield loss models have been derived from the data using mean yields from two plots which received the maximum number of fungicide applications (plots 1 and 2) at Croft; plots 41 and 42 at Haverfordwest).

RESULTS

At Croft, mildew (*Erysiphe graminis* f.sp. *hordei*) was first recorded on 2 November and it increased rapidly on leaf 2 from 1.3% on 8 December to 60% on 22 December. Mildew remained active (11-27% leaf 2) until GS30 (3 April) but remained <10% on the two upper leaves until GS69. Brown rust (*Puccinia hordei*) affected leaf 2 from February but increased in May and June to affect 22% of leaf 1 at GS77.

There was no consistent loss of yield compared with the full spray programme at Croft until plot14 (Fig.1) indicating that a spray at GS 30-31 (15 March) was critical to prevent yield loss. Good control of brown rust and mildew on the flag leaf at GS 77 was achieved by programmes which started up to GS 52(plot 19) and GS 61(plot 20) respectively (Fig.1). Regression analysis identified significant models for yield and disease or green leaf area from GS31-32. At GS31, the most appropriate yield model was mildew severity on leaf 3 (P = 0.008). Yield loss at Croft was attributable to both mildew and brown rust and a model based on green leaf area was considered appropriate : %yield loss (y) = 39.6 - 0.59 (mean % green leaf area on leaf 1 and leaf 2 at GS77). The maximum yield loss recorded was 39.9% of the yield from the full spray programme (10.91 t/ha).

At Haverfordwest, mildew, brown rust, leaf blotch (*Rhynchosporium secalis*) and net blotch (*Pyrenophora teres*) were active from February and together with halo spot (*Selenophoma donacis*) all affected the upper leaves from mid-May. Net blotch and brown rust were severe from GS 55 and affected 60% and 23% of the flag leaf in untreated plots at GS 77. At GS 71 (Fig.2),only programmes starting on or before 15 April (GS 31, plot 15) or completed by 8 May (GS 41, plot 38) gave c.90% control of net blotch (Fig.2). For comparable brown rust control, programmes could be initiated up to 15 May (GS 45,plot 19) or completed by 15 May. All treatments gave poor control of net blotch and brown rust on the flag leaf at GS 77 (19 June).

A large number of yield-disease or green leaf area models were identified from GS 31 onwards at Haverfordwest. Models based on green leaf area were often the most appropriate and they showed very high r^2 values (> 90%) from GS 55 onwards. At GS 71, a yield model (r^2 = 90.3%) for net blotch gave : yield (y) = 8.43 - 0.174 (mean % net blotch on leaf 1 and leaf 2 at GS 71). This is likely to over-estimate the importance of net blotch as other diseases were also present and controlled by the fungicide treatments. The best yield loss model (r^2 = 91.0%) at GS 71 was : % yield loss (y) = 113 - 1.14 (mean % green leaf area on leaf 1 and leaf 2 at GS 71). At GS 77 , the model was: % yield loss (y) = 35.6 - 0.84 (mean % green leaf area leaf 1 and leaf 2 at GS77) and had an r^2 value of 86.9%. The equivalent model at GS71 was y = 80.8 - 0.85 (mean % green leaf area on leaf 1 and leaf 2 at GS71) The maximum yield loss was 40.9% of the mean yield of 8.45 t/ha with the full spray schedule.

TABLE 1. Variables which gave significant ($P \leq 0.001$) single point models of yield against percentage foliar disease, green leaf area and eyespot index at various growth stages at Croft in 1989. (* see bottom of Table 2.)

Date assessed	GS	Significant variables * identified (r^2 % values in brackets)								DF
3.4.89	31-32	-								12
16.4.89	32	ML2	(56.8)							13
28.4.89	33	ML3	(64.5)	ML4	(68.0)	GL3	(50.2)			14
10.5.89	39-47	ML3	(54.2)	ML4	(63.6)	ML5	(80.0)	GL3	(47.4)	16
		GL4	(59.3)	GL5	(57.5)					
24.5.89	61-63	ML2	(60.1)	ML3	(82.3)	BRL1	(39.4)	BRL2	(54.3)	19
		BRL3	(63.3)	GL1	(42.9)	GL2	(60.6)	GL3	(79.4)	
		GL4	(49.8)							
14.6.89	71	ML2	(64.9)	BRL2	(49.0)	GL2	(49.8)	GL3	(58.2)	20
21.6.89	77	ML1	(68.8)	BRL1	(72.7)	EI	(46.4)	GL1	(68.2)	26
		GL2	(50.5)							

TABLE 2. Variables which gave significant ($P \leq 0.001$) single point models of yield against percentage foliar disease, green leaf area at various growth stages at Haverfordwest in 1992.

Date assessed	GS	Significant variables * identified (r^2 % values in brackets)								DF
16.4.92	31	ML3	(27.8)	NBL2	(21.9)	NBL3	(23.2)			42
21.4.92	32	ML4	(45.6)	RL3	(36.9)	RL4	(41.5)	GL4	(30.2)	42
29.4.92	37	ML3	(37.0)	RL2	(22.8)	RL3	(27.5)	NB1	(53.0)	42
		NBL2	(51.1)	NBL3	(28.2)	BRL3	(33.3)	GL1	(62.9)	
		GL2	(58.5)	GL3	(32.6)	GL4	(34.9)			
5.5.92	41	ML3	(42.2)	ML4	(40.3)	RL3	(39.0)	RL4	(33.5)	
		NBL2	(51.3)	NBL4	(32.0)	BRL4	(24.6)	GL2	(46.6)	
		GL3	(68.6)	GL4	(75.7)					
19.5.92	55	ML3	(41.7)	ML4	(36.0)	RL2	(49.6)	RL3	(49.8)	42
		NBL1	(53.6)	NBL2	(67.1)	NBL3	(61.8)	BRL1	(67.1)	
		BRL2	(81.0)	BRL3	(73.1)	BRL4	(59.8)	HSL1	(77.4)	
		HSPL2	(75.5)	GL1	(57.6)	GL2	(63.5)	GL3	(94.3)	
		GL4	(77.8)							
1.6.92	69-71	ML1	(72.8)	ML2	(71.7)	RL1	(69.2)	RL2	(80.6)	42
		NBL1	(77.2)	NBL2	(90.2)	BRL1	(75.5)	BRL2	(83.3)	
		GL1	(88.0)	GL2	(91.1)	GL3	(94.3)			
19.6.92	77	NBL1	(89.2)	BRL1	(21.4)	HSL1	(75.3)	GL1	(89.2)	42
		GL2	(72.1)							

* M - Mildew BR - Brown rust EI - Eyespot Index G - Green Leaf
L(1-5) - Leaf number assessed NB - Net Blotch R -Rhynchosporium HS - Halo Spot

FIG.1. Yield and severity of mildew and brown rust on leaf 1 at GS 77 in relation to plot number and growth stage at which fungicide spray programmes started, Croft, 1988/89. (C) Untreated control plot. (▪) Yield (+) Mildew (*) Brown rust.

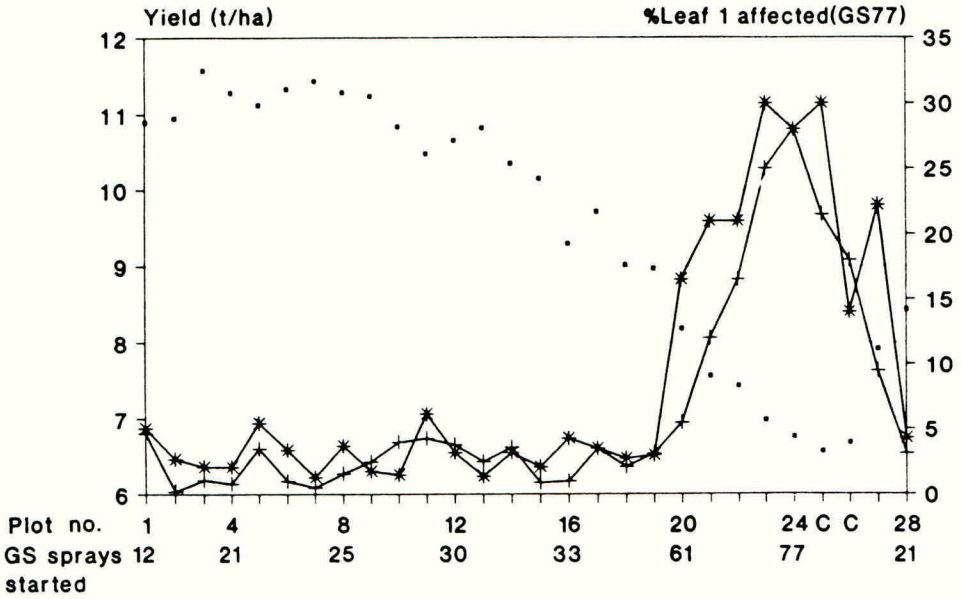
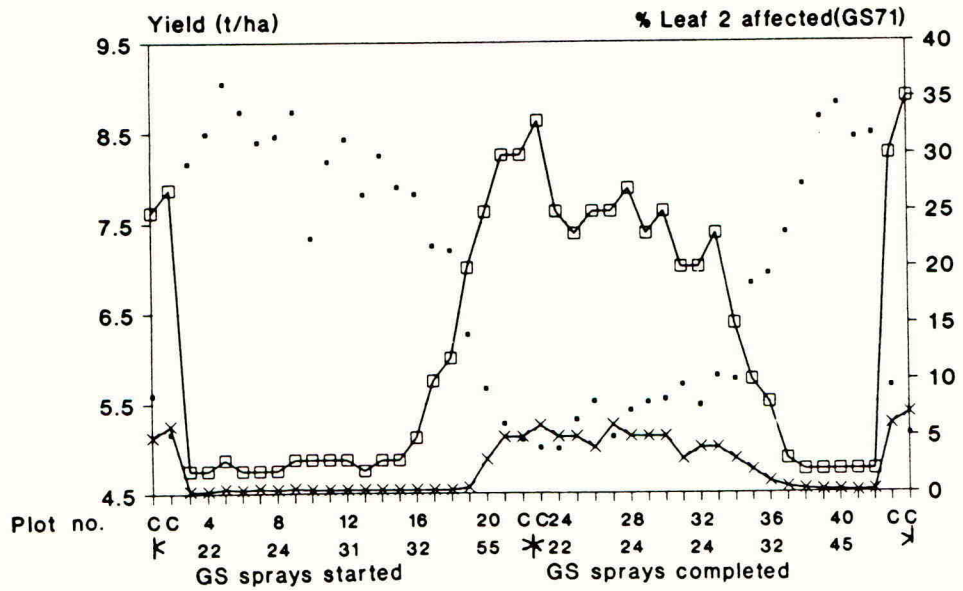


FIG. 2. Yield and severity of net blotch and brown rust on leaf 2 at GS 71 in relation to plot number and growth stage at which fungicide programmes were started or completed, Haverfordwest 1991/92. (C) Untreated control plot. (▪) Yield (□) Net blotch (×) Brown rust.



DISCUSSION

These experiments both confirmed the importance of sprays at GS 30-31 to prevent yield loss in winter barley and the progressive loss of yield with time if spray programmes were delayed. Sprays up to GS 41-45 were also critical for late season disease control but later timings were not worthwhile (Fig. 2). Highly significant yield-disease and green leaf area models were derived for individual sites from GS 31 onwards and these may reflect the contribution of inoculum to the subsequent disease epidemics rather than direct physiological effects alone (Wright & Gaunt, 1992). Yield responses at Croft, however, were associated with increased numbers of fertile tillers and with increased thousand grain weight.

As several diseases developed at these sites, yield-disease loss relationships for individual diseases were not calculated. General yield loss models based on green leaf area on the flag leaf and leaf 2 at GS 69-77 were appropriate in previous multi-site analyses (Gladders & Hims, 1994) and have been selected in preference to single leaf data. The models at GS 77 indicated that for each 1% green leaf area on the top two leaves contributed 0.84% and 0.59% to yield at Haverfordwest and Croft respectively, giving similar estimates for yield response which was equivalent to 0.07 and 0.06 t/ha respectively. The models at GS71 were considered less reliable as they gave very high estimates for potential yield loss (80.8 and 113%). Data from other sites in the series also indicated that fungicides applied between GS 30-31 and GS 39 were generally most critical for prevention of yield loss. More general models for disease-yield loss relationships which encompass different sites and seasons, are now being developed.

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