SESSION 3D

ADVANCES IN ARABLE CROP PROTECTION

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Poster Papers 3D-1 to 3D-20

DEVELOPMENT OF A MONITORING TRAP FOR SPRING AND SUMMER PESTS OF OILSEED RAPE

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ABSTRACT

Yellow sticky traps baited with lures of 2-phenylethyl isothiocyanate, were deployed at the edges of oilseed rape crops on several sites in 1996 during spring and early summer to detect movement of cabbage seed weevils, *Ceutorhynchus assimilis*, and pollen beetles, *Meligethes* spp., into the crop. At the same time, insect numbers within the crop were assessed on a transect across the field by beating the tops of a random selection of plants into a tray. Crop growth stage and weather data were also recorded. The results indicated that trap data collected in the early spring, could provide advanced warning of the main migration of seed weevils to oilseed rape, and showed that establishment of measurable numbers of pollen beetles within the crop was occurring simultaneously with their appearance on traps. It was not possible, from the limited data available, to attempt to correlate trap catches with population estimates made by tray beating.

INTRODUCTION

Because of concerns over the environmental effects of pesticides and the economics of their usage, there is increasing interest within the UK in obtaining accurate population estimates of the spring and summer pests of oilseed rape so that prophylactic spraying may be avoided (Walters and Lane, 1994a). Currently, populations of the cabbage seed weevil, *Ceutorhynchus assimilis*, and pollen beetles, *Meligethes* spp., are assessed by in-crop tray beating counts (*vide infra;* Lane and Walters, 1993; Walters and Lane, 1994a,b) but this method has drawbacks. Apart from the often considerable time and effort needed by crop consultants, the sizes of populations can be underestimated in some weather conditions, particularly if temperatures are low, or if counts are taken during windy weather, or if the crop canopy is wet. In addition, deceptively low counts may be obtained because insects are disturbed from the plants as the assessor forces a way through a dense crop. Thus it is worth investigating alternative methods of monitoring pest populations, such as the use of traps to intercept the pests as they invade the crop. Intensive sampling would then be done only when the crop was at susceptible growth stages, in the period when accurate counts are most likely to be obtained. The need to sample within the crop could also be eliminated, or, at the least, reduced.

C. assimilis and *Meligethes* spp. use both visual and olfactory cues to locate suitable host plants (Görnitz, 1953, 1956; Free and Williams, 1978) and this may be exploited for the

development of an attractant trap. The antennal sensory cells of both insects perceive volatile organic isothiocyanates, the glucosinolate catabolites which are characteristic of the Brassicaceae (= Cruciferae) (Blight *et al.*, 1989, 1995a,c). Field experiments showed that yellow sticky and water traps baited with a mixture of isothiocyanates attracted both pests (Smart *et al.*, 1993), and further field experiments (Blight *et al.*, 1995b; Smart *et al.*, 1995) demonstrated that the individual 3-butenyl, 4-pentenyl and 2-phenylethyl isothiocyanates were as attractive as the original mixture. The data suggested that a baited yellow sticky trap with a 45 ° orientation might be effective and convenient to use for routine detection of insect immigration to the oilseed rape crop.

This paper describes some of the initial results from an on-going investigation in which the relationships between insect numbers trapped during immigration into the crop and population numbers obtained from tray beating, are being studied.

MATERIALS AND METHODS

Insects were collected on baited sticky traps and monitoring assessments were made by tray beating at four sites during 1996. However, for clarity, data from only two of the sites, at ADAS Rosemaund (Herefordshire), and ADAS Drayton (Warwickshire), are described below. Sampling, which was done at ADAS Rosemaund from April 3 to June 18, and at ADAS Drayton from March 6 to June 7, was generally carried out at seven day intervals, but, where possible, sampling frequency was increased when insect populations increased.

Insect trapping

The angled sticky card trap (20 x 10 cm) consisted of an oblong piece of grey plastic sheet (0.3 cm thick ABS) glued at an angle of 45 ° to a 10 cm length of grey plastic tubing. The tubing fitted over the end of a rigid pole so that the trap was fixed in position at, or just above, crop height. A yellow sticky card coated with Oecotak A5 was clipped to the upper side of the angled face. The chemical bait, which was 2-phenylethyl isothiocyanate released at 2.5 mg/day from a sealed polythene bag, was suspended just above the trap from a wire loop.

Four traps were deployed at the edge of the crop at each site. Local operators judged the exact position, which depended on the prevailing wind, the position of the previous season's rape crops, and the contours of the site. Where possible, the traps were positioned to allow for easy access.

Population assessment by tray beating

This was done using the standard protocol described by Cooper and Lane (1991). On each sampling date the tops of 20 plants taken randomly on a transect across the field were beaten into a white tray. The number of adult cabbage seed weevils and pollen beetles collected were recorded, together with the growth stage of the crop (Sylvester-Bradley, 1985), the ambient temperature and prevailing weather conditions.

RESULTS

Figs. 1a & b show the mean numbers of seed weevils trapped per day on the two sites and Figs. 1c & d the mean numbers of pollen beetles. The mean number of insects per plant (measured by tray beating) is shown for each sampling date, together with the ambient temperature, and the growth stage of the crop. The data shown on the graphs are restricted to the periods during which insects were found.

Seed weevil data

The weevils were first detected on the traps on April 16 at Rosemaund when the crop was at the green bud stage (3.3) and on April 26 at Drayton at the yellow bud stage (3.7). This was 21 days (Rosemaund) and 19 days (Drayton) respectively before weevils were first detected on the crop by tray beating. By this time (growth stage 4.5 = 50 % of all buds opened) the mean catch per trap had dropped at Drayton though not at Rosemaund. The mean trap catch rose again at the time of pod development (growth stages 5.0 - 5.9) when the lack of young pods and flowers made the crop less attractive to the weevils. Throughout the trapping periods there were variations in the catches on individual traps at each site, but the numbers trapped were too low to draw any conclusions. Trap catches did not appear to be particularly temperature dependent. The weevils moved to the traps from overwintering sites when the temperature rose above 11-12 °C.

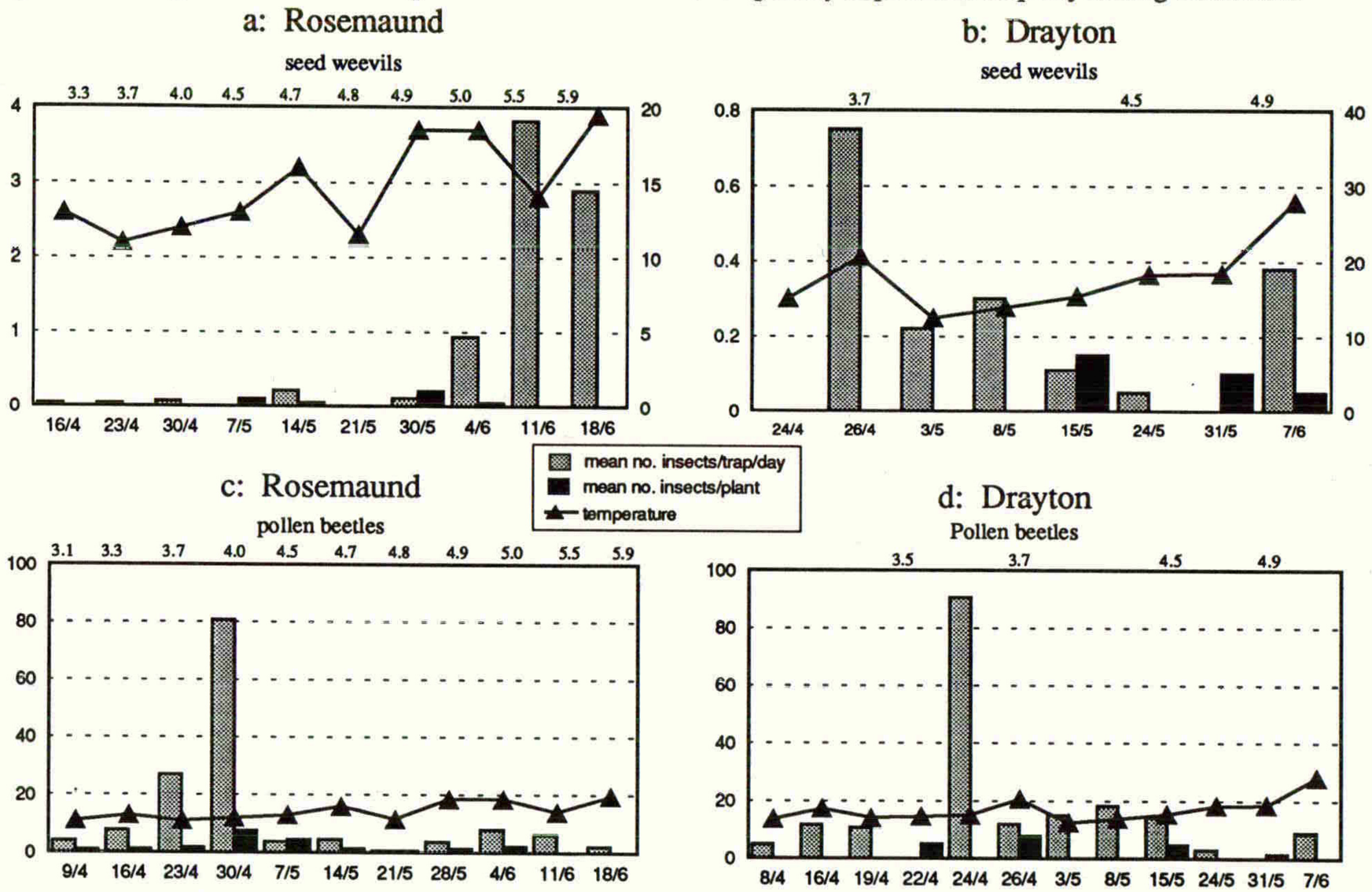
The population assessments made by tray beating indicated low populations at both sites. The maximum mean no. per plant was 0.2 at Rosemaund (May 30) and 0.15 at Drayton (May 15).

Pollen beetle data

Pollen beetles were first detected on traps on April 9 at Rosemaund when the mean daily catch per trap was 4.0, and on April 8 at Drayton, mean catch per trap per day, 4.8. The beetles were first detected on plants at Rosemaund on the same date (April 9), when flower buds were present but enclosed in leaves (growth stage 3.1). Since no tray beating or growth stage data were available from Drayton until April 22 the relationship between first detection on traps and plants at this site is not known. Maximum mean daily trap catches of 80.7 at Rosemaund and 90.6 at Drayton were measured on April 30 and April 24, respectively, when the growth stages were 4.0 (first flowers opening) and c. 3.6 (green bud stalks extending) respectively. Maximum numbers were recorded on plants at, or about the same time as the trap catches were at the maximum. The numbers detected - 7.4 per plant at Rosemaund on April 30, and 7.9 per plant at Drayton on April 26 - could have caused significant damage in a backward crop.

Catches on the four individual traps varied on both sites. Each trap position was at times favoured over all the others and there was a large range in the numbers of beetles caught at each trap position on a sampling date. At Rosemaund the maximum individual catch varied from 1.4 to 6 times the minimum, and at Drayton the variation was even greater - from 1.6 to 30.6 times the minimum.

Fig. 1 Monitoring of seed weevils and pollen beetles at two sites, using sticky traps and in-crop tray beating assessment.



Data are shown only for the period when insects were found (dates on x-axis). Figs. 1a & b: the left y-axis scale represents mean no. insects in traps and on plants, and the right y-axis, temperature (°C). Figs. 1c & d: the left y-axis scale represents insect numbers and temperature. The growth stage of the crop is shown along the top of each graph.

As with seed weevils, the trap catches did not appear to correlate closely with temperature. Pollen beetles were trapped when the temperature was ≥ 11 °C.

DISCUSSION

These are preliminary results, from the first year of a longer term study. They showed that for seed weevils, the trap catches gave early warning of population movement before the establishment of the pest in the crop, while for pollen beetles, the trap catches indicated that this insect was already entering the crop in measurable numbers. These results therefore suggest that monitoring seed weevils and pollen beetles using attractant traps could provide useful information on insect migration to oilseed rape. The deployment of several traps per site appeared to be essential because of variability in catches on individual traps. However, acquiring this type of data could facilitate decision making as to the need for, and timing of, pesticide application.

Catches of pollen beetles rose to a maximum in the spring and then remained low, but relatively steady, for the remainder of the trapping period, and thus did not suggest that any changes were taking place in either population numbers or in insect behaviour. However, the increase in activity of seed weevils in the early summer as evidenced by increased trap catches, could have indicated imminent dispersal to other crops such as spring rape or vegetable brassicas - information which could be useful when considering control on these crops.

From the data available it was not possible to relate numbers on traps to those detected on plants by tray beating. Data must be acquired from several sites over several years to answer this, and other questions.

ACKNOWLEDGEMENTS

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A MONITORING SYSTEM FOR THE PEA AND BEAN WEEVIL (*SITONA LINEATUS*)

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ABSTRACT

The synthetic aggregation pheromone, 4-methyl-3,5-heptanedione was used in cone traps to monitor the activity of the pea and bean weevil, *Sitona lineatus*, on overwintering sites in the spring of four consecutive years. In each year a legume crop in the vicinity of the overwintering site was also assessed for weevil damage. Monitoring indicated the imminent migration of the weevil to crops and thus could be used to time insecticide application efficiently. It could also show that pesticide application is unnecessary if the crop is not at a susceptible growth stage at the time of *S. lineatus* migration from the overwintering site.

INTRODUCTION

The pea and bean weevil (*Sitona lineatus*) is considered to be a significant pest in Europe, USA and the Middle East (Bardner *et al.*, 1983; Oschmann, 1984). It is polyphagous on many wild and cultivated legumes, but optimum reproduction occurs only on peas (*Pisum sativum*), beans (*Vicia faba*) and vetches (*Vicia sativa*) (Hans, 1959).

Adult weevils, after overwintering in grass near harvested pea and bean fields or in perennial legumes, migrate in the spring to annual legume crops where feeding is signalled by notches on the leaf margins. Most damage, however, is caused by larval feeding on the nitrogen-fixing root nodules (Oschmann, 1984).

Work in the UK has shown that effective control can produce yield increases for spring peas and spring field beans, (Bardner *et al.*, 1983; Biddle, 1985; Baughan *et al.*, 1985; Bennison, 1991). However, in winter field beans, such responses have been more difficult to detect (Bennison, 1991) and control is not normally recommended for this crop.

At present, pesticide application is either prophylactic or made in response to leaf notching. However, if weevil activity could be monitored on overwintering sites to give an indication of impending migration, it should lead to more accurately timed application, and reduced use of pesticides.

Following the identification of a male-produced aggregation pheromone for *S. lineatus* (Blight *et al.*, 1984) cone traps containing lures of the synthetic pheromone were shown to trap *S. lineatus* effectively on overwintering sites. These traps might therefore form the basis of a monitoring system (Glinwood *et al.*, 1993).

This report describes some further work towards the development of a monitoring system and considers its use in an integrated pest management regime in both spring sown peas and spring field beans.

MATERIALS AND METHODS

The study was carried out on sites at PGRO, Thornhaugh, Cambridgeshire, from 1993 to 1996 inclusive, and at ADAS High Mowthorpe, Yorkshire, in 1996. Pheromone-baited traps were used to monitor *S. lineatus* activity on each overwintering site, and a current seasons pea or spring bean crop in the vicinity was assessed for weevil damage.

Insect monitoring

Modified cotton boll weevil "Hardee" cone traps or "Scout" traps were deployed (Glinwood *et al.*, 1993). They were baited with the aggregation pheromone, 4-methyl-3,5-heptanedione, which was released at 250 µg/day by diffusion from polythene vials (Blight *et al.*, 1984). The synthetic pheromone was provided by Norsk Hydro a.s.

In each trial, five traps were placed 10 m apart, along the uncultivated grassy edge of a wheat field which had been cropped with peas (at Thornhaugh) or spring beans (at High Mowthorpe) the previous season. Traps were deployed from mid-February until migration from the site was complete, lures being replaced every six weeks. Insects were removed from the traps regularly and counted. When numbers began to increase noticeably, the traps were cleared daily. Daily maximum air temperatures were recorded using automatic weather stations.

Assessment of weevil damage to current crop

The crops used for damage assessment were peas cv. Progreta at Thornhaugh, and spring beans cv. Victor at High Mowthorpe. At Thornhaugh, from 1994-1996, the crop was separated from the overwintering site by at least one field, but in 1993, only a tree lined track separated the sites. At High Mowthorpe (1996) the crop and the overwintering site were contiguous, and the monitoring traps were placed on the boundary between the two. Weevil feeding was estimated by counting the leaf notches on 40 randomly selected plants within a 40 m^2 area of the crop. Assessments were made weekly at first leaf expansion (GS 101, Knott, 1987) untilmid-May.

RESULTS

Figs. 1-5 show the mean number of weevils caught per trap per day on each monitoring site together with the maximum daily temperature and the mean number of leaf notches per plant on the associated crop.

In each trial a few weevils were caught within 2-3 days of the traps being deployed on the overwintering site. Catches remained below a mean of 10 per trap per day on most sites (Figs. 1, 3, 4, 5) until the maximum daily temperature rose above 10° C. However, at Thornhaugh, 1994 (Fig. 2) the maximum temperature was between 10 and 15° C for approximately 21 days before there was a substantial increase in numbers trapped.

In general there were several peaks of weevil activity on each site from March to the end of April (Figs. 2, 3, 4). The numbers trapped then fell. The pattern of activity on each site was different, and at Thornhaugh 1993, and High Mowthorpe (Figs. 1, 5), there was only one major peak. The dates of peak activity also varied between sites and did not correlate closely with a pronounced rise in temperature except at Thornhaugh on March 13, 1993 (Fig. 1), Thornhaugh on April 11, 1996 (Fig. 4) and High Mowthorpe on April 10, 1996 (Fig. 5).

Figure 1. Thornhaugh 1993

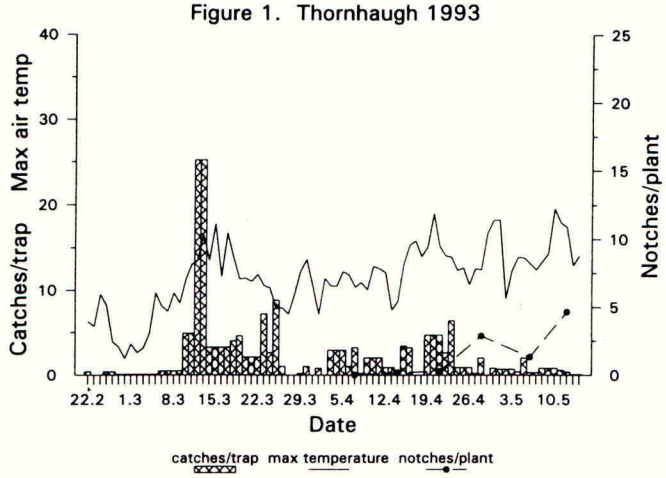


Figure 2. Thornhaugh 1994

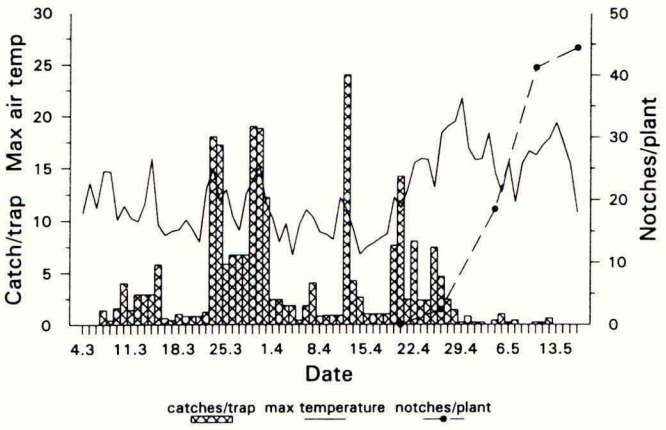


Figure 3. Thornhaugh 1995

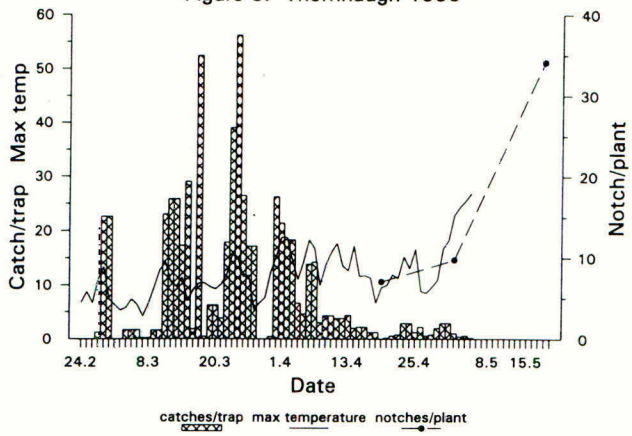


Figure 4. Thornhaugh 1996

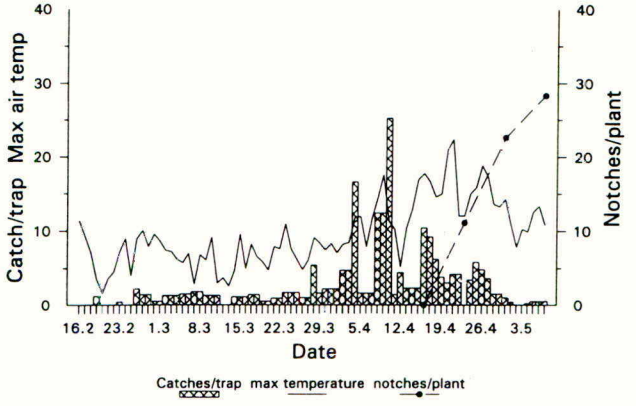
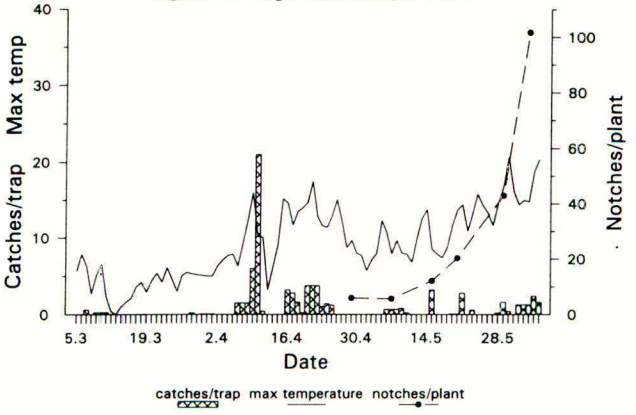


Figure 5. High Mowthorpe 1996



Reductions in trap catches in late April to early May usually occurred shortly before the onset of feeding in the crop, indicating that most of the population was migrating. In most trials, leaf notching on the current crop became apparent about one week after plant emergence, and in every trial, except that of 1993 (Fig. 1), damage was observed 6-20 days after a peak catch on the overwintering site. In the 1993 trial, leaf notching was first observed approximately 39 days after the only major peak of activity on the overwintering site, and remained low throughout. Table 1 shows the sowing date of the current crop, and the date of the last peak of activity on the overwintering site, and the interval between the two events. On the crops where there was significant notching (see Figs. 2-5) the interval ranged from 18 to 38 days. Where damage was insignificant (Fig. 1), the interval was only five days.

Table 1. Weevil activity on overwintering sites and crop sowing dates

Site	Last peak catch	Sowing date	Interval in days
Thornhaugh 1993	13-14 March	8 March	5
Thornhaugh 1994	21 April	14 March	38
Thornhaugh 1995	1 April	14 March	18
Thornhaugh 1996	17 April	18 March	30
High Mowthorpe 1996	10 April	18 March	23

No direct relationship was observed in any of the trials between the numbers of weevils trapped on the overwintering site and leaf notching on the crop.

DISCUSSION

The results suggest that monitoring *S. lineatus* activity on overwintering sites using pheromone-baited traps gives an indication of impending migration to crops and thus could be used to time insecticide application efficiently to a crop which has reached a susceptible growth stage. In addition, monitoring would indicate that spraying is unnecessary if migration from the overwintering site is occurring ahead of crop emergence, as at Thornhaugh in 1993.

In most trials there was some variation between catches in individual traps (data not presented) which may be due to various factors including localised weevil aggregation, shade and wind direction. Therefore, with a fully developed monitoring system, it will be necessary to use a minimum of three traps per site to monitor weevil activity efficiently.

Furthermore, the influence of factors such as rainfall, soil cultivations, minimum air temperatures and soil temperatures on weevil catches and the relationship between catches and crop damage requires further studies. However, the results clearly show that a fully developed pheromone-based monitoring system will be a useful component of an integrated pest management system for the pea and bean weevil.

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TOWARDS DECISION SUPPORT FOR CONTROL OF BARLEY YELLOW DWARF VECTORS.

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ABSTRACT

Outbreaks of barley yellow dwarf virus (BYDV) in autumn-sown cereals are erratic, varying temporally and spatially especially at field-scale. When they occur the damage can be devastating. In the absence of a reliable BYDV forecasting scheme, there is a tendency to minimise risk through routine application of insecticides to eliminate aphid vectors of the disease. Up to 60 % of winter cereal crops are sprayed each year at an estimated cost to UK industry of £10 million per annum. Much of this spraying is unnecessary and may be environmentally damaging. A model of BYDV spread during the autumn and winter is being developed, which will form the basis of a decision support system to assess the need for vector control. The model describes the population dynamics of immigrant winged aphids and their offspring, the interplant movement of these aphids and subsequent spread of virus. The model will be assessed in relation to field characteristics to ensure that the system will be applicable at field-scale nationwide.

INTRODUCTION

Barley yellow dwarf (BYDV) is an aphid-vectored, persistently transmitted viral disease, infecting a wide range of Graminaceous hosts, including cereal crops. The severity of outbreaks on autumn-sown cereals varies considerably, both temporally and geographically, but when epidemics occur the damage can be devastating with massive crop loss. Because chemical control of the aphid vectors in the autumn is easy and inexpensive, there has been a tendency to minimise risk by using insecticides routinely. Up to 60 % of the winter crop in Britain is sprayed at a total cost of approximately £10 million per year (Harrington *et al.*, 1994). However, many sprays may be unnecessary, even in years when the national incidence is high. With an accurate forecasting system, many farmers could reduce their use of insecticides, especially in winter wheat which tends to be less seriously affected by BYDV than does barley.

Because BYDV is not seed-borne, it is introduced into crops only by infective aphids feeding

on healthy plants. The main aphid vectors of BYDV are the bird cherry aphid *Rhopalosiphum padi* (Linnaeus) and the grain aphid *Sitobion avenae* (Fabricius). Both *R. padi* and *S. avenae* have clones which are holocyclic (having a sexual phase) and clones which are anholocyclic (having no sexual phase). In Britain the majority of *R. padi* clones display holocycly, producing their winter egg on *Prunus padus*, while the majority of *S. avenae* clones display anholocycly reproducing parthenogenetically on *Gramineae* through the autumn and winter (Blackman and Eastop, 1984). In the autumn all aphids migrate to their winter hosts. Those of holocyclic clones (primarily *R. padi*) are searching for winter hosts on which to lay their eggs and therefore are of little consequence to BYDV infection. By contrast, those of anholocyclic clones colonize Gramineae and if carrying BYDV, have the potential to introduce virus (primary infection). These colonizers remain in the crop, producing wingless offspring which reproduce and move through the crop during the winter, transmitting BYDV for as long as the weather permits (secondary spread). The amount of secondary spread during the winter is critical in determining whether or not BYDV infections in the autumn develop to damaging levels the following spring.

As a measure of the potential threat of BYDV infection in the autumn, Plumb *et al.*, (1981) developed an infectivity index (I.I.) calculated from the total number of each species of cereal aphid caught in the 12.2 m Insect Survey suction trap at Rothamsted, multiplied by the proportion of individuals of those species caught alive in a neighbouring trap which were found to be infective. Values of this index, accumulated weekly from planting date, were correlated positively with yield loss in crops grown in fields in the immediate area (Plumb, 1986). The index did not, however, predict BYDV accurately in other regions (Kendall and Chinn, 1990; Foster *et al.*, 1993), even when holocyclic *R. padi* migrants in the autumn were discounted (Tatchell *et al.*, 1988); the proportion of anholocyclic clones in the autumn differs markedly both geographically and from year to year. Predictions were inaccurate partly because no account was taken of the amount of secondary spread occurring during the autumn and winter (Harrington *et al.*, 1994). Kendall and Chinn (1990) evaluated crop exposure to BYDV on the basis of accumulated infectious-aphid days (later elaborated by Kendall *et al.*, 1992) and obtained a closer correlation with crop damage than using the I.I of Plumb *et al.*, (1981). The main problem with the Kendall *et al.* (1992) method of assessing the need to control BYDV vectors, was that it required regular field sampling during the winter which has been deemed impractical by most farmers. An improved forecast using a system of remote monitoring is needed.

Work at SAC, Auchincruive, has shown that there is large variation in the number of aphids, and subsequent level of BYDV infection, recorded in cereal fields during the autumn. Through analysis of data collected over a six year period (Masterman *et al.*, 1993), a number of field/farm characteristics were identified which explain a major part of between field and between region variation. This information is needed to correct regional forecasts down to a field/farm scale.

The approach being developed builds on prior knowledge and experience to provide decision support in assessing the need to control BYDV vectors on autumn-sown crops. This system aims firstly to be sufficiently accurate to eliminate the need for routine insecticidal application, secondly, to be based on remote monitoring and therefore avoid difficulties involved in field sampling throughout the winter and thirdly, to take account of field characteristics to ensure applicability at field-scale throughout the UK.

DECISION SUPPORT COMPONENTS

An assessment of BYDV risk requires a measure of the number and timing of aphids colonizing autumn-sown cereals, and an estimation of the amount of secondary spread occurring during the autumn and winter months by wingless aphids. Precision at field-scale requires that field characteristics are accounted for in estimating both primary infection and secondary spread.

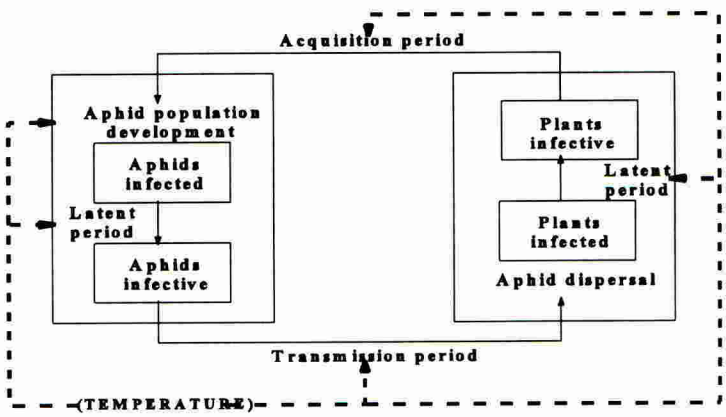
Suction traps and aphid colonization

The Rothamsted Insect Survey (Woiwod and Harrington, 1994) operates a network of 11 suction traps in England and a further 4 are operated in Scotland by the Scottish Agricultural Science Agency. These provide data throughout the year on the timing and number of all aphid species migrating. Each suction trap gives a standardized measure of the aerial density of aphids representative up to 50 to 80 km away (Taylor, 1979). Using data provided by this network, estimates can be made of the size and timing of migrations of winged anholocyclic cereal aphids which may colonize autumn-sown crops throughout Britain. In addition it is now possible to detect the presence of BYDV in single aphids using an amplified ELISA system, which enables data on the infectivity of colonizing aphids to be collected quickly and cost-effectively (Torrance, 1987). These data will be used alongside data on the movement of winged aphids within the crop to estimate the level of primary infection.

Model structure to assess virus spread

The amount of secondary spread which occurs in autumn-sown crops depends on the survival of aphids during the autumn and winter, on their inter-plant movement during this period and on the extent to which aphids are able to acquire virus from infected plants and subsequently inoculate uninfected plants. A model is being developed to simulate virus epidemiology to act as the basis for decision support. This considers the spatial component of aphid dispersal and virus epidemiology (Morgan and Morse, 1996) as well as temporal aspects of virus spread which have been the basis of previous models (Morgan *et al.*, 1988; Kendall *et al.*, 1992). The model being developed contains five inter-related components; immigration; development; survival; reproduction and morph determination.

Figure 1. Conceptual diagram of BYDV model.



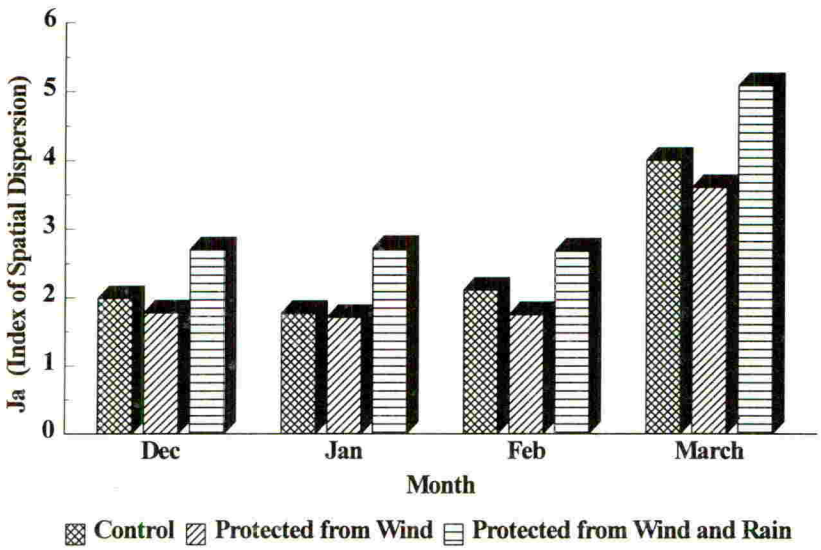
The model assumes that winged aphids are deposited randomly within a field, while wingless aphids are more likely to move to a new host plant within a row than between rows. If the aphid moves beyond the boundaries of a field it is assumed to be lost and not considered further by the model. In the model, after aphid dispersal has taken place, the virus status of each aphid and plant is updated. A plant or aphid exhibits one of three states: uninfected, latent (has virus but is uninfective), or infectious.

Weather and BYDV epidemiology

Weather is probably the single most important factor determining the incidence and levels of BYDV in autumn-sown crops. Temperature in particular, as well as rain and wind, determine aphid flight into the crop, the survival and movement of aphids in the crop, and the transmission of BYDV.

Basic data of survival and movement by overwintering *S. avenae* and *R. padi* have been obtained from an outdoor experiment following Mann *et al.*, (1995). Adult wingless *S. avenae* and *R. padi*were released on trays of barley seedlings, planted at field density, placed under three levels of protection: from wind and rain, from wind alone and unprotected. One week later, the plant-positions of all aphids on the tray were noted. The experiment was repeated monthly from November to March. Rain increased levels of mortality for both species and had a greater impact on whether or not aphids moved away from a plant than did wind (Figure 2). Consistently more *S. avenae* were retrieved than *R. padi* supporting previous evidence that the former are more winter hardy than the latter. Weather factors monitored at local meteorological stations will be used to drive the model of secondary spread.

Figure 2. Dispersal of aphids as measured by an Index of Spatial Dispersion (SADIE) based on moves to crowding (Perry, 1995). Higher values represent more aggregated distributions. Aphids were more aggregated when protected from both wind and rain than protected from wind alone or when unprotected ($F_{2,16} = 28.54$, $P < 0.001$).



Data concerning the aquisition access period, the aphid latent period and the inoculation access period (Lowles, 1996) have been collected in laboratory experiments for *S. avenae* and *R. padi* at temperatures common in the autumn in Britain. These will be incorporated into the model to estimate the amount of virus spread resulting from a known amount of aphid movement.

Field characteristics

A number of field characteristics have been identified which explain a large part of the spatial variation in BYDV outbreaks. Sowing date is critical, as later sown crops often emerge after the major part of the autumn migration of aphids is over, and they are therefore protected from BYDV infection. Previous cropping influences the number of aphids in a field, with large numbers of aphids following oil-seed rape, winter barley, or undesiccated ploughed-in grass which can act as a "green-bridge". Land-use of each farm has also been shown to affect the aphid species present in farmland. On mainly cereal growing farms, winter barley stubble fields which favour *S. avenae* are common. In contrast, *R. padi* are associated with mainly livestock farms due to the greater area of ryegrass on these farms, which is the principal host of *R. padi*. Shelter is another contributory factor, with fields partially or totally enclosed by mature woodland more likely to be infested by aphids than those exposed to strong winds. Therefore, field specific factors will be an important component of the decision making process. Field data is currently being collected from approximately 600 winter cereal sites throughout the UK (150 barley and 150 wheat each, for early and late drilled crops) to quantify the influence of local factors in determining crop-specific BYDV incidence.

DISCUSSION

The epidemiology of barley yellow dwarf is complex and for this reason predictions of outbreaks have proved to be difficult. The decision support system is being developed with this in mind. Using the suction trap network, migrating aphids and their infectivity are monitored to give a measure of levels of primary infection on a regional basis which can be adjusted according to farm/field characteristics. The model of virus spread is a mechanistic simulation model incorporating spatial aspects of the interactions between plant, aphid and virus. At present, it is a research tool which provides a method for assessing the relative contributions of different biological processes in determining the extent of virus spread, although through a process of refinement it will eventually form the underpinning of a practical BYDV decision support system.

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STRATEGIES FOR CONTROLLING APHIDS AND VIRUS YELLOWS IN SUGAR BEET

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ABSTRACT

Imidacloprid, applied as a film-coating to sugar beet pellets at 90 g a.i./unit of seed, gave the best control of aphids and, consequently, reduced virus yellows infection by 72% in four trials carried out in sugar beet in 1994 and 1995. The efficacy of the product against aphids persisted for up to 12 weeks, compared to just 8 weeks for aldicarb applied as a granule at drilling at 760 g a.i./ha. Aldicarb reduced virus infection by 44% on average. Two or three sprays of pirimicarb (at 140 g a.i./ha), or pirimicarb plus deltamethrin (at 100 g a.i. and 7.5 g a.i./ha respectively) or triazamate (at 56 g a.i./ha + oil at 500 ml/ha), applied when aphid numbers exceeded a threshold of one green wingless aphid per four plants, gave better reduction than aldicarb of virus yellows infection (*c.*55-50%), but there were few differences between spray treatments. Triazamate was occasionally more persistent than the other two sprays, but not enough to further reduce virus yellows infection. All treatments significantly increased sugar yield in 1994 at the Broom's Barn site, but only imidacloprid did so at that site in 1995. None of the treatments significantly improved sugar yields at the Sandy site in either year. On average over the four trials, imidacloprid increased yields by 19%, the three spray treatments by around 12% and aldicarb by only 7%.

INTRODUCTION

Farmers in the UK now have a choice of several insecticides and three different methods of application to control aphid vectors of virus yellows in sugar beet crops. These can be used alone or in combination with each other, but the cost-benefit of the strategy adopted will depend on the risk of infection. The choice is between imidacloprid applied as a film-coating to pelleted seed, aldicarb granules applied at drilling, and aphicidal sprays applied when aphid numbers exceed thresholds. This paper describes results from four trials carried out in 1994 and 1995 to compare the efficacy of each treatment in areas with high and low risk of infection.

MATERIALS AND METHODS

Trial sites

Two trials were conducted each year at Broom's Barn and at Portabello Farm, Sutton, Sandy Bedfordshire. The latter site was chosen for its location in an area prone to high levels of virus yellows, while Broom's Barn normally has low levels of infection (see map in Dewar, 1994). Seed, cv. Saxon in both years, was sown on 22 April and 29 March in 1994 at Broom's Barn and Sandy respectively, and on 2 April and 21 March in 1995. Plots were large (18 rows by 12 m at Broom's Barn, and 12 rows by 20 m at Sandy) to allow better assessment of the effects of treatments on virus infection by reducing the risk of spread from neighbouring plots.

Treatments

Imidacloprid ('Gaucho', Bayer plc) was applied at 90 g a.i./unit of pelleted seed (1 unit = 100,000 seeds) by Germain's U.K. of King's Lynn, to the outside of previously pelleted seed (Asher & Dewar, 1994). Aldicarb ('Temik'; Rhône-Poulenc Agriculture Ltd.) was applied as a granule at 760 g a.i./ha using a Stanhay Webb Granyl G40 applicator. Sprays were applied using a two-man Oxford precision sprayer delivering 200 l/ha through Lurmark 02F 80 nozzles at 2.0 bar. Pirimicarb ('Aphox'; Zeneca Crop Protection) at 140 g a.i./ha, a formulated mixture of pirimicarb at 100 g and deltamethrin at 7.5 g a.i./ha respectively ('Evidence'; Agrevo UK Crop Protection Ltd.) and triazamate (not yet registered; Cyanamid Agriculture UK) at 56 g a.i./ha plus mineral oil ('Swirl') at 500 ml/ha, were applied on three occasions in both years at Broom's Barn, but only twice in both years at Sandy, when numbers of aphids exceeded the threshold of one green wingless aphid per four plants. Thus at Broom's Barn, sprays were applied on 30 May, 16 June and 7 July in 1994, and 23 May, 16 June and 7 July in 1995; at Sandy corresponding dates were 23 May and 16 June in 1994, and 9 May and 2 June in 1995. The results for selected treatments only are reported here.

Assessments

Aphid numbers were assessed approximately weekly in all treated plots after the first aphids were recorded in untreated plots, until the end of June at Sandy, or until mid-July at Broom's Barn. Virus yellows infection was assessed visually in 10 m lengths of the central six rows of each plot on several occasions in each year. At Broom's Barn this was done on 15 July and 30 August in 1994, and 4 July and 15 August in 1995; at Sandy, assessments were made on 29 June, 28 July and 15 September in 1994, and 28 July and 21 August in 1995. Assessments were done when the crop was not wilting. Sub-samples of yellow leaves were tested by ELISA in the laboratory (Smith & Hinckes, 1987) to determine which of the two viruses which cause yellowing was present, and in what proportion. The effect of treatments on yield and quality of sugar beet was measured by harvesting rows from 8 m lengths of the central four rows (16 m^2) in October or November. Root weight, sugar concentration and levels of impurities were measured in Broom's Barn tarehouse using standard techniques. Data from the trials were analysed using GENSTAT V. Aphid numbers were transformed logarithmically ($\log_{10}(n+1)$), and the percentage of virus infection transformed using angles (arcsin $\sqrt{\%}$), prior to analysis.

RESULTS

Aphid numbers

In both years aphids appeared at least a week earlier at Sandy than at Broom's Barn confirming that site as high risk. However, numbers there never reached more than a total of 3 aphids per plant in 1994, and were less than one per plant in 1995; thus only 2 sprays were applied in each year. Both *Macrosiphum euphorbiae* and *Myzus persicae* were present in both years at Sandy, but black aphids, *Aphis fabae*, were relatively scarce.

In contrast, at Broom's Barn in 1994, aphid numbers rose to over 200 per plant by July but these were mostly *A. fabae* (95%). Green aphid numbers, largely *M. persicae*, peaked at around 15 per plant in early July. Numbers were much less in 1995 and black aphids were very few; peak numbers were at only 6.4 aphids per plant in untreated plots, most of which were *M. persicae*.

Effect of treatments on aphids

Only a selection of the large amount of data collected is presented in Table 1 to show particular aspects of the comparative efficacy of treatments. For example, at Sandy in 1994, 65 days after sowing, imidacloprid was still giving excellent control of aphids, significantly better than aldicarb; 21 days later, although numbers were still significantly less than in untreated plots, it was clear that imidacloprid-treated plants were beginning to be colonised and differences between the two treatments were less obvious (Table 1A). At Broom's Barn in the same year there were no significant differences between the two treatments 46 and 62 days after sowing, but the number of wingless green aphids on aldicarb-treated plots (5 aphids per plant) on 28 June (67 days after sowing) was significantly greater than on imidacloprid-treated plots (0.7 per plant). Of the spray treatments, triazamate gave significantly better control of aphids than pirimicarb plus deltamethrin nine days after the first spray at Sandy, but there were no differences between treatments after the second spray. At Broom's Barn there were no differences between spray treatments on any sampling occasion, except on the last one when all treatments were swamped by large numbers of black aphids in the first week of July. Then, triazamate gave better control than either primicarb or pirimicarb plus deltamethrin.

In 1995 numbers of aphids in all plots at Sandy remained very low throughout the summer and, although numbers were less on treated plots than on untreated, there were no significant differences between treatments (Table 1B). At Broom's Barn, imidacloprid and aldicarb gave equivalent control up to 7 June, 66 days after sowing, but two weeks later, aldicarb-treated plants had been significantly infested by aphids while imidacloprid was still effective. All three spray treatments gave excellent knockdown immediately after application, but plots were recolonised within a week and there were no consistent differences between treatments.

Table 1. Effect of treatments on the total number (log_{10} (n+1) of aphids per sugar beet plant.

A) 1994	Sandy		Broom's Barn	
Treatment	65 DAS 9 DAT 1	86 DAS 4 DAT 2	46 DAS 7 DAT 1	62 DAS 7 DAT 2
Untreated	0.418 (1.6)	0.699 (4.0)	0.238 (0.7)	1.129 (12.5)
Imidacloprid	0.050 (0.1)	0.351 (1.2)	0.048 (0.1)	0.448 (1.8)
Aldicarb	0.200 (0.6)	0.448 (1.8)	0.077 (0.2)	0.540 (2.5)
Pirimicarb	0.145 (0.4)	0.117 (0.3)	0.184 (0.5)	0.399 (1.5)
Pir + Delt	0.228 (0.7)	0.233 (0.7)	0.234 (0.7)	0.342 (1.2)
Triazamate + oil	0.074 (0.2)	0.168 (0.5)	0.061 (0.2)	0.214 (0.6)
LSD (5%)	0.1344	0.3100	NS	0.2627

B) 1995	Sandy		Broom's Barn	
Treatment	57 DAS 7 DAT 1	85 DAS 11 DAT 2	66 DAS 15 DAT 1	81 DAS 6 DAT 2
Untreated	0.199 (0.6)	0.122 (0.3)	0.544 (2.5)	0.717 (4.2)
Imidacloprid	0.095 (0.2)	0.065 (0.2)	0.134 (0.4)	0.270 (0.9)
Aldicarb	0.112 (0.3)	0.148 (0.4)	0.293 (1.0)	0.693 (3.9)
Pirimicarb	0.087 (0.2)	0.171 (0.5)	0.210 (0.6)	0.407 (1.6)
Pir + Delt	0.104 (0.3)	0.103 (0.3)	0.439 (1.7)	0.605 (3.0)
Triazamate + oil	0.091 (0.2)	0.062 (0.2)	0.316 (1.1)	0.378 (1.4)
LSD (5%)	0.0976	NS	0.2429	0.1939

DAS = Days after sowing; DAT 1/2 - Days after spray treatment 1 or 2; NS = not significant. Figures in brackets are back-transformed minus 1.

Effect of treatments on virus yellows

Virus infection was much higher at Broom's Barn than at Sandy in both years. Most infection (90%) in all four trials, was caused by beet mild yellowing virus (BMYV); beet yellows virus (BYV) was relatively scarce. All treatments significantly reduced infection in three of the four trials, but not at Sandy in 1995 where infection was less than 10% on untreated plots. Imidacloprid gave significantly better control of virus than aldicarb in the other three trials, giving an average reduction in infection of 72% compared to only 44% for aldicarb (Table 2). The three spray treatments on average reduced virus levels by 53-58%, with triazamate being significantly better than pirimicarb at Broom's Barn in 1994, while pirimicarb plus deltamethrin was more effective than pirimicarb in 1995.

Table 2. Effect of treatments on incidence of virus yellows (arcsin √% plants infected) in sugar beet.

Treatment	Broom's Barn		Sandy		Mean 4 trials
	1994 30 Aug.	1995 15 Aug.	1994 28 July	1995 21 Aug.	
Untreated	45.55 (50.9)	31.25 (26.9)	42.87 (46.3)	17.65 (9.2)	(33.3)
Imidacloprid	12.47 (4.7)	14.29 (6.1)	27.41 (21.2)	12.55 (4.7)	(9.2)
Aldicarb	27.40 (21.2)	26.59 (20.0)	31.62 (27.5)	14.93 (6.6)	(18.8)
Pirimicarb	20.02 (11.7)	22.96 (15.2)	32.56 (29.0)	15.50 (7.1)	(15.8)
Pir + Delt	17.28 (8.8)	21.34 (13.2)	27.41 (21.2)	14.29 (6.1)	(14.5)
T'zamate + oil	14.21 (6.0)	24.91 (17.7)	30.39 (25.6)	15.61 (7.2)	(14.1)
LSD (5%)	5.409	8.093	4.006	NS	-

Figures in brackets are back-transformed. LSD= Least Significant Difference at P>0.05; NS = no significant differences.

Effect of treatments on sugar yield

All treatments significantly improved sugar yields at Broom's Barn in 1994, but only imidacloprid did so at that site in 1995 (Table 3). There were no significant differences in yield at Sandy in either year. In 1994 this was due to large variability within blocks caused by uneven irrigation using a raingun, while in 1995, when no irrigation was applied, virus infection was not high enough to cause a measurable loss in yield. Uneven emergence of seedlings in one corner of the trial at Broom's Barn in 1995 contributed to the relatively high variablity in yield data there, leading to lack of significance between some treatments and untreated plots. Nevertheless, all treatments did give some substantial and important increases in yield. On average in the four trials, imidacloprid gave the greatest increase in sugar yield (19.3%) followed by the three spray treatments (11-12%); aldicarb gave the lowest increase at 7.1%.

Table 3. Effect of treatments on sugar yield (t/ha) of sugar beet.

Treatment	Broom's Barn		Sandy		Mean 4 trials	% increase
	1994	1995	1994	1995		
Untreated	9.75	11.60	6.26	10.35	9.49	-
Imidacloprid	12.18	14.43	7.64	11.03	11.32	19.3
Aldicarb	11.72	11.99	6.34	10.57	10.16	7.1
Pirimicarb	11.88	12.10	7.38	11.18	10.61	12.1
Pir + Delt	11.74	13.02	7.23	10.52	10.63	12.0
T'zamate + oil	12.21	11.87	7.12	11.01	10.55	11.2
LSD (5%)	0.868	1.804	NS	NS	-	-

LSD =Least Significant Difference at P>0.05; NS=no significant differences

DISCUSSION

In the face of continuous migrations of aphids over a prolonged period (*c.* 10 weeks) the best strategy for controlling these pests is with a systemic insecticide which has long persistence. Until the introduction of imidacloprid in 1994, best control of virus-carrying aphids was given by aldicarb granules applied at drilling at a reasonably high rate. However, the results reported here have shown that imidacloprid seed treatment gives significantly better control of aphids for more than 7 weeks after drilling, and significantly greater reduction of virus yellows in late July or August than aldicarb, resulting in substantially higher root and sugar yields at harvest, confirming previous results (Dewar, 1992). It was necessary to supplement aldicarb with at least one aphicidal spray to give good control of virus in the two years in which the trials were carried out. The use of sprays alone gave better reduction in virus levels than aldicarb, but they were consistently poorer than imidacloprid. Yields of all treatments were substantially, but not always significantly, improved when virus levels were above 20% in untreated plots. No significant yield benfits were observed with any treatment when virus levels were less than 10%.

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ASSESSMENT OF RISK AND CONTROL OF FLAX FLEA BEETLES

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ABSTRACT

Potential strategies for the control of flax flea beetles on linseed were compared at four sites in 1995. At two sites where flea beetles invaded the crop at an early growth stage, a gamma-HCH seed treatment or phorate granules applied in furrow, provided better control than spray treatments applied after emergence. At one site where flea beetles invaded the crop after plant emergence seed treatment was ineffective compared to sprays applied at full crop emergence. Gamma-HCH sprays provided a more effective control than esfenvalerate at two sites, but at one site when sprays were applied early, esfenvalerate was more effective than gamma-HCH. Yield responses to treatment were recorded at three of the four sites with a seed treatment plus a single spray post-emergence providing the most cost-effective control. In 1996 a similar experiment was conducted at one site comparing control strategies on Antares linseed with Coniston Linola and Klasse flax. The Linola was slower to establish and proved to be more susceptible to damage than the other crop types. Bifenthrin was tested as a seed treatment in 1996 and was more effective than a gamma-HCH seed treatment.

INTRODUCTION

The incidence of the large and small flax flea beetles (*Aphthona euphorbiae* and *Longitarsus parvulus*) has increased in response to an increase in the area of linseed grown since the early surveys conducted by Walters & Lane (1991), to cause severe crop damage from 1993 onwards. Severe damage was initially confined to southern England, but has spread progressively outwards from this initial focus to affect the whole of England and Wales by 1995. Damage has frequently resulted in total crop loss, with attempts at control with gamma-HCH or pyrethroid-based sprays, as recommended for oilseed rape crops, giving inconsistent and frequently inadequate control. It has been observed that in the more severe cases of damage, flea beetles invaded the crop soon after sowing, feeding on germinating seedlings and destroying many plants before they could emerge from the soil. In addition to shot holing of the cotyledons of emerged seedlings, flea beetles frequently fed on the stem causing additional loss of plant stand. Both *A. euphorbiae* and *L. parvulus* overwinter as adult beetles which migrate to crops in the spring when maximum daily temperatures exceed 15°C. Activity is enhanced when temperatures reach 20°C, allowing long distance flight

(Fritzsche, 1958). An experiment was done at four sites in 1995 comparing a range of potential control strategies to determine the most effective against the prevailing flea beetle attack in relation to activity of both species through the susceptible growth stages of linseed. A similar experiment was conducted in 1996 comparing the susceptibility of linseed, Linola and flax.

MATERIALS AND METHODS

In 1995, plots were sown with the same batch of linseed cv. Antares seed treated with procloraz and carboxin, lindane and thiram applied by commercial seed treatment apparatus. The tefluthrin seed treatment was applied with a mini-rotostat seed treatment machine by Zeneca Crop Protection. The seed rate was adjusted according to the estimated field factor to achieve a target plant population of 350 plants per m^2. Sites were established at ADAS Bridgets, Martyr Worthy, Winchester, Hampshire; ADAS Drayton at Billesley Manor Farm, Billesley, Stratford-on-Avon, Warwickshire; ADAS Rosemaund, Preston Wynne, Hereford and at ADAS Arthur Rickwood, Mepal, Ely, Cambridgeshire. Treatments were arranged in a randomised blocks design with four replicates. The dates of sowing and treatment application are shown in Table 1. Sprays were applied by Oxford Precision sprayer using flat fan nozzles and medium droplet size in 300 litres of water per ha.

Table 1. Dates of sowing and treatment application in 1995

	Bridgets	Drayton	Rosemaund	Rickwood
seed sown	3 April	11 April	12 April	21 April
first spray	20 April	2 May	5 May	5 May
second spray	4 May	18 May	19 May	21 May

1995 treatments

A) prochloraz seed treatment ('Prelude 20LF' at 200 ml/100 kg of seed)
B) prochloraz ('Prelude 20LF' at 200 ml per 100 kg) + caboxin + lindane + thiram ('Vitavax RS' at 10 litres/tonne of seed) seed treatment
C) seed treatment A plus a gamma-HCH spray at 540 g a.i./ha (0.7 litres 'Gamma-Col'/ha) within one week of first plant emergence
D) seed treatment A plus a gamma-HCH spray at the same rate within one week of first plant emergence and a second spray two weeks later
E) seed treatment A plus an esfenvalerate spray at 7.5 g a.i./ha (0.3 litres 'Sumi-Alpha'/ha) within one week of first plant emergence
F) seed treatment A plus an esfenvalerate spray at the same rate within one week of first plant emergence and a second spray two weeks later
G) seed treatment B plus a gamma-HCH spray within one week of first plant emergence
H) seed treatment B plus a gamma-HCH spray at the same rate within one week of first plant emergence and a second spray two weeks later
I) seed treatment B plus an esfenvalerate spray within one week of first plant emergence
J) seed treatment B plus an esfenvalerate spray within one week of first plant emergence and a second spray two weeks later

K) seed treatment A plus tefluthrin seed treatment at 2.5 g a.i. per kg seed.
L) seed treatment A plus phorate granules at 1,700 g a.i./ha (17.0 kg 'BASF Phorate' /ha) applied through the drill at drilling

In 1996, recleaned seed of Antares linseed, Coniston Linola and Klasse flax was used and the seed treatments were applied by Uniroyal Chemicals. Sowing rates were set to achieve target plant populations of 350 plants /m^2 for Antares and Coniston and 1,250 /m^2 for Klasse. The experiment was sown at ADAS Bridgets on 3 April and spray treatments were applied on 25 April. Treatments were arranged in a split plot design.

1996 treatments

A) prochloraz ('Prelude 20LF' at 200 ml per 100 kg of seed) seed treatment
B) caboxin + lindane + thiram ('Vitavax RS' at 10 litres per tonne of seed) seed treatment
C) bifenthrin + carboxin + thiram (40 + 80 + 80 g a.i. per 100 kg) seed treatment
D) seed treatment A plus a gamma-HCH spray at 540 g a.i./ha (0.7 litres 'Gamma-Col'/ha) within one week of first plant emergence
E) seed treatment A plus an esfenvalerate spray at 7.5 g a.i./ha (0.3 litres 'Sumi-Alpha'/ha) within one week of first plant emergence
F) seed treatment B plus a gamma-HCH spray within one week of first plant emergence
G) seed treatment B plus an esfenvalerate spray within one week of first plant emergence
H) seed treatment C plus a gamma-HCH spray within one week of first plant emergence
I) seed treatment C plus an esfenvalerate spray within one week of first plant emergence

Assessments

One 165 mm diameter yellow water trap was positioned at ground level at each of two opposite corners of each experimental site. The traps were filled with water with sufficient wetter added to break the surface tension, installed immediately after drilling and emptied weekly until the end of May. The flea beetles *A. euphorbiae* and *L. parvulus* were identified and counted in the laboratory. At Arthur Rickwood, where the catches were greatest, a 20% sub-sample was taken for identification between 3 May and 7 June.

First crop emergence date was recorded and plant populations were measured two and four weeks after first emergence, growth stage being assessed before each treatment application. The whole plot area was harvested by combine harvester. A sub-sample of seed was taken from each plot, cleaned to remove and estimate the weight of any contaminants and the moisture content measured. Harvest yields were corrected to 91% dry matter content.

RESULTS

In 1995 the phorate treatment caused a marked cupping and marginal scorch to the cotyledons, but this had no apparent affect on the rate of emergence or on yield. At Bridgets flea beetles were active from the time the crop was sown. An apparent peak of activity in the first week of May corresponded to a particularly warm spell with temperatures regularly exceeding 20°C. *A. euphorbiae* represented 19% of the total catch of 4,482 flax flea beetles. Treatments affected plant numbers (P 0.004) (Table 2). Yields differed considerably between

treatments (P <0.001) with yield responses relative to the fungicide only control treatment ranging up to 0.6 tonnes per ha (Table 3). At Drayton, flax flea beetles first invaded the crop during the warm week in early May, *A. euphorbiae* representing 68% of the catch of 2,530 flax flea beetles. Plant establishment was less affected by treatment (P 0.005) (Table 2). Yields were significantly affected by treatment (P 0.005) with yield responses ranging up to 0.25 tonnes per ha (Table 3).

Table 2. Plants per square metre at full emergence in 1995.

Treatment Date assessed:	Bridgets 16 May	Drayton 24 May	Rosemaund 5 June	Arthur Rickwood 24 May
A control	325	365	203	130
B HCH st	548	418	255	206
C HCH spray	388	374	231	104
D " " twice	374	366	264	140
E esfenvalerate	462	396	256	150
F " twice	386	340	265	155
G B + C	495	398	210	233
H B + D	454	436	261	255
I B + E	532	396	254	241
J B + F	496	404	271	239
K tefluthrin st	437	479	245	152
L phorate	427	438	173	218
SEM (33 df)	37.0	13.0	33.5	16.2
CV%	16.7	6.5	27.9	17.5
P	0.004	<0.001	0.576	<0.001

Table 3. Yields in 1995 (tonnes/ha at 91% dm).

Treatment	Bridgets	Drayton	Rosemaund	Arthur Rickwood
A control	0.85	1.09	1.55	0.33
B HCH st	1.29	1.08	1.54	0.62
C HCH spray	0.90	1.25	1.49	0.18
D " " twice	0.87	1.19	1.51	0.31
E esfenvalerate	1.24	1.12	1.56	0.38
F " twice	1.16	1.18	1.58	0.37
G B + C	1.26	1.28	1.70	0.73
H B + D	1.41	1.32	1.46	0.66
I B + E	1.34	1.22	1.63	0.53
J B + F	1.46	1.34	1.52	0.65
K tefluthrin st	1.05	1.29	1.54	0.40
L phorate	1.32	1.31	1.44	0.74
SEM (33 df)	0.101	0.050	0.086	0.045
CV%	17.2	8.2	11.1	18.4
P	<0.001	0.005	0.741	< 0.001

At Rosemaund, flax flea beetles first invaded the crop in the same warm week in early May, *A. euphorbiae* representing 56% of the total catch of 1,750 flax flea beetles. Crop establishment was poor relative to the other sites, with no significant treatment effects in terms of plant populations (Table 2) or yield (Table 3). At Arthur Rickwood, flax flea beetles

were present in the field before sowing. Crop emergence also coincided with the warm period in early May resulting in a very high pest pressure on the crop at that time, *A. euphorbiae* representing 33% of the total catch of 8,484 flax flea beetles. Plant populations differed significantly between treatments (P <0.001), but none of the treatments raised the plant population above the target of 350 plants per m^2. Yield responses were obtained from the gamma-HCH seed treatment with an additional yield response from supplementary sprays. Spray treatments did not provide a yield response on plots which had not received a gamma-HCH seed treatment (P <0.001). Phorate granules provided a similar yield response to the best seed treatment and spray combination. Yields were low and it was concluded that none of the treatments applied was sufficiently effective against the high pest pressure at this site.

In 1996, the flea beetle activity at Bridgets was less with *A. euphorbiae* representing 11% of the total catch of 1,463 flax flea beetles. The attack had no significant effect on plant populations of Antares or Klasse, although the population of Klasse was reduced by apparent phytotoxicity from the gamma-HCH seed treatment (Table 4). The Coniston Linola was slower to establish and suffered significant plant loss; both the gamma-HCH and bifenthrin seed treatments increased plant stand with bifenthrin significantly superior to gamma HCH (P < 0.05).

Table 4. Plant populations as a percentage of target populations in 1996

Treatment Target population:	Antares linseed 350 plants/m^2	Coniston Linola 350 plants/m^2	Klasse flax 1250 plants/m^2
A control	116.3	81.1	101.1
B gamma-HCH st	117.4	109.9	66.1
C bifenthrin st	111.3	121.0	108.9
D gamma-HCH spray	104.5	88.3	100.7
E esfenvalerate spray	117.0	85.1	103.0
F B + D	116.8	98.0	70.3
G B + E	94.6	109.8	72.1
H C + D	124.9	113.8	122.3
I C + E	125.6	102.5	124.6
SEM (72 df)		5.65	
CV%		10.9	
P		< 0.001	

DISCUSSION

The experiments demonstrated that against early attacks, only seed or soil treatments were sufficiently effective to protect the crop from plant loss. Gamma-HCH seed treatments had little effect in reducing damage beyond the cotyledon stage and at Drayton where migration to the crop was later, they were relatively ineffective. The tefluthrin seed treatment was applied to the crop at one rate only for the purposes of widening the range of strategies tested, it was not supposed that this would be the rate used for a commercial treatment and further development would be required to determine the most appropriate rate for this use. In terms of yield response there was a distinct inconsistency between sites as to the most effective spray treatment. At Bridgets, the first sprays were applied in a cooler period, the mean temperature over the four days after treatment being 8.2°C which may have favoured

esfenvalerate and over the three days after the treatments were applied 13.5 mm of rain fell which may have washed the residues of gamma-HCH off the soil surface. At Drayton and Arthur Rickwood the sprays were applied later in a period of warm, dry weather with mean temperatures of 15.5°C following treatment. No further yield response was obtained to the additional second spray applications applied at the four true-leaf stage and it was concluded that the crop was less susceptible to damage at this later growth stage. In view of continuing reports of varying efficacy in the field, further work is needed to define the parameters affecting the performance of the available treatment options.

Previous workers have concluded that sprays of gamma-HCH worked into the seed bed were more effective than seed treatment (Fritzsche, 1958; Kirkov, 1958). The soil treatment is not currently approved in the UK and such an approval, leading to an increased, prophylactic use of the active ingredient in a method prone to leaching, is justified neither economically nor environmentally. Other seed treatments have been developed in Romania where Gheorge, Brudea, Enica & Luca (1990) found carbofuran and furathiocarb to be effective. The good control obtained with bifenthrin in 1996 mirrors earlier work with this product. The phytotoxicity observed with Klasse in this experiment was not observed elsewhere and was thought to be associated with the particular batch of seed used.

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MANAGED APPLICATIONS OF MOLLUSCICIDES FOR SLUG CONTROL IN WINTER WHEAT

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ABSTRACT

A series of four replicated, plot-scale trials was undertaken on winter wheat in the harvest years 1994-1996. The objectives were to assess the potential for improving slug control by applying molluscicides managed in relation to assessments of slug numbers and by reference to meteorological forecasts for conditions favouring slug activity. Results obtained from pilot studies at two sites in 1993/1994 were further developed to investigate the efficacies of single or multiple managed treatments of metaldehyde applied at peak slug activity. During the three cropping seasons, the timing of peak slug activity varied from late-October to early-December. The greatest slug activity and subsequent plant damage was recorded in 1994/1995 with a well-defined activity peak 14 days after drilling (DADr) on 31 October when the managed applications were applied. At 21 DADr, the single managed inputs significantly reduced slug numbers under bran-baited refuge traps compared with the untreated mean of 11.3 slugs per six traps. At GS 12, all metaldehyde treatments applied at drilling or as managed inputs significantly reduced damage compared with the untreated mean plant damage of 20.7 %. In the final year of the study, similar data were obtained, although slug activity and incidence of crop damage were lower.

INTRODUCTION

Slugs are considered by many farmers to be one of the most significant pests of autumn-sown wheat (Glen, 1989); in 1993/1994, 27 % of the area of wheat grown in Great Britain was treated with molluscicide (Garthwaite,Thomas & Hart, 1994). For effective slug control, pelleted formulations of molluscicides are normally applied at or soon after drilling before the extent of damage is known. Many methods of risk assessment are used to target inputs, for example by obtaining evidence of slug activity pre-drilling (Glen *et al.*, 1993; Spaull, 1990). Yet, slug activity at drilling, when most treatments are applied, is often poorly related to subsequent crop damage (Glen *et al.*, 1993) as dry soils at the time of drilling often restricts slug activity on the soil surface. Such slug behaviour patterns are important to the success of control measures using surface-applied pelleted molluscicides as slugs need to be active on the soil surface to locate baits and initiate feeding.

The potential for improved targeting of inputs in response to studies of slug behaviour (Young *et al.*, 1991) led to the development of models to predict slug activity using measurements of air and soil temperatures and soil moisture status (Young, Port &

Green, 1993). Variations in slug activity in the post-drilling period also indicated potential for better targeting of molluscicides in relation to slug activity (Green *et al.*, 1992). This paper describes how aspects of these studies were developed to assess the potential for a more managed approach to slug control by applying slug pellets post-drilling, with treatments timed to coincide with peak slug activity.

MATERIALS AND METHODS

In the cropping years 1993/94, 1994/95 and 1995/96, a total of four replicated plot-scale trials were established within crops of winter wheat (Table 1). All experiments were sited in fields with a history of slug damage and with evidence of slug activity pre-drilling. The preceding crop at all sites was oilseed rape.

Table 1. Details of trial sites

Site		Cultivar	Drilling date	Replicates	Plot size (metres)
1.	Nottinghamshire	Hussar	22.10.93	4	12 x 10
2.	Warwickshire	Soissons	24.10.93	4	12 x 8
3.	Warwickshire	Hunter	17.10.94	3	12 x 8
4.	Warwickshire	Brigadier	12.10.95	3	12 x 10

Treatments

In the pilot studies at sites 1 and 2, winter wheat was treated with metaldehyde ('Metarex RG') at 8.0 kg/ha broadcast at drilling or 4-5 days after drilling or as a managed input comprising metaldehyde at 8.0 kg/ha at drilling followed by a second treatment of 8.0 kg/ha applied in response to assessments of slug activity. Comparisons were made with methiocarb ('Draza') at 5.5 kg/ha broadcast at drilling and with an untreated control. Treatments were applied using a pepperpot technique.

At sites 3 and 4, treatments were: metaldehyde at 5.0 or 8.0 kg/ha applied at drilling or as a managed application at peak slug activity; metaldehyde at 5.0 kg/ha 7 days after drilling; repeat applications of metaldehyde at 5.0 + 5.0 or 8.0 + 5.0 kg/ha at drilling followed by a managed treatment in response to assessments of slug activity and by reference to meteorological forecasts. At site 3 only, metaldehyde at 8.0 kg/ha was broadcast at the onset of crop damage; at site 4 only, methiocarb at 5.5 kg/ha was broadcast at drilling. Treatments were compared with untreated controls. With the exception of the experimental treatments, the winter wheat crops were managed according to current good-farm practice.

Slug Activity

Post-drilling slug activity in the pilot studies at sites 1 and 2 was monitored on five occasions during autumn 1993 using three bran-baited refuge traps (plant-pot saucers 15 cm diameter) per plot. Such traps were shown by Young (1990) to provide a good

indication of slug activity, notably at sites where the field slug *(Deroceras reticulatum)* is present. As a result of these pilot studies, the experimental design for autumn 1994 and 1995 was expanded to investigate the efficacies of single managed inputs.

At sites 3 and 4, six bran-baited traps per plot were used to assess slug numbers on 4 or 5 occasions (Tables 2 & 4). In autumn 1994, high levels of slug activity were noted with a single, well-marked peak of activity of short duration in late-October which enabled the timing to be determined for the managed treatments. In autumn 1995, the slug activity profile was very different from the previous year. Increasing slug catches on 27 October were used as the basis for the application of the managed inputs but were followed by a second smaller peak in mid-November.

Damage Assessments

Plant counts were made on 10 x 0.5 metre lengths of drill per plot at the two-leaf stage (Zadoks GS 12). Plant damage (grazed or shredded leaves) was assessed on the same dates and recorded as percentage damaged plants. Data were subjected to analysis of variance after angular transformation, at sites 3 and 4 (Tables 3 & 5).

RESULTS AND DISCUSSION

In the pilot studies at two sites in 1993/1994, assessments of slug activity using bran-baited refuge traps indicated good potential for application of molluscicide treatments in relation to slug activity. Although data from the pilot studies are not presented in this paper, post-drilling slug activity at both sites was low during the period after drilling when the soil surface was dry. As soils grew progressively wetter, slug numbers under traps increased to a well-defined peak in early-December, more than a month after the winter wheat crops were drilled.

In year 2 (site 3), single managed treatments were applied by reference to assessments of slug activity and by closer reference to weather forecasts considered likely to provide conditions favourable for continuing slug activity. Moderate post-drilling slug activity, seven days after drilling, increased substantially to a mean of 39.3 slugs per six bran-baited traps on the untreated plots following a two-day rainfall total of 36 mm in late-October (Table 2). The managed, peak activity treatments were applied on 31 October. On this date, treatments applied at drilling had significantly (P=0.001) reduced slug numbers compared with the untreated mean of 39.3 slugs per six bran-baited traps. Slug numbers on plots treated with metaldehyde on 31 October were then similar to those on the untreated plots. By 22 DADr (8 November), some recovery of slug numbers on plots treated at drilling was noted, whereas the single managed treatments had significantly (P=0.05) reduced slug catches compared with the untreated mean of 11.3 slugs per six traps.

Table 2. Site 3: post-drilling slug activity. Mean numbers of slugs under 6 bran-baited traps per plot.

	Treatment	Rate (kg/ha)	Timing	Drilling + 7 days 24.10.94	Drilling + 14 days 31.10.94	Drilling + 22 days 08.11.94	Drilling + 29 days 15.11.94
1.	untreated	-	-	7.7	39.3	11.3	4.3
2.	metaldehyde	5.0	Drilling	1.0*	4.3***	9.7	2.7
3.	metaldehyde	8.0	Drilling	1.0*	4.7***	9.7	2.7
4.	metaldehyde	5.0	7 DADr	7.0	5.7***	5.7*	7.7
5.	metaldehyde	8.0	Damage	13.7	39.7	18.3*	4.3
6.	metaldehyde	5.0	Peak	9.0	42.3	5.0*	2.0
7.	metaldehyde	8.0	Peak	6.3	36.7	5.7*	3.7
8.	metaldehyde	5.0 + 5.0	Drilling + Peak	1.0*	2.7***	2.3**	5.3
	SED			2.76	4.82	2.44	2.20
	df			14	14	14	14
	CV(%)			57.9	26.9	35.3	66.1

7DADr: 7 days after drilling.
*, **, *** significantly different from untreated mean at $P = 0.05, 0.01, 0.001$ respectively.

Table 3. Site 3: mean percentages of plants grazed or severed by slugs at GS 12 (angular transformed data in brackets).

	Treatment	Rate (kg/ha)	Timing of application	Mean % damaged plants on 24.11.94 at GS 12	
1	untreated	-	-	20.7	(26.8)
2	metaldehyde	5.0	Drilling	7.7	(15.7)**
3	metaldehyde	8.0	Drilling	9.2	(17.3)*
4	metaldehyde	5.0	7 DADr	6.5	(14.7)**
5	metaldehyde	8.0	Crop damage	16.9	(24.3)
6	metaldehyde	5.0	Peak activity	6.4	(14.2)**
7	metaldehyde	8.0	Peak activity	9.6	(17.6)**
8	metaldehyde	5.0 + 5.0	Drilling + peak	3.8	(11.1)***
	SED				(3.20)
	df				14
	CV (%)				(22.1)

*, **, *** significantly different from untreated mean at $P = 0.05, 0.01, 0.001$ respectively.

Analysis of percentages of plants damaged by slugs at GS 12 (Table 3) on 24 November (38 DADr) showed that all metaldehyde treatments, except that applied at the onset of crop attack, significantly reduced damage compared with the untreated mean of 20.7 % damaged plants.

In the final year of the study at site 4, identical core drilling and managed single-input treatments to those at site 3 were retained. Slug activity was again low in the dry weather after drilling (Table 4) but increased substantially to a mean of 9.0 slugs per

six traps 15 DADr (27 October) at the end of a 7-day period with 19 mm rain. Activity was anticipated from models relating soil moisture assessments to slug activity, as described by Young *et al.*, (1991) and Young, Port & Green (1993). Peak activity treatments were therefore applied on this date.

Table 4. Site 4: post-drilling slug activity. Mean numbers of slugs under 6 bran-baited traps per plot.

	Treatment	Rate (kg/ha)	Timing	Drilling + 8 days 20.10.95	Drilling + 15 days 27.10.95	Drilling + 22 days 03.11.95	Drilling + 29 days 10.11.95	Drilling + 43 days 24.11.95
1	untreated	-	-	1.0	9.0	3.0	7.7	5.7
2	metaldehyde	5.0	Drilling	0	2.7	1.7	2.7*	3.3
3	metaldehyde	8.0	Drilling	0	2.3	0.3*	1.7*	2.3
4	metaldehyde	5.0	7 DADr	0.3	1.7	1.0*	0.7*	3.0
5	metaldehyde	5.0	Peak	0.7	10.0	0.7*	1.0*	1.3*
6	metaldehyde	8.0	Peak	2.0	11.0	0.7*	0.3*	2.3
7	metaldehyde	5.0+ 5.0	Drilling + Peak	0.3	2.0	2.0	1.3*	1.0*
8	metaldehyde	8.0 + 5.0	Drilling + Peak	0	2.0	1.0*	1.7*	1.7*
9	methiocarb	5.5	Drilling	0.3	2.3	1.7	0.7*	3.7
	SED			1.06	3.56	0.77	1.87	1.87
	df			16	16	16	16	16
	CV(%)			249.0	90.7	69.8	116.4	84.6

*, **, *** significantly different from untreated mean at $P = 0.05, 0.01, 0.001$ respectively.

Table 5. Site 4: mean percentages of plants grazed or severed by slugs at GS 12 (angular transformed data in brackets).

	Treatment	Rate (kg/ha)	Timing of application	Mean % damaged plants on 24.11.95 at GS 12	
1	untreated	-	-	12.4	(20.6)
2	metaldehyde	5.0	Drilling	2.5	(8.9)***
3	metaldehyde	8.0	Drilling	2.1	(8.1)***
4	metaldehyde	5.0	7 DADr	3.5	(10.7)***
5	metaldehyde	5.0	Peak activity	2.1	(8.3)***
6	metaldehyde	8.0	Peak activity	2.1	(8.2)***
7	metaldehyde	5.0 + 5.0	Drilling + peak	1.7	(7.2)***
8	metaldehyde	8.0 +5.0	Drilling + peak	1.8	(7.5)***
9	methiocarb	5.5	Drilling	1.5	(6.6)***
	SED				(1.77)
	df				16
	CV (%)				(22.7)

*, **, *** significantly different from untreated mean at $P = 0.05, 0.01, 0.001$ respectively.

Single managed treatments reduced slug activity at site 4 as effectively as treatments applied at drilling (Table 4). Differences were significant (P=0.05) compared with the untreated mean of 7.7 slugs per six traps 29 DADr. Mean percentage damaged plants at GS 12 (Table 5) were significantly (P=0.001) reduced by all treatments compared with the untreated mean of 12.4 %. The managed applications reduced damage as effectively as treatments applied at drilling. Additional benefits from multiple applications of metaldehyde were smaller in 1995/1996 than in the previous season.

CONCLUSIONS

These trials demonstrate that a managed approach to the application of slug pellets can achieve similar or more effective reductions in slug surface activity and reductions in plant damage compared with treatments applied at drilling. This technique, however, requires more detailed monitoring of slugs and closer reference to weather forecasts for conditions favourable to slug activity.

ACKNOWLEDGEMENTS

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THE IMPACT OF INSECTICIDE RESISTANCE IN INSECT PESTS ON INTERACTIONS WITH NATURAL ENEMIES

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ABSTRACT

The effect of insecticide resistance in insect pests on interactions with natural enemies has received little attention in the ecotoxicological literature. However, there is growing evidence that resistance within pest populations can have a substantial impact on the survival of parasitoids in the aftermath of spraying. This has not been taken into account by the groups who have traditionally worked on non-target organisms. This paper provides evidence that the resistance status of the host is extremely important in governing the effect of insecticides on non-targets, in particular parasitoids, and that this should be investigated more thoroughly.

INTRODUCTION

In recent years, increasing attention has been paid to evaluating the side effects of pesticides on beneficial organisms, including parasitoids (e.g. Hassan *et al.*, 1994). This has also become obligatory in several countries for the registration of plant protection products (Anon., 1991). Such evaluations are undertaken initially in small scale bioassays with strains that may have been kept in the laboratory for a long time. No account is taken of the resistance status of the host which may have significant implications, particularly for the survival of immature stages of internal parasitoids. Very often the effect, or lack of effect, of the pesticide on the non-target species in the field can be masked because the insecticide kills the pest leaving a parasitoid with no means of reproducing. It is possible therefore that the direct effect of a pesticide on a parasitoid may be less significant than its indirect effect due to host depletion.

In this paper we describe experiments conducted under simulated field conditions in the laboratory investigating the effects of two insecticides, dimethoate and pirimicarb, on interactions between the peach-potato aphid, *Myzus persicae* and its aphidiid parasitoid *Diaeretiella rapae*. Results are presented and compared for both an insecticide-susceptible strain of *M. persicae* and a strain exhibiting strong resistance to a wide range of chemicals including carbamates and organophosphates.

MATERIALS AND METHODS

Field simulators

The design of the field simulators used for this work has been described elsewhere (Rowland *et al.*, 1990). The cage containing the plants was 1.7 x 1.2 x 1.0 m with glazed side panels and insect proof mesh at each end. The mesh allowed a fan mounted at one end to draw air uniformly through the cage containing the plants. Banks of fluorescent and tungsten lights

above the cage gave a photoperiod of 16 hours. The room containing the simulators was kept at 21-22 °C.

Insect strains

Both strains of *M. persicae* were maintained as parthenogenetic clones derived from a single adult in the original field collection. The clone 1076A (S) originated from oilseed rape at Rothamsted in 1992, and in bioassays exhibited baseline susceptibility to insecticides. Clone 946E (R) originated from sugar beet in Norfolk in 1991 and exhibited R2 levels of resistance conferred by overproduction of a carboxylesterase (E4) capable of detoxifying carbamate, organophosphate and pyrethroid insecticides (Devonshire and Moores, 1982). The strain of *D. rapae* originated from *M. persicae* on oilseed rape at Rothamsted and was maintained in the laboratory on the S clone of *M. persicae*. The host plant used for aphid rearing and field simulator experiments was winter oilseed rape (*Brassica napus*) cv. Falcon. Aphids and parasitoids were reared at 21-22 °C under a light : dark regime of 16:8 h.

Establishment of experimental populations

Eight oilseed rape plants at the 4-5 node stage were distributed in two rows of four plants on the floor of each simulator. The rows were isolated from each other by a strip of 'Oecotak A-10' (Oecos Ltd) applied to the cage floor, and then inoculated with different clones. Each plant was inoculated with fifteen, 2-3 day old adult *M. persicae*. Newly emerged adult female parasitoids were added two days later, at a starting density of 72 per cage (i.e. 9 per plant), to coincide with the first cohort of aphid nymphs becoming second instars, the preferred stage for parasitism.

Application of insecticide

The insecticides used were dimethoate ('BASF Dimethoate 40' EC) and pirimicarb ('Aphox' SG; Zeneca). They were applied using a hand sprayer at a rate and volume calculated to correspond to the field rates of 340 g a.i. ha^{-1} and 140 g a.i. ha^{-1} respectively. Formulated products were diluted with distilled water and sprayed once only, four days after the initial inoculation of aphids into the simulators.

Design of the experiments

Each individual experiment consisted of a single compound and utilized four simulators. One cage had no parasitoids added and was unsprayed, one was sprayed with the insecticide but had no parasitoids added, another had parasitoids but no insecticide and the final one had both. This design was intended as far as possible to distinguish the effects of an insecticide on both host and parasitoid. The experiments ended on day 14 after aphid inoculation when the plants were removed from the simulators, and examined to determine the number of aphids and parasitoid mummies on a leaf by leaf basis. Day 14 corresponded with the F1 generation of parasitoids being present as mummies and therefore being easy to distinguish from unparasitised aphids.

RESULTS AND DISCUSSION

Effects of parasitoids alone

In the absence of parasitoids or an insecticide application, aphid populations of both clones showed a characteristic exponential build up to about 10, 000 individuals of all ages per four plants by day 14 (Fig. 1). This proved extremely repeatable between clones and replicate experiments.

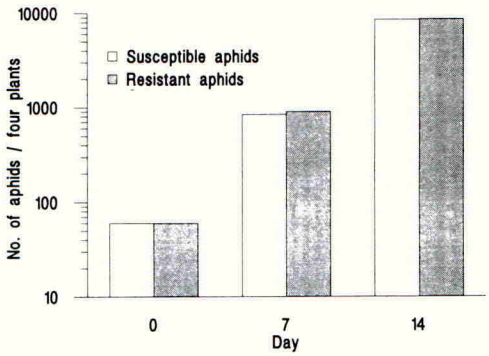

Figure 1. Characteristic build up of the clones of *M. persicae* in the field simulators in the absence of parasitoids

With the addition of parasitoids this increase was substantially suppressed (Fig. 2); counts on day 14 seldom exceeded 1500 aphids per four plants. The level of suppression was dependant on the starting density of parasitoids, optimised at 72 adult females per cage to suppress the aphid population sufficiently so that any effect of the insecticide on parasitoid success would be readily discernable, without reducing aphid numbers to such an extent that the population was in danger of extinction. Both clones were parasitised to the same degree.

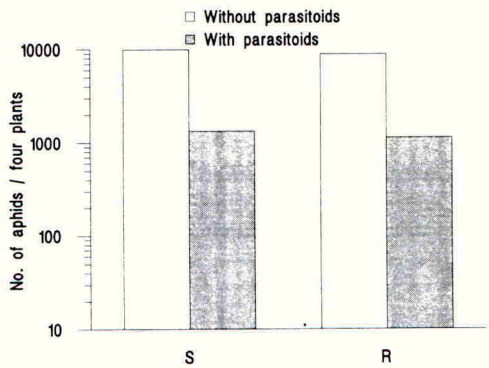

Figure 2. The effect of adding 72 adult female parasitoids on aphid numbers in field simulators on day 14 after inoculation

Effect of insecticicides alone

As expected, single sprays of insecticide differed in their ability to control the two aphid clones (Fig 3). Both compounds gave > 95 % control of the susceptible aphids, but neither reduced the R2 clone to less than 50 % of the unsprayed control. It was notable that parasitoids alone gave a greater level of control than either compound against the R2 clone. The increased survival of R2 aphids to dimethoate compared with pirimicarb was consistent with the greater resistance recorded to dimethoate in bioassays (Sawicki and Rice, 1978).

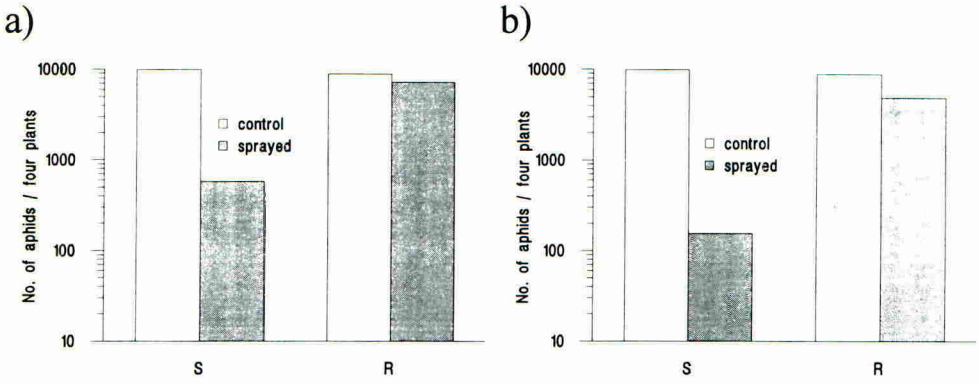

Figure 3. The effect of dimethoate (a) and pirimicarb (b) on aphid numbers of two clones of *M. persicae* assessed on day 14 of field simulator experiments

Combining insecticides with parasitoids

When pirimicarb was applied to cages containing parasitoids the cumulative effect of insecticide and parasitoids eradicated the sprayed S aphids entirely (Fig. 4b). When pirimicarb was sprayed onto parasitised R2 aphids a number of hosts remained, allowing the survival of some parasitoids.

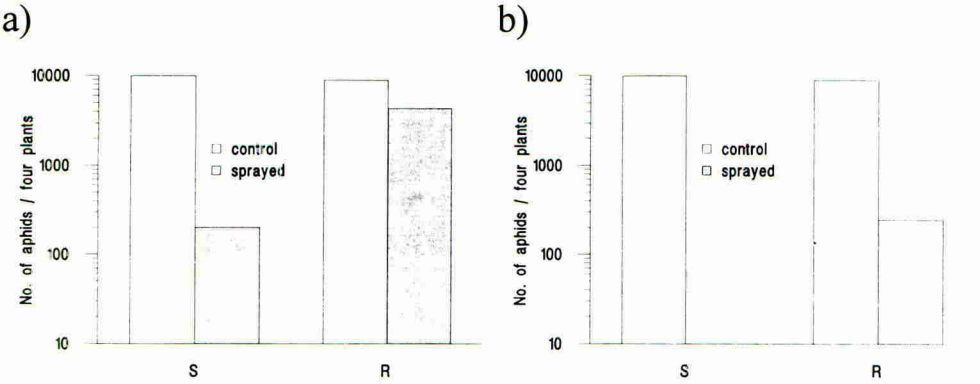

Figure 4. The effect of dimethoate (a) and pirimicarb (b) on two clones of *M. persicae* parasitised by *D. rapae* in the field simulators

When dimethoate was sprayed against either clone in conjunction with parasitoids, the suppression was greater than with dimethoate alone (Fig. 4a). However, against the S clone there was a severe effect on the parasitoids leaving only two mummies in the sprayed cage. Against the R2 clone, the combination of dimethoate and parasitoids was also less effective than parasitoids alone, implying that dimethoate exhibited a marked effect on parasitism.

Aphid : parasitoid ratio

On plants containing the S clone, very few or no mummies were detected after spraying. The primary reason for this was the poor survival of susceptible hosts. By exploiting the greater survival of R2 aphids, it was possible to compare the direct effects of pirimicarb and dimethoate on parasitoids, using the aphid : parasitoid ratio on day 14. This ratio is an important measure of likely control in the successive generation. The larger the ratio, the more likely that aphids will outstrip parasitoids. As seen in Fig. 5 both insecticides significantly increased this ratio compared to the unsprayed control. Pirimicarb almost doubled the ratio and dimethoate increased it five fold. This indicates that in the subsequent generation, suppression due to parasitism would be less than in the previous one, perhaps leading to "flaring" of the aphid population.

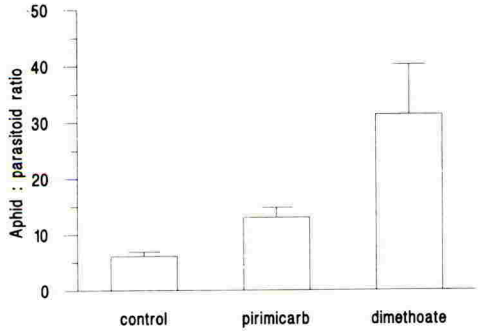

Figure 5. Aphid : parasitoid mummy ratio in R2 clone on day 14 of field simulator experiments treated with different insecticides

CONCLUSIONS

Where resistance is present, parasitoids, either on their own or in combination with insecticides, can give better control than insecticides alone. In some circumstances it may be advantageous to have resistant aphids in the population i.e. where there are parasitoids present and insecticide is to be sprayed then resistant aphids can act as a refugia for parasitoids, thus providing hosts in which to develop. These results highlight the possibility of exploiting resistant pests when assessing the side effects of pesticides on natural enemies.

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THE INFLUENCE OF NITROGEN FERTILISER APPLICATIONS ON THE CEREAL APHIDS *METOPOLOPHIUM DIRHODUM* AND *SITOBION AVENAE*

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ABSTRACT

The effects of five different rates of nitrogen fertiliser applications on the abundance of the cereal aphids *Metopolophium dirhodum* and *Sitobion avenae* in winter wheat were investigated over three years. At the time of peak aphid populations, higher densities of the two species were associated with plots which received the highest spring applications of fertiliser. *M. dirhodum* was more markedly influenced than *S. avenae* but the differences were only apparent and significant when natural populations of aphids were relatively high.

INTRODUCTION

Since the Second World War there has been a marked increase world-wide in the use of nitrogenous fertilisers (Anon., 1979). An average of 185 kg ha^{-1} was applied to winter wheat in 1993 (Burnhill *et al.*, 1994) and recommendations for this crop from the Ministry of Agriculture, Fisheries and Food rise to 265 kg ha^{-1}, depending on soil type and crop yield potential (Anon, 1994). The effects of this nutrient on cereal plants is well documented. It promotes stem extension and can encourage tiller survival and boost grain yield and protein content (Wibberley, 1989). Leaves also remain green for longer, senescence is delayed and the leaf area duration is extended (Woolhouse, 1981).

The evidence for effects of crop nitrogen on aphids, particularly at the higher recommended rates is unclear. That the enhanced supply of nitrogen in the soil was possibly a relevant factor to explain the increase in cereal aphids since the mid 1940s, was first suggested by Baranyovits (1973). Schaefer, Bent and Cannon (1979) noted that the increase in the numbers of *M. dirhodum* in the UK in 1978 and 1979 was coincident with an increase in the applications of nitrogen fertiliser and a noticeable increase in the greenness of leaves. Dewar (1980) found that wheat plants receiving high rates of nitrogen fertiliser had 48 % more aphids than those receiving a lower rate. Hönek (1991) studied the effects in the field of nitrogen treatments on the abundance of *M. dirhodum* and *S. avenae* in winter cereals over two years at application rates of up to 160 kg ha^{-1} and concluded that the abundance of *M. dirhodum* increased with nitrogen input but that the numbers of *S. avenae* were little affected.

The aim of this study was to examine more closely the influence of nitrogen fertiliser applications on the two most frequently found cereal aphid species in eastern England (Carter *et al.*, 1980), the grain aphid *Sitobion avenae* and the rose-grain aphid *Metopolophium dirhodum*.

MATERIALS AND METHODS

In each year an experimental site was located in a commercial winter wheat crop (cv. Haven in 1992 and cv. Riband in 1993 and 1994) on the estate of Writtle College, Chelmsford, Essex. The experiment was laid out using plots of 12 m long x 12 m (the width between tramlines) in a randomised block design of five treatments in each of four blocks. Each block was separated by a 10 m 'discard' strip to facilitate fertiliser spreading. Land preparation and cultivations were carried out according to normal farm practice. The plots received varying levels of 34.5 % ammonium nitrate fertiliser ('Norsk Hydro ExtraN') which was applied at G S 31 (Zadoks *et al.*, 1974), using a tractor mounted air-assisted spreader. The rates applied were 100, 125, 150, 175 and 200 kg ha^{-1} in 1992; 75, 100, 125, 150 and 175 kg ha^{-1} in 1993 and 50, 100, 150, 200 and 250 kg ha^{-1} in 1994. All other inputs apart from insecticide treatments were carried out according to normal commercial farm practice. However, an aphicide was applied in April of each year prior to the start of the spring migration, to eliminate aphids that may have colonised the crop in the autumn. In 1993 and 1994 a plant growth regulator programme was included to reduce the risk of crop lodging.

Aphid counts were begun in each year as soon as reasonable numbers of natural populations of *S. avenae and M. dirhodum* were found in the plots. From this time onwards, the plots were inspected at approximately seven to ten day intervals and aphids counted initially on 100 tillers per plot, selecting twenty groups of five tillers at random. As aphid populations increased during the season, the number of shoots sampled was reduced to fifty and then to twentyfive. Aphids were identified to species in the field and sampling continued throughout the season until populations declined to very low levels.

To evaluate the effects of treatments on aphid populations, analysis of variance (ANOVA) was done on transformed ($\log_{10}$ (n+1)) numbers of aphids per 100 shoots using an SPSS (Statistical Package for the Social Sciences) for Windows, Release 6.0 computer program. When the F-test in ANOVA was significant, Tukey's Honestly Significant Difference (HSD) test was used to identify means which were significantly different.

RESULTS

M. dirhodum - 1992

Numbers of this species remained at less than five per shoot until July (Table 1). On 1 July the highest density was found in the plots receiving the 150 kg ha^{-1} nitrogen application; it was also higher at the 175 and 200 kg ha^{-1} application rates than at the 125 and 100 kg ha^{-1} rates. Statistical analysis showed that the differences in aphid numbers between the 100 and

150 kg ha^{-1} application rates and also between the 125 and 150 kg ha^{-1} rates were significant ($p< 0.05$; F value = 4.547 with 4,11 degrees of freedom - one plot lodged). On the last sampling date on 11 July, the highest numbers of *M. dirhodum* were recorded in all of the plots, except for those which had received 150 kg ha^{-1} nitrogen, but none of the differences between treatments were significant. Because of crop lodging problems this year, sampling was discontinued after this date.

S. avenae - 1992

As with *M. dirhodum*, numbers of this species were very low during May and June (Table 1). Although numbers increased during July, there were no significant differences between the numbers of aphids on different treatments on any sampling date.

Table 1. Mean numbers of aphids per shoot for each sampling date in 1992 at five rates of nitrogen fertiliser application.

	M. dirhodum					*S. avenae*				
	Nitrogen application kg ha^{-1}									
Sampling date	100	125	150	175	200	100	125	150	175	200
22 May	0.06	0.02	0.06	0.11	0.09	0.09	0.28	0.21	0.09	0.23
26 May	0.01	0.08	0.10	0.14	0.07	0.08	0.16	0.23	0.15	0.07
03 June	0.19	0.22	0.35	0.31	0.19	0.70	0.50	0.38	0.41	0.55
10 June	0.28	0.25	0.35	0.43	0.22	0.77	0.61	0.70	0.70	0.72
16 June	0.68	0.96	0.97	0.85	0.65	0.54	0.66	0.58	0.71	0.69
24 June	2.00	2.50	2.56	2.25	2.40	0.56	0.65	0.69	0.62	0.61
01 July	5.32	6.10	12.41	7.50	6.92	2.32	2.42	3.10	3.02	2.94
11 July	5.34	8.29	11.66	8.75	7.35	4.42	5.35	4.70	4.70	5.17

M. dirhodum and *S. avenae* - 1993

Numbers of both species were low throughout this year and never reached 1 per shoot on any sampling date. For *M. dirhodum*, the highest mean number for the year (0.55 per shoot) was found on the 150 kg ha^{-1} rate plots on 23 June. On each sampling date the lowest density occurred at the lowest nitrogen rate and higher densities generally occurred in the plots with the two highest nitrogen rates, but the differences were not significant.

The highest mean number of *S. avenae* for each treatment was recorded on 23 June for the 75, 125 and 175 kg ha^{-1} rate plots (with the overall highest mean of 0.69 per shoot on the 75 kg ha^{-1} plots) and on 21 July for the 100 and 150 kg ha^{-1} plots. Again, differences between the numbers found on any sampling date were not significant.

M. dirhodum - 1994

Few aphids were found in any of the plots until 22 June (Table 2) when there was an increase in the size of the population with each increase in the rate of nitrogen fertilisation, the highest

numbers found on the plots with the 250 kg ha^{-1} application rate. The differences in numbers between the plots with the lowest rate of nitrogen application and those with the 150, 200 and 250 kg ha^{-1} application rates were highly significant ($p < 0.01$)(Table 3). On 30 June, numbers of *M. dirhodum* peaked at just over 5 per shoot on the plots with the highest rate of nitrogen. As on the previous sampling date, peak numbers followed exactly the increasing rate of fertiliser application and the differences were very highly significant ($p < 0.001$)(Table 3) between the numbers found on plots which received 50 kg nitrogen ha^{-1} and those with 150, 200, and 250 kg ha^{-1}; between those which received 100 and those with 200 kg ha^{-1} and also between those which received 100 and those with 250 kg ha^{-1}.

By the following week (7 July), numbers of this species had declined with no significant differences between treatments, although there were still more aphids on the high rate nitrogen plots compared with the low rate plots. The populations declined rapidly to very low levels by the next sampling date and had all but disappeared by 21 July (Table 2).

Table 2. Mean numbers of aphids per shoot for each sampling date in 1994 at five rates of nitrogen fertiliser application.

	M. dirhodum					*S. avenae*				
	Nitrogen application kg ha^{-1}									
Sampling date	50	100	150	200	250	50	100	150	200	250
01 June	0.11	0.16	0.09	0.20	0.12	0.02	0.07	0.06	0.07	0.03
11 June	0.27	0.48	0.40	0.59	0.58	0.02	0.03	0.08	0.06	0.04
15 June	0.40	0.79	0.84	0.72	0.96	0.07	0.10	0.05	0.14	0.19
22 June	0.76	1.35	1.85	2.10	2.29	0.42	0.35	0.30	0.20	0.66
30 June	1.45	2.34	3.65	5.07	5.34	1.35	2.17	1.82	2.36	2.45
07 July	0.45	1.61	1.04	1.01	1.47	1.61	2.18	2.12	2.56	2.72
14 July	0.02	0.01	0.04	0.02	0.05	0.36	0.29	0.33	0.25	0.22
21 July	0.01	0.03	0.00	0.01	0.01	0.06	0.04	0.05	0.03	0.05

S. avenae - 1994

As with *M. dirhodum*, few aphids were present in any of the plots until 22 June when the highest density was found in the plots with the highest nitrogen application rate (Table 2). On 30 June, numbers had risen considerably and followed the increasing rate of fertiliser application in the plots except for the 100 and 150 kg ha^{-1} rates. However, the differences were just outside the 5% level of significance ($p = 0.06$).

On 7 July the highest numbers were recorded in all of the plots, with the peak for the year at just under 3 aphids per shoot on the plots with the highest rate of nitrogen. Numbers again followed the increasing rate of fertiliser application except for the 150 kg ha^{-1} rate. Statistical analysis showed that the differences in numbers between the plots with the lowest rate of nitrogen application and those with the 200 and 250 kg ha^{-1} rates were significant ($p < 0.05$)(Table 3).

Table 3. 1994 ANOVA results for numbers of *M. dirhodum* and *S. avenae* on three sampling dates for 50, 100, 150, 200 and 250 kg ha^{-1} nitrogen application rates.

	M. dirhodum						*S. avenae*		
Sampling date	22 June			30 June			7 July		
Variation source	SS	MS	F	SS	MS	F	SS	MS	F
Fertiliser	.666	.167	10.793**	.902	.225	16.825***	.162	.041	3.555*
Replicate	.071	.024	1.537	.061	.020	1.526	.027	.009	.778
Within	.185	.015		.161	.013		.137	.011	
Total	.922	.049		1.12	.059		.326	.017	

Listed are sum of squares (SS), mean square (MS) and F values. In each case there are 4, 12 degrees of freedom. * = $p< 0.05$, ** = $p< 0.01$, *** = $p< 0.0001$.

DISCUSSION

In 1992, differences between treatments were not detectable until numbers of aphids increased in July. Although most *M. dirhodum* were found on the mid-nitrogen application rate plots, there were some significant differences between the lower and higher rates. These results gave an indication of the effects of crop nitrogen applications on the abundance of this species. No such influence, however, could be detected on *S. avenae.*

Throughout 1993, low numbers of aphids were recorded and, although treatment differences were not significant, higher densities of both species were generally at the two highest nitrogen application rates.

In 1994, numbers of *M. dirhodum* at peak densities followed exactly the increased rate of nitrogen application to the crop. These results are similar to those of Hönek (1991) when lower rates (up to 160 kg ha^{-1}) were used. When in the current study, the increase in fertiliser application rate between treatments was doubled to 50 kg ha^{-1} in 1994 (using rates approaching the maximum recommended for the crop), the increase in numbers of this species was significantly different for each increase of 100 kg ha^{-1} applied. For *S. avenae,* the lowest density present was at the lowest nitrogen application rate and the two highest densities at the two highest application rates.

These data show that abundance of these two cereal aphid species in winter wheat is related to the amount of nitrogen fertiliser applied to the crop, up to rates approaching the maximum recommended in the UK. The influence is more marked on *M. dirhodum* than on *S. avenae*. However, these differences are only apparent and significant when infestations are relatively high and are greater when there is a sufficient difference in the levels of fertiliser applied.

The number of aphids recorded in a crop is the product of the numbers entering and the numbers of offspring produced on it. The overall relationship, however, is not a simple one, with various factors such as weather and natural enemies affecting the population dynamics after immigration (Walters & Carter, 1981). Whether alate spring immigrants initially enter a crop in response to factors related to the plant's nitrogen content, or choose to remain in these crops after some preliminary feeding, is still unclear. As nitrogen fertiliser applications in cereals influence crop colour and the number of shoots per unit area, these factors may play a part in attracting these immigrants.

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DEVELOPMENT OF A RAPID ON-SITE TEST FOR THE PRESYMPTOMATIC DETECTION OF *SEPTORIA TRITICI* AND *SEPTORIA NODORUM*

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ABSTRACT

An immunological based test for the presymptomatic detection of the cereal foliar pathogens *Septoria tritici* and *Septoria nodorum* has been developed. The test is specific for *Septoria* and can distinguish between *S. tritici* and *S. nodorum*. The test itself employs a novel technology which allows it to be accomplished within 2 minutes in the field, requires no laboratory equipment, includes positive and negative controls in each test and requires only very minimal training to perform. The entire test can be stored at ambient temperature for at least 6-12 months. Simultaneous testing of samples is reported between the field test and our '*Septoria Watch*' laboratory based ELISA.

INTRODUCTION

The foliar pathogens *Septoria tritici* and *Septoria nodorum* can be quite devastating to a number of wheat cultivars (Gilbert & Tekauz, 1993). Their early detection is often difficult and requires a very trained eye to distinguish between *Septoria* and several other foliar pathogens. Distinction between *S. tritici* and *S. nodorum* at early stages is virtually impossible without the aid of microscopy. Although fungicide application can be effective in control of the disease, early detection of these pathogens is critical in determination of timing of application. Therefore, detection of the disease presymptomatically is extremely advantageous to control of the disease and to maximize crop yields.

With the introduction of polymerase chain reaction (PCR) technology into diagnostics there is great potential for the development of very selective and sensitive tests in a relatively short period of time as compared to the time needed to develop antibodies conventionally. Although there are certainly many advantages to PCR and it may someday dominate the diagnostic field, it is still somewhat unknown outside the scientific community and does require some prior training. On the other hand, immunological diagnostic tests have been used in agriculture for a number of years and have a proven track record of utility. A variety of formats have been developed using antibodies and

include enzyme linked immunosorbent assay (ELISA), dot-blot (Bausher & Sweeney, 1991), dip-stick (Cahill & Hardham, 1994), and agglutination (Takahashi, 1991). Although the majority of these tests must be performed in a laboratory, several field based assays have been developed (Miller *et al.*, 1988; Sherald & Lei, 1991; Benson, 1991) and several are available commercially (Neogen, USA and Adgen, UK). These field assays are primarily dot-blot whereby, a capture antibody is adsorbed onto a membrane, blocked, and dried. Although this type of test is quite successful in medical applications where the sample is provided in a soluble buffered form such as blood or urine, there are a number of problems when the sample is in the form of a plant leaf, stem or root. We have developed a novel immunoassay format which has a design specific for plant diagnostics and therefore alleviates many of the problems commonly associated with agricultural field assays. We report on the comparison of field data between this on-site assay and the complimentary rapid laboratory-based ELISA.

MATERIALS AND METHODS

Polyclonal antibody development

The polyclonal antibodies against both *Septoria tritici* and *Septoria nodorum* were produced and affinity purified as described by Petersen et al. (1990). Subsequent affinity purified antibodies were additionally conjugated to alkaline phosphatase (Voller *et al.*, 1980).

ELISA and on-site development

The ELISA used throughout is part of our 'Septoria Watch' program (Mittermeier *et al.*, 1990) and has been shown to be species specific. The same polyclonal antibodies were employed to develop the on-site test. Affinity purified antibody was covalently bound to cellulose acetate filters by a number of methods (Stults *et al.*, 1989; Dean *et al.*, 1985). The same affinity purified polyclonal antibody was used as the secondary enzyme conjugate in the on-site test. Each on-site test consists of three filters; one assay positive control consisting of alkaline phosphatase covalently bound to the filter, one test filter consisting of the specific test antibody covalently bound to the filter, and one negative control filter consisting of 1.0% BSA (bovine serum albumin) covalently bound to the filter. The filters were dried and loaded into the syringe and immediately capped to minimize any deterioration due to moisture.

Sample harvest

Leaf samples were harvested from wheat crops in both Switzerland and the USA with various levels of disease severity. Each sample consisted of 25 leaves from the same leaf position randomly harvested within a test plot. Duplicate samples were harvested for simultaneous comparison of the ELISA and on-site tests.

Sample preparation

The samples were extracted in a proprietary buffer specifically developed for simultaneous extraction and antibody-antigen reaction in the presence of enzyme conjugate. Exactly 10ml of the extraction buffer was added to each sample and the tissue was homogenized with a hand held homogenizer (Bioreba AG, Switzerland) until a green slurry was produced. This slurry was then used directly in both the ELISA and on-site tests.

Test performance

The ELISA was accomplished as described by Mittermeier *et al.* (1990). The On-site test was accomplished by transferring approximately 500µl of the test sample to a small tube and then adding one drop (approximately 50µl) of secondary antibody enzyme conjugate to the sample. This mixture was then aspirated into the test and pumped up and down three times to allow capture of the antigen/antibody complex. The sample was then discharged and approximately 1ml of wash buffer (PBS, 0.5% Tween 20, 0.5% BSA, 0.3% sodium azide, pH 7.4) was aspirated and discharged to remove any residual unbound sample and antibody conjugate. One volume of a one component alkaline phosphatase substrate solution (Kirkegaard & Perry, USA) was aspirated to allow visualization of the reaction. The results were recorded after three minutes as either positive or negative for pathogen presence.

RESULTS AND DISCUSSION

On-site development

Development of the on-site test specifically addressed many issues which typically hinder the effectiveness of "field" assays. When dealing with crude plant tissue samples there is often the problem of large pieces of plant debris being trapped within the test material and causing high background, non-specific binding, and cross-reaction. Miller *et al.* (1988) attempted to eliminate this problem by adding a pre-test filtering step. While this can be effective it does add an additional piece of equipment and an additional step to the test. Since the on-site is effectively built into a filtering mechanism, simply performing the test pre-filters the sample. Additionally by locating the positive control filter as the first filter through which the sample flows, any background signal due to trapped debris is masked by the strong positive signal. The negative control will detect any true cross-reaction or background within the test. Another advantage of the filter format of the on-site is the exceptional surface area available for analyte coupling. When compared to nitrocellulose based membranes the binding capacity is several magnitudes greater for the same sized diameter disk. The opportunity for capture as analyte passes through the filter is greater than for a typical thin membrane based test because there is an additional 3-4 millimeters of depth. Finally, the covalent binding and orientation of capture antibody

within the filter matrix assures that all antibody bound will remain bound and optimally positioned for antigen capture, thus increasing sensitivity by maximizing capture.

The development of a buffer system which allows for plant tissue extraction and simultaneous antigen-antibody/enzyme conjugate binding not only minimizes assay steps and time but also increases sensitivity (Naser, 1990). This is a critical component of the assay.

Field sample testing

The *Septoria* on-site was developed to detect presymptomatic levels of disease within a defined plot and was calibrated with our ELISA using field samples and visual assessment. Through testing with our ELISA program we determined that the best method to test and report this was by sampling various leaf positions but only one leaf position per sample. Therefore any level of disease severity at or above presymptomatic levels as defined by the ELISA and visual assessment should be determined to be positive by the On-Site. Any noticeable difference between the negative control and the test filter is considered positive and is not rated according to intensity. Although the human eye can detect differences in intensity of the on-site test corresponding to differences in disease level, this type of visual rating would be too subjective and potentially defeat the purpose of the test.

The results in Table 1. demonstrate that the correlation between the ELISA and the on-site test is 100%. Samples with a full range of disease severity of both pathogens show absolutely no cross-reaction between species and the sensitivity of the on-site test was as good as for the ELISA. Initial optimization of the on-site test determined that the sensitivity potential significantly exceeded that of the double antibody sandwich (DAS) when using the same enzyme detection method. This can be seen in Table 1. In which the only difference between ELISA and on-site tests was for sample BA-6, which gave a *S. tritici* ELISA value of 0.23, which is considered a negative ELISA rating, but gave a positive signal when tested with the on-site test. However, samples BA-5 and BA-33 were both positive for *S. nodorum* by ELISA and on-site and both negative for *S. tritici* by ELISA and on-site.

To date, over 5,000 *Septoria* on-site tests have been field trialed with similar results. The sensitivity, ease of use, quickness, storage qualities, reliability, and suitability for field use should result in the on-site test becoming a valuable tool for crop disease diagnostics and integrated pest management.

Table 1. Field samples analyzed by ELISA versus on-site tests for *S. tritici* (SET) and *S. nodorum* (SEN).

Sample	ELISA rating[a]/OD[b] values				On-site rating	
	SEN		SET		SEN	SET
	Rating	OD	Rating	OD		
BA-5	+	0 .63	-	0.09	+	-
BA-6	+++	2.18	-	0.23	+	+
BA-11	+++	3	+++	2.28	+	+
BA-26	-	0.2	-	0.09	-	-
BA-33	+	0.81	-	0.12	+	-
BA-35	+	0.55	-	0.09	+	-
NC-2	-	0.19	+	0.35	-	+
NC-4	-	0.21	++	0.98	-	+
NC-13	+++	1.9	++	1.23	+	+
NC-3	+++	3.01	+++	2.4	+	+

[a] ELISA rating is determined by standard controls with pre-determined cut-off values for -, +, ++ or +++ A single + by ELISA is generally indicative of being visually presymptomatic.
[b] OD equals optical density at 405 nm.

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COLORIMETRIC PCR AND ELISA DIAGNOSTICS FOR THE DETECTION OF *PSEUDOCERCOSPORELLA HERPOTRICHOIDES* IN FIELD SAMPLES

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ABSTRACT

We have developed oligonucleotide primers for use in polymerase chain reaction (PCR) diagnostic assays for the detection of *Pseudocercosporella herpotrichoides*, the causative agent of eyespot on cereals. One set of primers amplifies a PCR product from *P. herpotrichoides* R-type DNA only. The other primer combination produces an amplification product from *P. herpotrichoides* W-type DNA only. The assays discriminate from other common cereal diseases including the stem diseases caused by *Fusarium* spp. and *Rhizoctonia solani*. These assays have been incorporated into a colorimetric microtiter plate format using enzyme-linked immunosorbent assay (ELISA) technology permitting quantification of the PCR products. We have also developed polyclonal antibodies to *P. herpotrichoides* and integrated them into a rapid double antibody sandwich (DAS) ELISA. These assays are being developed to provide an effective tool for optimizing applications of fungicide and to support the introduction of Unix™ for eyespot control.

INTRODUCTION

Pseudocercosporella herpotrichoides (telemorph = *Tapesia yallundae*) is the causal agent of eyespot on wheat, rye and barley in temperate regions (reviewed by Fitt *et al.*, 1988). *P. herpotrichoides* has been subclassified into two major pathotypes. Based on pathogenicity, the W-type is more pathogenic on wheat than on rye and certain grasses. The R-type tends to be equally pathogenic on wheat and rye (Lange de la Camp, 1966; Scott *et al.*, 1975). Potential yield losses occur when the fungus infects the stem, decreasing sap circulation. Depending on the meteorological conditions and the field's terrain, the yield losses can be 4-30% of total production if the crop is untreated (Lagneau *et al.*, 1986). Early detection and quantification of the fungus would be of considerable use to growers and cereal pathologists permitting the optimal use of fungicides to control the pathogen.

MATERIALS AND METHODS

Development of pathotype-specific PCR primers

We have previously reported (Beck & Ligon, 1995) on the development of PCR assays for the detection of *Stagonospora nodorum* and *Septoria tritici*. Using the same methods we developed pathotype-specific PCR primers for *P. herpotrichoides*. The internal transcribed spacer (ITS) regions were amplified by PCR, cloned and sequenced from R-pathotype and W-pathotype isolates of *P. herpotrichoides*. Diagnostic primers were designed to the most polymorphic regions of the aligned ITS regions. PCRs were done, testing different primer combinations for their ability to amplify a pathotype-specific fragment from purified fungal DNA. The primers were also tested for their ability to detect the pathogen in infected wheat and for their inability to cross-react with fungal DNA from other cereal pathogens.

Colorimetric quantification of PCR products

The eyespot diagnostic primers were integrated into a quantitative colorimetric microtiter plate format as described by Holmstrøm *et al.* (1993). The 5' PCR primer was modified to contain biotin, and digoxigenin-11-dUTP was used in the dNTP mix. A titration of the concentration of biotin-labeled primer and the amount of digoxigenin-11-dUTP produced the optimum amounts for the highest signal to noise ratio. After amplification, PCR products are immobilized on a streptavidin-coated microtiter well. Alkaline phosphatase-labelled antibody specific for digoxigenin binds the immobilized PCR product. Subsequently, *p*-nitrophenyl phosphate is added to produce a colorimetric response which can be quantified in a conventional ELISA plate reader.

Development of antibodies

Preparations of the W-pathotype of *P. herpotrichoides*, American Type Culture Collection #44643, were grown in PDB (potato dextrose broth), homogenized, and used for the immunization of rabbits and chickens. Subsequent IgG was immunoaffinity purified through a column prepared with an extract of *P. herpotrichoides*, ATCC #44643. The purified antibodies from both species recognize both pathotypes of *P. herpotrichoides* with no cross-reaction to other cereal pathogens tested.

Preparation of wheat extracts

Ten wheat plants were randomly sampled from a minimum of 25 acres. Samples of 4 cm length were cut directly above the basal culm of each stem. Wheat stems were macerated in plastic bags using a Homex 6 homogenizer (Bioreba) in a proprietary extraction buffer.

Preparation of samples for ELISA

Samples assayed by ELISA were derived from the field and also from fungal liquid cultures in PDB to characterize the specificity and sensitivity of the ELISA. Fungal samples were filtered, frozen at -80°C, then thawed and homogenized in 75 mM sodium carbonate, 25% ethylene glycol, 20 mM 2-mercaptoethanol, 0.5 mM AEBSF (4-2-aminoethyl-benzenesulphonyl fluoride hydrochloride), pH 8.9. The homogenate was centrifuged at 31,000 x g for 20 min. The resulting supernatant was diluted 1:2 in an ELISA diluent of PBS, 0.05% Tween-20, 1.0% BSA (bovine serum albumin), 0.03% sodium azide, pH 7.4, for use in the ELISA. Field samples were prepared as outlined for PCR and then diluted 1:2 in ELISA diluent.

Detection of *P. herpotrichoides* by DAS ELISA

Immunoaffinity purified rabbit antibodies were coated onto polystyrene microplates, blocked with BSA, and then dried and sealed for storage at room temperature. Prepared samples were then added directly to the microplate without any additional washing or hydration of the microplate. The DAS is completed with the addition of immunoaffinity purified chicken antibodies and followed by alkaline phosphatase labeled anti-chicken antibody. The substrate *p*-nitrophenylphosphate was added for visualization.

Diagnostic PCRs

Diagnostic PCRs were run with 1 µl of 1:10 or 1:50 diluted wheat extract, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 2 µM digoxigenin-11-dUTP (Boehringer Mannheim), 100 µM dGTP, 100 µM dATP, 100 µM dCTP, 50 µM dTTP, 25 pmol 3' primer, 24.5 pmol unlabelled 5' primer, 0.5 pmol biotin-labeled 5' primer and 2.5 units of Taq polymerase (Perkin-Elmer) in a final volume of 50 µl. Reactions were run for 35 cycles, each consisting of 15 s at 94°C and 1 min at 75°C in a Perkin-Elmer Model 9600 thermal cycler. Ten of the 50 µl of PCR products were analyzed on an ethidium bromide-stained gel. The remaining 40 µl of PCR products were processed in the colorimetric assay.

RESULTS AND DISCUSSION

Determination of PCR primer specificity

The PCR primers JB540 (5'-GGG GGC CAC CCT ACT TCG GTA A-3') and JB542 (5'CCA CTG ATT TTA GAG GCC GCG AA-3') amplified a 413 bp fragment from all of the *P. herpotrichoides* R-type isolates tested. These primers did not produce any amplification products from any of the W-type isolates tested nor from any of the

following cereal pathogens: *Fusarium culmorum, Fusarium graminearum, Microdochium nivale, Rhizoctonia solani, Stagonospora nodorum, Septoria tritici, Drechslera sorokiniana, Cercospora arachidicola, Cladosporium herbarum* and *Pseudocercosporella aestiva.*

The *P. herpotrichoides* W-type specific primers JB537 (5'-GGG GGC TAC CCT ACT TGG TAG-3') and JB541 (5'-CCA CTG ATT TTA GAG GCC GCG AG-3') produced a 413 bp PCR product from W-type isolates of *P. herpotrichoides* only. These primers did not cross-react with R-type isolates of *P. herpotrichoides* nor with any other cereal pathogens tested.

Colorimetric quantification of PCR products

A series of purified genomic *P. herpotrichoides* R-type DNA ranging from 2.5 pg to 10 ng was amplified by PCR using the R-type specific primers, JB540 and JB542. A similar genomic DNA titration was done with *P. herpotrichoides* W-type DNA and the W-type specific primers, JB537 and JB541. With both assays, as little as 2.5 pg of genomic DNA was detectable. Colorimetric analysis of the PCR products resulting from the amplifications produced the colorimetric results in Table 1. PCR analyses of these dilution series demonstrate that the response is relative to the amount of DNA at the start. Increasing initial template DNA increased the PCR product. All of the amplification products that were detected on an ethidium bromide-stained gel produced colorimetric values which were above the background value (water control = 0 ng DNA in Table 1).

Table 1. Colorimetric quantification of PCR products.

	Rye-pathotype PCR		Wheat-pathotype PCR	
ng DNA[a]	EtBr Gel[b]	Colorimetric[c]	EtBr Gel[b]	Colorimetric[c]
0	-	0.076	-	0.033
0.0025	+	0.109	+	0.106
0.005	+	0.127	+	0.125
0.01	+	0.182	++	0.196
0.1	+++	0.391	+++	0.512
1	++++	0.668	++++	0.837
10	+++++	0.945	+++++	1.102

[a]R-type genomic DNA used for R-type PCRs;
W-type genomic DNA used for W-type PCRs.
[b]-, no visible band; + to +++++, increasing order of fragment intensity.
[c]Colorimetric values are in OD_{405} units.

Analysis of field samples

Field samples were tested by both methods for the presence of eyespot (Table 2). Preliminary testing of field samples by PCR and ELISA has shown good correlation between the two assays. Although the ELISA was unable to differentiate between the pathotypes of *P. herpotrichoides*, it was able to validate both the presence and the relative quantification of eyespot by PCR. Neither PCR assay amplified DNA from the healthy wheat extract (RA 5.1). The W/R ELISA also produced a very low absorbence value (0.134) for the healthy extract. This indicates that none of the assays cross-reacted with healthy wheat.

Table 2. Analysis of field samples for eyespot.

	Rye-pathotype PCR		Wheat-pathotype PCR		W/R
Extract #	EtBr Gel[a]	Colorimetric[b]	EtBr Gel[a]	Colorimetric[b]	ELISA[b]
R-type PCR control (10 ng)	+++++	1.241	NA[c]	NA[c]	NA[c]
R-type PCR control (0 ng)	-	0.047	NA[c]	NA[c]	NA[c]
W-type PCR control(10 ng)	NA[c]	NA[c]	+++++	1.869	NA[c]
W-type PCR control (0 ng)	NA[c]	NA[c]	-	0.061	NA[c]
ELISA positive control	NA[c]	NA[c]	NA[c]	NA[c]	1.058
ELISA negative control	NA[c]	NA[c]	NA[c]	NA[c]	0.045
RA 5.1	-	0.049	-	0.069	0.134
RA 7.2	-	0.045	+	0.052	0.117
RA 8.4	-	0.05	++	0.561	0.619
RA 8.6	-	0.073	+++	1.212	0.873
RA 5.2	+	0.1	-	0.078	0.218
RA 6.7	++	0.254	-	0.053	0.418
RA 8.2	+++	0.678	-	0.058	0.772

[a]-, no visible band; + to +++++, increasing order of fragment intensity.
[b]Colorimetric and ELISA values are in OD_{405} units.
[c]Not applicable.

Extracts RA7.2, RA8.4 and RA8.6 all produced amplification products with the W-type specific PCR primers as determined by visualization on an agarose gel and quantified colorimetrically. There were no amplification products produced from these extracts when they were tested using the R-type specific primers. These extracts produced W/R ELISA values between 0.117 and 0.873, respectively.

The R-type specific primers amplified a diagnostic fragment from extracts RA5.2, RA6.7 and RA8.2. There were no diagnostic amplification products produced from these extracts when they were tested with the W-type specific primers. The W/R ELISA produced values between 0.218 and 0.772 for these extracts. We have also been able to

differentiate R- and W-type infected wheat using the PCR assays when testing samples which were infected with both pathotypes of eyespot.

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DETECTION AND QUANTIFICATION OF INDIVIDUAL FUNGAL SPECIES IN DISEASE COMPLEXES BY POLYMERASE CHAIN REACTION (PCR)

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ABSTRACT

Until recently, no method was available to identify and quantify individual fungal species present within plant tissue. Specific DNA markers have been identified for the majority of the components of the 'stem-base complex' and 'ear blight complex' which are two disease complexes affecting cereals. Assays based upon the polymerase chain reaction (PCR) have been developed for detection of these fungi directly in extracts from plant tissue. These assays have been refined to enable quantification of each species, allowing the relative contribution of each component to the disease of the plant to be estimated. Detection and quantification of *F. poae* and other *Fusarium* species in samples of wheat seed is reported as an example of the use of these PCR techniques. Some potential uses for these systems are discussed.

INTRODUCTION

Two economically important disease complexes affect cereals in the U.K.. The 'stem-base complex' involves *Fusarium culmorum, F. graminearum, F. avenaceum* and *F. poae* along with *Microdochium nivale* (varieties *majus* and *nivale*), all of which cause foot rot. In addition the complex may contain, *Tapesia yallundae* and *Tapesia acuformis* (W and R-type of *Pseudocercosporella herpotrichoides*) which cause eyespot and *Rhizoctonia cerealis* which causes sharp eyespot. The *Fusarium* and *Microdochium* species are also involved in the 'ear blight complex' which is of additional concern because of the potential of the *Fusarium* species to produce mycotoxins within the grain which are harmful to humans and livestock.

Visual diagnosis of stem base disease, where several fungi may be present in the same plant is difficult. particularly during the early growth stages (Goulds & Polley, 1990). The symptoms of eyespot, sharp eyespot, and *Fusarium* and *Microdochium* foot rots can be indistinguishable. The inability to diagnose disease correctly may result in the adoption of inappropriate or poorly timed disease control measures leading in inadequate disease control and subsequent yield, and/or quality losses. Even where disease is correctly diagnosed problems occur because of the different species, or pathotypes, which may be the cause of the disease. For example, the W- and R-type of *P. herpotrichodes* both cause eyespot but the fungal type cannot be determined on the basis of visual symptoms which may be of importance as they have been shown to respond differently to several fungicides (Gallimore *et al.*, 1987; McNaughton, pers comm). This may also be true of foot rot caused by *Fusarium* species and *Microdochium* varieties where, even if visual diagnosis was correct

at the disease level, inappropriate control measures may be taken because of the inability to identify which fungal species, or varieties, were present in the plant.

In an attempt to overcome such problems, the pathogens within the diseased material can be isolated into axenic culture and identified on cultural and/or morphological criteria. However, in the absence of selective media the relative amounts of each pathogen may not be accurately determined. For example *F. culmorum* tends to outcompete *M. nivale* which may result in an underestimation of the prevalence of the latter in mixed infections (Pettitt *et al.*, 1993). Whatever the method, the isolation of pathogens from plant tissues can only reveal what may be grown out of the plant rather than what is growing in the plant.

Molecular techniques are being developed to overcome many of the problems associated with the study of disease complexes. Among the most sensitive techniques available is the polymerase chain reaction (PCR). Assays based upon this technique have been developed for several plant pathogenic fungi and bacteria, including *Gaeumannomyces graminis* (Schesser *et al.*, 1991) and *Erwinia amylovora* (Bereswill *et al.*, 1992) and have been used to detect the pathogens in infected plant material. More recently, similar assays have been developed for components of the stem-base and ear blight complexes of cereals (Nicholson *et al.*, 1996; Parry & Nicholson, 1996; Nicholson & Parry, 1996; Schilling *et al.*, 1996), and this paper reports aspects of this work and some preliminary results achieved using these systems.

Although PCR is an extremely sensitive technique it has generally been used only to reveal the presence of pathogens and not to quantify pathogen biomass. However quantitative PCR has been developed for pathogens involved in a number of disease complexes including *Verticillium* species (Moukhamedov *et al.*, 1994) and *Microdochium* varieties (Nicholson *et al.*, 1996). An example of the use of these techniques to detect and quantify infection of wheat seed by *F. poae*, *F. culmorum*, *F. graminearum* and *F. avenaceum* is presented below.

MATERIALS AND METHODS

Plant material

A total of 10 seed samples were obtained from several sources (Table 1). All were suspected of being contaminated by *Fusarium* species, but only two (CSL, NIAB) were confirmed as contaminated by *F. poae* above 1%. Two samples, each consisting of 96 randomly selected seeds, were taken from each seed lot. One sample was used for DNA extractions and the other assayed for the presence of pathogens by conventional methods (see below). DNA was extracted from sub-samples (48 seeds) of seed using the CTAB method described by Nicholson *et al.* (1996), after grinding seeds for 15 min in a mill (Glen Creston Ltd, Stanmore; mixer/mill 8000) using steel ball bearings.
For conventional assay, 96 seeds were randomly selected, surface sterilised and plated out directly onto potato dextrose agar containing antibiotics. Emerging fungi were identified *in situ* or following sub-culture onto nutrient media.

PCR amplification

Amplification reactions were carried out in volumes of 50μl containing 10-20ng of fungal DNA or DNA from 2-5mg dry weight of plant material. The reaction buffer consisted of 100μM each of dATP, dCTP, dGTP and dTTP, 10 pM each of forward and reverse primer for PCR reactions, and 0.8 units of *Taq* polymerase in reaction buffer (10 mM Tris; HCl (pH 8.3), 1.5 mM $MgCl_2$, 50mM KCl), with the following reagents added to give final concentrations of 100μg ml^{-1} gelatine, 0.05% Tween 20 and 0.05% Nonidet P-40. Reaction mixtures were overlaid with mineral oil prior to PCR.

Specific PCR was performed using primer pairs selected for each of four ***Fusarium*** species; *F. culmorum, F. graminearum, F. poae* and *F. avenaceum* (Nicholson ***et al.***, unpublished, Parry & Nicholson, 1996). All primers were designed to have a theoretical melting temperature of 62°C to facilitate multiplex PCR if required. The primer pairs were generated following selection of specific products from Random Amplified Polymorphic DNA (RAPD) assays of each fungal species (results not shown). Primer pairs were tested for their specificity against DNA of a range of isolates of the species of interest along with related species and other fungi associated with the stem-base and ear blight disease complexes of cereals. Primer pairs were also tested against DNA from uninfected plant material to ensure that there was no cross-reaction to the host.

DNA extracted from fungal cultures and from infected plant material was amplified using 'touchdown' PCR to ensure specificity of product amplification. In this process the annealing temperature was 66°C for the first 5 cycles, and 64°C for the next 5 cycles. For amplification of DNA from infected plant material, this was followed by twenty-five cycles at 62°C. The temperature cycle used consisted of denaturation (95°C) for 30 sec, annealing (as described above) for 20 sec and extension (72°C) for 45 sec with maximal ramping rates between temperatures. A final extension step of 5 min was incorporated followed by cooling to 10°C until recovery of samples. Identical amplification conditions were used to detect *F. culmorum, F. graminearum, F. avenaceum*, and *F. poae*.

Quantitative PCR

Quantification of fungal biomass as measured by DNA content of tissues was achieved using competitive PCR for each of the four *Fusarium* species. In this system a known quantity of 'competitor' template DNA is added to the sample before PCR. The 'competitor' template has annealing sites identical to the primer sites in the fungal 'target' DNA (thus four competitor templates were required) but the distance between them differs from that in the fungal 'target' DNA. Therefore, the PCR product of the 'competitor' differs in size from that of the fungal 'target' so that the two fragments may be separated following electrophoresis through an agarose gel. In plants where no fungus is present only the 'competitor' DNA is amplified. In infected plants the ratio of fungal 'target' DNA to 'competitor' DNA amplified increases with increasing amounts of fungus. The more fungus that is present, the more 'target' copies are made compared to 'competitor' copies. By estimating the ratio of fungal 'target': 'competitor' product, and relating the ratio to a standard curve, the quantity of fungal DNA in the sample may be calculated. Competitor templates were designed and produced for each of the four *Fusarium* species and used to estimate the quantity of fungal DNA of each species in the ten samples of wheat seed.

RESULTS

Of the 10 seed samples selected for investigation, *F. poae* was detected in seven using an agar plate method (Table 1). Only a single seed was found to be contaminated by *F. poae* from four of the eight samples ('HAAC', '3/4766', '3/3155' and '4/3021'). No *F. poae* was isolated from three samples ('CSL-poae', 'Riband A' and 'Riband B'). There was no apparent correlation between the degree of contamination by *F. poae* at harvest and frequency of isolation, although where contamination by *F. poae* was low at harvest ('HAAC', 'Riband A', and 'Riband B') there was a concomitant low frequency of isolation of *F. poae* subsequently.

Table 1. Code, origin and *Fusarium* contamination of wheat seed samples.

Code & Origin	% *Fusarium* contamination (at 1994 harvest)	% *F. poae*, (March 1995) (agar plate)	Detection of *Fusarium* species by PCR (ng fungal DNA/ mg dry weight plant tissue)			
			F. poae	*F. avenaceum*	*F. culmorum*	*F. graminearum*
CSL-poae Herts.	10% *F. poae*	0	+ (1.559)	-	-	-
NIAB-poae Lincs.	9.5% *F.poae*	6	+ (0.320)	-	-	-
HAAC Suffolk	<1% *F. poae*	1	-	+ (0.002)	+ (0.130)	-
Riband A Lincs.	<1% *F. poae*	0	+ (<0.001)	+ (0.016)	+ (0.001)	-
Riband B Yorks.	<1% *F. poae*	0	+ (<0.001)	+ (0.234)	+ (0.003)	-
4/4343 Suffolk	25.5% *Fusarium*	9	+ (0.012)	+ (0.007)	+ (0.011)	-
4/3155 Glam.	16.5% *Fusarium*	1	+ (<0.001)	-	-	-
4/3084 Surrey	18% *Fusarium*	4	+ (<0.001)	-	-	-
4/3069 Dorset	12.5% *Fusarium*	2	+ (<0.001)	-	-	-
4/3021 Cambs.	12.5% *Fusarium*	1	+ (0.019)	-	+ (0.001)	-

-, No *Fusarium* detected; +, *Fusarium* detected.

DNA from the seed sub-samples was subjected to PCR using primer pairs specific for *F. poae* as well as for the other three *Fusarium* species. Two reactions were carried out on each sample. The first was conventional PCR in which only DNA extracted from the sample was included in each reaction. The second was competitive PCR in which all reactions included the relevant competitor fragment to permit quantification of the amount

of fungal DNA present in each sample.

Following conventional PCR a fragment of similar size to that of the control *F. poae* DNA was amplified from nine of the ten seed samples (not 'HAAC'), indicating the presence of this species (Table 1). The level of amplification was high (a bright band) in four samples ('4/3021', '4/4343', 'NIAB-poae' and 'CSL-poae') and moderate/low in the remaining samples (results not shown). *Fusarium avenaceum* was detected in four samples ('HAAC', 'Riband A', 'Riband B' and '4/4343') and *F. culmorum* was detected in five samples ('HAAC', 'Riband A', 'Riband B', '4/4343' and '4/3021'). PCR analysis did not reveal the presence of *F. graminearum* in any of the seed samples.

Quantitative PCR indicated that both 'CSL-poae' and 'NIAB-poae' contained high levels of *F. poae* (1.559 and 0.32ng fungal DNA/mg dry weight of plant tissue (Table 1) and moderate levels were detected in '4/3021' and '4/4343' (0.019 and 0.012ng respectively). The levels in '4/3069', '4/3084', '4/3155', 'Riband A' and 'Riband B' were below the minimum quantifiable level using the selected competitor concentration. A high level (0.130ng fungal DNA/ mg dry wt.) of *F. culmorum* was detected in 'HAAC', a moderate level (0.011ng) in 4/4343 and low levels in the three remaining samples in which *F. culmorum* was detected by PCR. A high level (0.234ng fungal DNA) of *F. avenaceum* was detected in 'Riband B' and moderate/low levels in the other three samples (Table 1).

DISCUSSION

Primers specific for four *Fusarium* species have been tested on DNA from contaminated wheat seed, which had previously been conventionally assayed for the presence of *F. poae* by agar plate tests (Parry & Nicholson, 1996). The PCR assay detected the presence of *F. poae* where conventional isolation, at the time of harvest, had indicated that less than 1% of seed was contaminated (Riband A and B). Competitive PCR indicated that the level of *F. poae* in both these samples was very low (<0.001), being below the level for which reliable quantification was possible.

High levels of *F. poae* were detected in the 'CSL-poae' and 'NIAB-poae' samples, both of which had been found to contain a high percentage of contamination by this species at harvest. Significantly, *F. poae* was not isolated from 'CSL-poae' in the present work. There was a high degree of contamination by *Epicoccum nigrum* on the 'CSL-poae' sample and it is suggested that this fungus may have inhibited the isolation of *F. poae*. Sample '4/4343' had over 25% *Fusarium* contamination at harvest and the quantitative PCR results indicate that this may have been due to a combination of *F. poae* and *F. culmorum*. In contrast, the contamination of samples 'Riband B' and 'HAAC' appeared to have been predominantly due to a single species in each case, *F. avenaceum* and *F. culmorum* respectively.

In our laboratory we have developed specific PCR assays for nine species/varieties involved in the 'stem-base' and/or 'ear blight' complexes. These assays will prove to be a valuable tool in epidemiological studies, providing a rapid and reliable means of detecting and quantifying pathogens in cereal tissues, and other plants, throughout the season. This may lead to an improved understanding of the significance of individual fungal species, pathotypes and varieties in the 'stem-base' and 'ear blight' disease complexes and their

impact on yield. The techniques may also be used for seed testing and quarantine purposes.

The combination of specificity and sensitivity of these techniques allows precise detection and identification of fungi present in crops very early in the season. This is useful for providing early warning of a particular disease so that correct action can be taken to reduce the impact of disease. This technique also provides a way of evaluating the effect of factors such as crop variety, fungicide treatment and environment on each of the fungi involved in a disease complex. Using this knowledge, it may be possible to develop strategies to ensure that all the fungal species are effectively controlled and that control of one fungus does not merely lead to it being replaced by another.

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TOWARDS THE PREDICTION OF FUSARIUM EAR BLIGHT EPIDEMICS IN THE UK - THE ROLE OF HUMIDITY IN DISEASE DEVELOPMENT

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ABSTRACT

Examination of historical data from the CSL/ADAS national wheat disease survey for the years 1987 to 1995 indicated a relationship between rainfall during anthesis and the incidence of fusarium ear blight. To investigate this further, small scale field experiments were carried out to determine the effect of increasing relative humidity on the development of the five major species of *Fusarium* known to cause ear blight in the UK. A mist irrigation system, controlled by an in-plot relative humidity sensor, was used to manipulate relative humidity levels in plots artificially inoculated with single species of either *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* or *Microdochium* (formerly *Fusarium*) *nivale*. Visual and cultural monitoring, both pre- and post-inoculation, indicated dry, cool spring conditions favoured natural stem-base infection by *M. nivale*, and that the degree of ear infection by all five inoculated species was related to relative humidity, with evidence of differing optima. Yield data indicated that losses occurred even under conditions of low relative humidity, and that *F. culmorum* and *F. graminearum* were the most aggressive pathogens, reducing thousand grain weight by 25% and 20% respectively under conditions of high relative humidity.

INTRODUCTION

The annual winter wheat disease survey carried out at the Central Science Laboratory in collaboration with ADAS, has shown the incidence of fusarium ear blight (FEB) to be sporadic, being high in some seasons, particularly 1982, 1992, and 1993, but low in others.

Several *Fusarium* species have been associated with ear blight of wheat. However, those most commonly recorded are *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *Microdochium nivale*. Although there are limited field data regarding the development of the disease it seems that a dry spring, allowing the development of inoculum (Parry *et al.*, 1994), followed by warm, wet, humid conditions around anthesis (Inglis & Cook, 1981; Snijders, 1990) are likely to favour infection.

This paper reports on the analysis of historical disease incidence data, obtained from the CSL/ADAS wheat disease survey, to examine the relationship between disease development and rainfall, and the results of field experiments to investigate the effect of variation of relative humidity on the development of disease on the ear.

MATERIALS AND METHODS

Analysis of historical data - effect of rainfall on disease development

Historical data from the CSL/ADAS winter wheat disease survey was analysed to determine the incidence of fusarium ear blight (expressed as percentage plants affected) in England and Wales for the years 1987 to 1995. Rainfall data, for the corresponding area, for the periods March-May (spring) and the period around anthesis (June-July), obtained from the Meteorological Office, were plotted against disease incidence from the corresponding years.

Field experiment on the effect of humidity on disease development

Two plots (12m x 9m) were drilled with winter wheat cv. Avalon (a variety susceptible to fusarium ear blight) on 4 October 1994. Each plot was divided into four replicate blocks, these were further divided into six micro-plots (1m x 1.5m). Meteorological data were collected from drilling until the end of harvest using in-crop sensors at ear height and recorded by a datalogger.

Plants were inoculated at early to mid-anthesis (GS62). Five micro-plots per replicate block were sprayed with a 200 ml suspension containing 10^5 conidia per ml of either *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* or *M. nivale*. An equivalent amount of water was sprayed onto the sixth micro-plot as a control. Two relative humidity regimes were implemented immediately following inoculation, (i) ambient and (ii) high: where in crop air humidity (at ear height) was maintained at greater than 80% using a mist irrigation system switched via an in crop humidity sensor. This resulted in ears being maintained at constant wetness. The difference between ambient and high plot humidity was maintained until caryopsis watery ripe (GS70).

Disease development was monitored by isolation from lesions on the stem-base at first node detectable (GS31), flag leaf ligule just visible (GS39), medium milk (GS75) and soft dough (GS85) and at the latter two stages from lesions on the ear. Yield loss data was generated from single ear analysis of ear grain number and weight in the form of thousand grain weights.

RESULTS

Analysis of historical data - effect of rainfall on disease development

The rainfall, calculated as a percentage of the average, during spring and around flowering were both important in the development of fusarium ear blight with that occurring around flowering appearing to be of greater importance (Figure 1).

Results from 1990 and 1992, when ear blight incidence was, respectively, lower and higher than expected , suggest other factors may also be important in disease development.

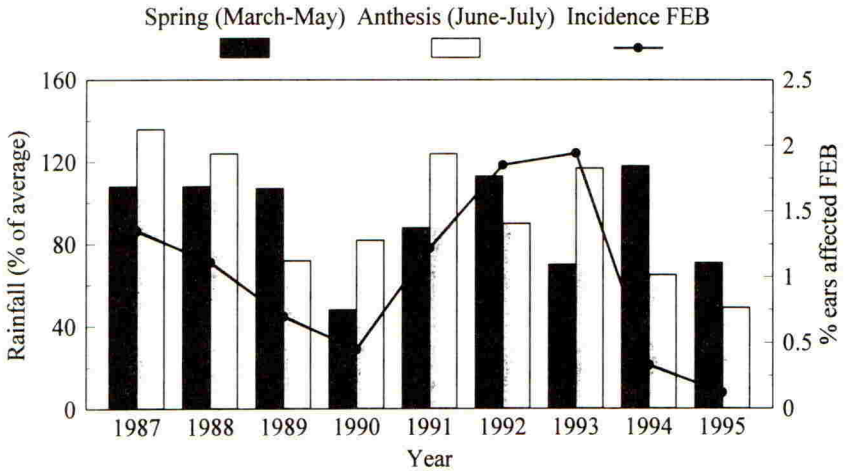

Figure 1. Relationship between rainfall during spring, rainfall during anthesis and the incidence of fusarium ear blight (FEB)

Field experiment on the effect of humidity on disease development

The incidence of natural infections of *Fusarium* species on the stem-base at GS 31, 39, 75 and 85 in experimental plots is shown in Figure 2. A high level of stem-base infection was seen throughout the season, with 30% and 10% stems affected at GS31 on experimental plots prior to treatment, increasing to more than 50% at the subsequent growth stages. *M. nivale*, the dominant species isolated at all stages, was present on 100% of infected stem-bases up to GS75 and on 90% of infected stems at GS85.

The effect of increased humidity and ear surface wetness on the frequency of isolation of *Fusarium* species from the ear at GS85 is shown in Figure 3. Differences in the levels of *Fusarium* species isolated were found to be dependent upon humidity and the species inoculated. Natural infection caused by *F. poae* and *M. nivale* was also present (Figure 3). *F. poae* was isolated from all micro-plots at both ambient and high humidity, with higher levels present under ambient conditions. *M. nivale* was isolated from all micro-plots at high humidity, but from none of the micro-plots under ambient conditions; the widespread infection raised the possibility of natural infection by both species. *F. avenaceum*, *F. culmorum* and *F. graminearum* were primarily isolated from micro-plots to which they had been inoculated, with earlier and increased levels of infection resulting from the high humidity plot. At high humidity, recovery of *M. nivale* and *F. poae* from natural infection was reduced in micro-plots where *F. avenaceum*, *F. culmorum* or *F. graminearum* had been inoculated previously (Figure 3).

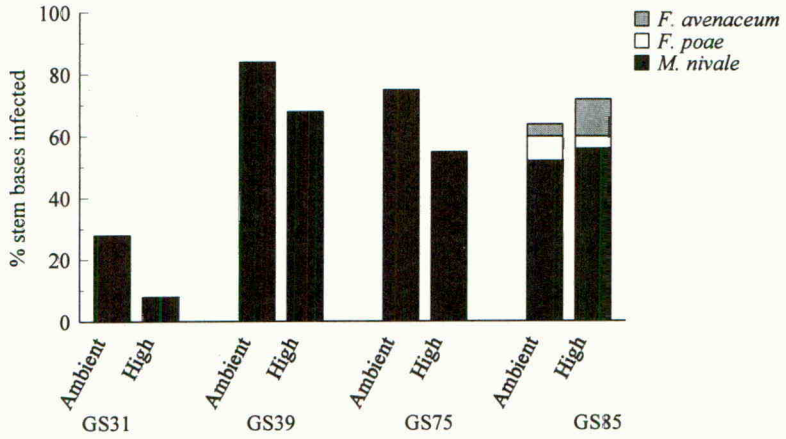

Figure 2. Incidence of *Fusarium* species isolated from stem-base lesions at growth stages 31, 39, 75 and 85.

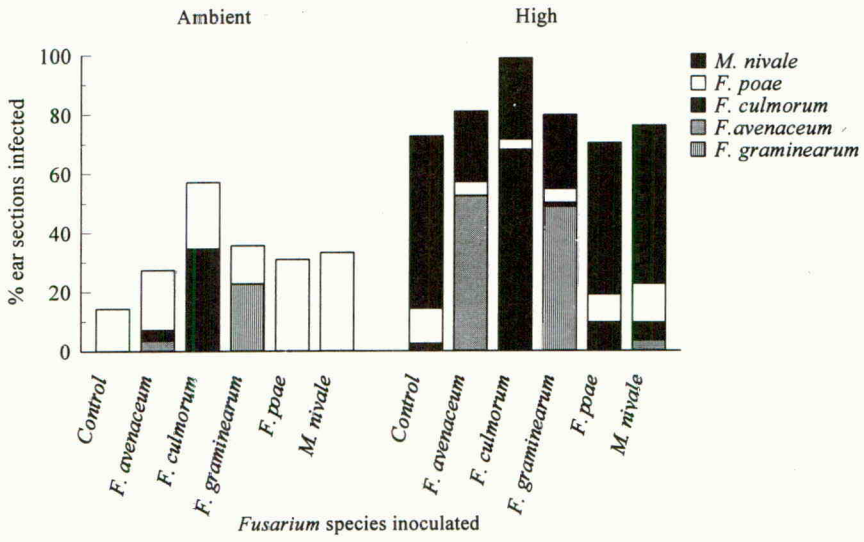

Figure 3. Effect of increased humidity and *Fusarium* species inoculation on *Fusarium* species isolated from ear lesions at GS85

The effect of treatment on yield is shown in Figure 4. Significant reductions in thousand grain weight occurred on all inoculated micro-plots under ambient conditions, with *F. culmorum* causing a significantly greater loss than the other treatments. When compared to the ambient, all treatments under high humidity produced highly significant yield losses ($p=0.001$); however, only *F. graminearum* and *F. culmorum* produced losses that were

significantly greater than those of the high humidity control. The losses on the high humidity control micro-plot were associated with natural infection by *M. nivale* on the ear.

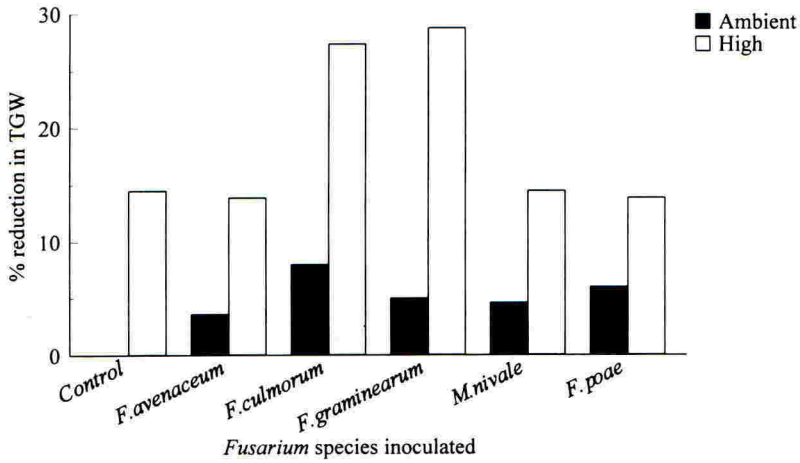

Figure 4. The effect of humidity and *Fusarium* species inoculation on thousand grain

DISCUSSION

Historical data analyses confirmed the role of rainfall in the development of fusarium ear blight. Rainfall during anthesis was the most important factor with drier than average springs enhancing this effect. Rainfall during anthesis is important not only in promoting spore dispersal but also in subsequent infection and disease development from associated elevated humidities. However, there is clearly an initial requirement for adequate levels of inoculum, the development of which appears to be enhanced in dry springs.

The important role of humidity during infection and disease development has been clearly illustrated by the experimental results. Increasing humidity produced earlier development and increased incidence of disease for all species inoculated, except for *F. poae*. The most aggressive of the ear blight pathogens, producing the highest disease levels and yield loss, were *F. culmorum* and *F. graminearum*. *F. graminearum* is currently thought to be of minor importance in the UK, but data suggest that under favourable conditions this species could cause losses similar to those caused by *F. culmorum*.

The widespread development of *M. nivale* on the stem-base was consistent with the cool, dry weather recorded during the spring of 1995. Heavy rainfall which occurred during anthesis may have transferred inoculum to the ear, accounting for the widespread ear infection by *M. nivale* on control plots maintained at high humidity. Under ambient conditions, natural ear infection by *M. nivale* was not observed. This may suggest a humidity threshold level below which *M. nivale* will not infect and develop on the ear. In

contrast to *M. nivale*, natural infection by *F. poae* occurred at both humidity levels. The absence of *F. poae* on the stem-base, prior to anthesis, makes the source of inoculum less clear than that for *M. nivale*. Data suggest that *M. nivale* and *F. poae* require similar conditions for inoculum development. However, they differ in their humidity requirements for ear infection.

Reductions in thousand grain weight on control plots under high humidity appeared to result primarily from natural infection by *M. nivale*, which under favourable conditions produced yield losses of up to 12%, a figure similar to that obtained by Hani (1981). Losses caused by *F. culmorum* and *F. graminearum* were significantly higher at 25 and 20% respectively, comparing favourably with the work of Saur (1991) and Wong *et al.* (1992). Significantly reduced levels of natural infection by *M. nivale* on plots inoculated with *F. culmorum*, *F. avenaceum* and *F. graminearum* indicate that the latter species are more competitive than *M. nivale* under favourable conditions for infection.

The importance of rainfall in the development of fusarium ear blight indicated by the historical data is supported by experimental results. Other factors such as temperature, cultivar and relative crop development are also likely to be important and these will be further investigated to elucidate apparent anomalous years such as 1992 when high disease levels resulted despite a wet spring and little rainfall during anthesis. Further work is already in progress to generate the additional data necessary to construct a preliminary forecasting system for prediction of ear blight epidemics in the UK.

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PREDICTING RISK OF SEVERE LIGHT LEAF SPOT (*PYRENOPEZIZA BRASSICAE*) ON WINTER OILSEED RAPE IN THE UK

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ABSTRACT

Survey data indicate that seasonal patterns in use of fungicides against light leaf spot do not relate well to the seasonal variation in disease severity. A provisional scheme for forecasting light leaf spot severity has been constructed, with three components. 1. A seasonal risk index with two stages; prediction of % crops with light leaf spot on the leaves in March from survey data collected in the previous July and prediction of the % crops with light leaf spot on pods in July from the March survey data. 2. An initial crop risk index at the beginning of the season in October, which depends on cultivar susceptibility, sowing date and regional climate. 3. An improved crop risk index at monthly intervals from October to March, to be based on disease assessments. A suggested protocol for assessment of light leaf spot in crops combines regular inspection of transects across crops with assessment of symptoms on plants sampled from the crops.

INTRODUCTION

Light leaf spot (*Pyrenopeziza brassicae*) is a serious disease of winter oilseed rape in the UK, causing losses estimated to cost > £30M per annum by calculation from severity/yield loss relationships (Sansford *et al.*, 1996), despite expenditure of > £5M per annum on fungicides to control the disease. ADAS/CSL oilseed rape survey data suggest that the severity of light leaf spot epidemics, as indicated by the incidence (%) of plants with infected leaves in March or with infected pods in July, differs between seasons and that patterns in fungicide use against the disease do not relate well to this seasonal variation in disease severity (Fig. 1).

To optimize use of fungicides for control of light leaf spot, avoiding unnecessary applications to crops which do not need them and improving the accuracy of timing to crops which warrant treatment, it is necessary to develop a scheme for forecasting the severity of light leaf spot epidemics (Fitt *et al.*, 1994; Gladders *et al.*, 1995). The objective has been to construct a provisional forecasting scheme. This has three components:-

1. A seasonal risk index to identify high risk seasons.
2. An initial crop risk index to identify high risk crops at the start of the season.
3. An improved crop risk index, based on disease assessments at monthly intervals.

This paper reports work to develop a provisional scheme to forecast the incidence of plants with light leaf spot on leaves in March (an indication of the severity of winter light leaf spot epidemics) or on pods in July (an indication of disease severity at the end of the season).

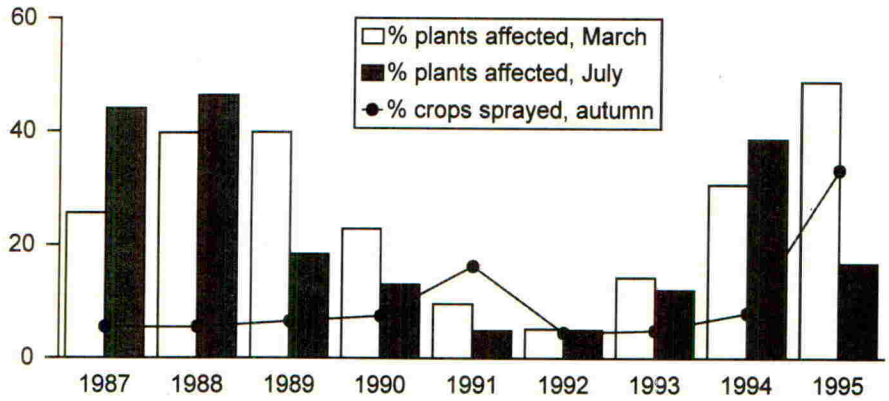

Figure 1. Incidence (%) of plants affected with light leaf spot on leaves in March or on pods in July and fungicide use (% crops sprayed in autumn) in each season (data from oilseed rape disease surveys for England and Wales, 1987-1995).

MATERIALS AND METHODS

Seasonal risk index

Data from winter oilseed rape disease surveys in England and Wales (harvest years 1977-1986, eastern England only; 1987-1995, all six ADAS regions) and Scotland (1993-1995) were used to estimate seasonal and initial crop risk indices. Samples of plants were taken from commercial crops at early stem extension (GS 2.1-2.5) in March and at maturity (GS 6.4) in July and assessed for light leaf spot. To estimate a seasonal risk index, step-wise regression techniques were used to analyse the relationships between incidence (% crops affected) of light leaf spot on leaves in March or on pods in July and factors such as previous disease incidence on pods, stems or leaves, monthly rainfall and temperature, alone or in combination, using data for eastern England for 1977-1995. These analyses were used to predict incidence of light leaf spot (% crops with disease) in eastern England. Then they were used to develop a seasonal index for risk of light leaf spot for England and Wales using survey data for 1987-1995.

Initial crop risk index

Data from the winter oilseed rape disease survey for England and Wales for the harvest years 1987-1995 (e.g. Hardwick & Turner, 1995) were used to estimate the influence of proximity to a previous season's crop, sowing date, cultivar resistance and regional climate on incidence (% plants affected) of light leaf spot on leaves in March or on pods in July. These analyses were used to estimate initial crop risk indices (0-100% scale) for indicating in October the risks that light leaf spot will develop on leaves by March or on pods by the following July.

Improved crop risk index

Experiments were done to develop a protocol for sampling crops and assessing light leaf spot

incidence to produce monthly improved crop risk indices during the period October - March. To investigate the spatial variability of the disease (% leaves with light leaf spot on each plant), on 14 February 1996 a structured sample of 360 plants in total was taken from different areas in eight unsprayed plots of a field experiment on winter oilseed rape (cv. Envol). To determine the optimum temperatures at which to incubate samples from crops for enhancing symptom development, four experiments were done. In experiments 1 and 2 (starting on 4 and 11 March 1996) five groups of 10 plants each, sampled from the field experiment, were incubated in polyethylene bags at 2, 5, 10 15 or 20°C and the % of leaves with light leaf spot was recorded on each plant 1, 2, 3, 4 and 6 days after sampling. In experiments 3 and 4 (starting on 22 March and 7 April 1996) five groups of 10 plants each, were grown in the glasshouse, exposed in the infected crop for 2 weeks, incubated in the glasshouse for 1 week, and then incubated at 2, 5, 10, 15 or 20°C before disease assessment.

RESULTS

Over the period 1977-1995, >30 % of the 61 regions (x seasons) had >80 % of crops affected with light leaf spot in both March and July (Fig. 2a). By contrast, only 10% of regions (x seasons) had <20 % of crops affected in March. More than 60 % of the 1021 crops had <20 % of plants with light leaf spot and only 10 % had >80 % of plants affected in March and July (Fig. 2b). These data were used to divide epidemics into categories (slight, moderate, severe) for developing the seasonal risk and crop risk indices.

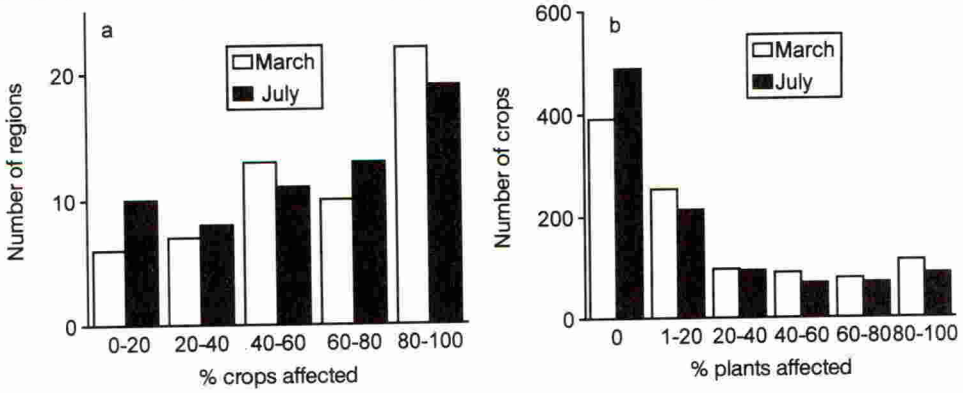

Figure 2. Frequency of light leaf spot epidemics with different incidences of disease on leaves in March or on pods in July: a) numbers of regions (x seasons) in relation to % crops affected; b) numbers of crops in relation to % plants affected. Data from oilseed rape disease surveys for England and Wales (1977-1995) (a,b) and Scotland (1993-1995) (a).

Seasonal risk index

Regression analyses on winter oilseed rape survey data for eastern England indicated that it was best to predict the % crops with light leaf spot on pods in July in two stages: predict % crops with light leaf spot on leaves in March from survey data for the previous July (% crops with light leaf spot on pods) (43.8% variance accounted for); predict % crops with light leaf spot on pods from disease incidence in March and rainfall in May (48.2% variance accounted for). In autumn or spring, respectively, the best predictors of risk for subsequent spring or summer disease incidence (% crops affected) were the % crops affected in the previous July or % crops affected in March. Tabulation of 1987-1995 survey data for England and Wales

then shows the long-term risk of a slight, moderate or severe epidemic (Table 1).

Table 1. Seasonal risk: prediction of light leaf spot incidence (% crops with leaves affected in March or pods affected in July) using survey data for ADAS regions in England and Wales, 1987-1995.

% crops in region with light leaf spot in previous July	Expected risk (%) of y% crops in region affected in March			
	y=0-30%	y=30-60%	y>60%	Number of regions
0-30%	63.6	27.3	9.1	22
30-60%	46.7	33.3	20.0	15
>60%	22.2	22.2	55.6	9

% crops in region with light leaf spot in March	Expected risk (%) of y% crops in region affected in July			
	y=0-30%	y=30-60%	y>60%	Number of regions
0-30%	60.0	24.0	16.0	25
30-60%	28.6	50.0	21.4	14
>60%	36.4	36.4	27.3	11

Initial crop risk index

There was evidence from England and Wales survey data that early sowing and cultivar susceptibility both increased incidence of light leaf spot, which was greatest in the northern (mean annual rainfall 64.4 mm) and south-western (65.1 mm) regions and smallest in the south-east (59.6 mm), but proximity to previous oilseed rape crops did not. However, analysis of % plants affected in spring with respect to cultivar resistance rating, sowing date and regional differences accounted for only 18.5% of the variation in the data. Predicted disease incidences for an August sowing date are given in Table 2.

Table 2. Predicted incidence of light leaf spot (% plants affected) on leaves in March (± standard error) for an August sowing date, classified by region and NIAB cultivar resistance rating, using survey data from England and Wales, 1987-1995.

Region	Light leaf spot resistance rating			
	<5	5-6	6-7	>7
East	37±5.1	23±4.2	23±2.5	13±3.2
Midlands & West	65±7.6	37±8.1	33±3.4	24±5.0
North	57±4.6	58±5.9	44±3.2	27±8.1
South East	8±10.1	15±7.8	22±3.9	14±5.6
South West	66±10.1	16±10.1	21±6.3	6±10.7
Wales	28±21.4	no data	58±8.4	30±10.8

Sowing dates of 1-14 September or after 14 September decreased predicted incidence by 8 or 12 %, respectively. These predictions give an autumn risk factor used to produce an initial crop risk index for predicting light leaf spot incidence (% plants affected) on leaves in March

(Table 3). For individual crops, analysis of effects of these factors on disease incidence on pods in July accounted for only 9.2 % of the variation in the data and gave little indication of risk.

Table 3. Relationship between initial crop risk (predicted from sowing date, cultivar susceptibility, ADAS region) and incidence (% plants affected) of light leaf spot on leaves in March, using data from oilseed rape disease surveys for England and Wales, 1987-1995.

Initial crop risk	Expected risk (%) of y% plants infected with light leaf spot in March				
	y=0-10%	y=10-25%	y=25-50%	y>50%	Number of crops
0-10%	81.2	11.6	2.9	4.3	69
10-25%	60.3	17.6	7.4	14.7	484
25-50%	34.6	9.3	17.5	38.6	246
>50%	19.2	13.7	6.8	60.3	73

Improved crop risk index

Analyses of data on the spatial distribution of light leaf spot suggested that the disease occurred initially in small patches (smaller than the plot size of 3 x 20 m); visual inspection of crops confirmed this suggestion, with patches of diameter < 1m frequently observed. In all four experiments on incubation temperature, the incidence of light leaf spot (% leaves infected) increased with incubation time at all temperatures, whilst maximum disease incidence differed between experiments. The rate of development of symptoms and the maximum disease incidence were generally greatest at 15°C (Fig. 3) but initial differences between temperatures were less in experiments 2 and 4 than in experiments 1 and 3.

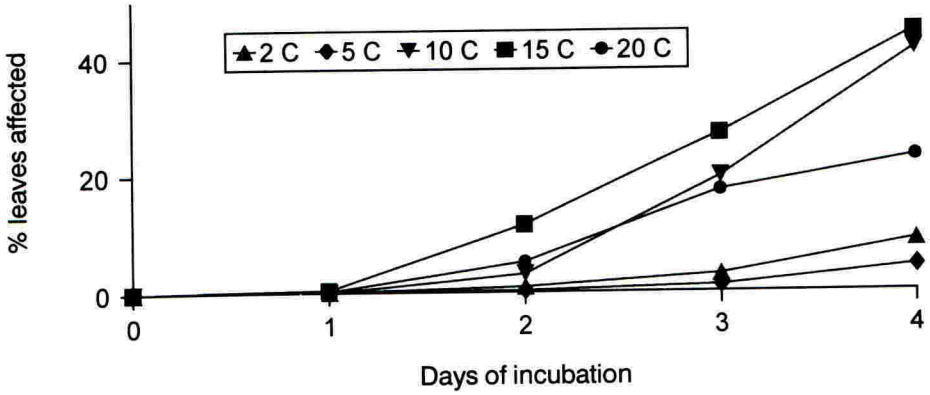

Figure 3. Development of light leaf spot symptoms (% leaves affected) on winter oilseed rape plants sampled from an unsprayed crop and incubated at temperatures of 2, 5, 10, 15 or 20°C (experiment 1).

These data, together with existing information, were used to develop a provisional protocol for disease assessment to confirm the presence of disease in crops predicted to be at risk. To assess the incidence of light leaf spot in a crop accurately, a combination of crop walking and incubation of samples before disease assessment is recommended, because the disease distribution is initially patchy and accurate diagnosis is difficult without incubation. The

procedure that farmers/consultants should consider provisionally is:

(i) inspect crops at monthly intervals between October and March, looking for patches of plants with light leaf spot.

(ii) collect 100 plants in a diagonal transect across the crop.

(iii) incubate plants (which should be reasonably dry) in polyethylene bags at 10-15°C (for example in a closed barn) for 4-5 days and assess the incidence of light leaf spot to confirm the presence of disease.

DISCUSSION

This work provides provisional risk indices for assessing seasons with risk and crops at risk of developing a high incidence of light leaf spot. However, considerable further work is required to develop risk indices for predicting light leaf spot severity and potential yield loss, as well as incidence. Furthermore, the initial crop risk indices might be improved by including other factors (e.g. previous cropping). Methods for producing the improved crop risk indices at monthly intervals from October to March still need to be developed; relationships between disease incidence and yield loss need to be established more clearly so that threshold incidences of disease can be determined for each month. Furthermore, the indices could be modified to include factors such as the occurrence of infection periods (Fitt *et al.*, 1994) and the use of fungicides on crops, besides incorporating data on the occurrence of the disease from the winter oilseed rape disease surveys in autumn and from inspection and sampling of individual crops.

ACKNOWLEDGEMENTS

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OCCURENCE OF CLUBROOT (*PLASMODIOPHORA BRASSCAE*) IN SPRING OILSEED RAPE; ESTIMATION AND PREDICTION OF CROP LOSS

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ABSTRACT

Clubroot was detected in 148 (78%) out of 190 fields assayed for soil-borne inoculum with a bioassay method in a Swedish oilseed growing area. Subsequent assays of fields where no *Brassica* crops were grown in the following years indicated a significant decrease in disease incidence. Using the rate of decline observed, it was estimated that the level of soil infestation declines to below the detection level after a period of 17 years. The half-life of the spore inoculum was estimated as 3.6 years.

In field experiments where increasing amounts of infested soil were supplied as inoculum and distributed prior to planting, relationships between yield and disease incidence and between yield and soil infestation measured by the bioassay were observed. A good relationship between disease incidence and degree of infestation according to the bioassay was also observed. Results from a field experiment site naturally contaminated with *Plasmodiophora brassicae* showed that the highest level of infestation (90% plants infected plants) decreased yield by 50%. At lower levels of infestation (<20% plants infected), yield decreases were estimated as 300 kg ha^{-1}.

INTRODUCTION

Clubroot, caused by *Plasmodiophora brassicae*, is an important disease in Swedish cruciferous crops, and in certain areas limits successful oilseed production. Following the introduction of oilseed rape in Sweden in the early 1940 s, it has become a valuable component in crop rotations. Thus, during the 1970 s rotations with an oilseed rape crop every fourth year were encouraged. In the early 1980 s severe attacks of clubroot were observed to decrease yields, (Wallenhammar, 1986).

This paper reports work started in 1986, on the occurence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels (Wallenhammar 1996*a*), yield loss from clubroot infections in spring oilseed rape and strategies for control of clubroot (Wallenhammar,1996*b*).

MATERIALS AND METHODS

Soil sampling and bioassay method

Soil samples were taken from 190 fields on 18 farms in the county of Örebro, with a total acreage of about 1800 hectares. The fields were sampled diagonally with a soil auger (type "Trekanten") during May and June.

The level of *P. brassicae* contamination of the soil samples was investigated by a modified bioassay method (Colhoun, 1957; Clarkson & Brokenshire, 1984) assessing the percentage of bait plants affected. The universally susceptible Chinese cabbage (*Brassica campestris* spp. *pekinensis*) cultivar Granaat (Buczacki *et al.*, 1975) was used as bait plant and planted pots were kept in a greenhouse at a temperature of c. 21°C (min temperature 18°C), with 16-18 hours of daylight. Daily watering to bring soil to field-capacity in the early stages of each experiment was followed by normal watering during the last two weeks of the experiment. The plants were assessed after 5-6 weeks and divided into two categories: (a) healthy plants and (b) plants showing macroscopically visible symptoms.

Assessment of infection in a field experiment with added soil-borne inoculum

A biennial field experiment, in which infested soil was added to give soil inoculum, was done in 1993-94 west of Örebro. Increasing amounts of infested soil were distributed prior to planting. The experiment was arranged in a randomized block design with four replications.

The plots were sampled and the infection capacity of the soil was measured by assessing the percentage of bait plants infected. A final evaluation of clubroot infection was done after harvest in the stubble. Plants were assessed as healthy or infected with clubbed roots.

Assessment of infection in a field experiment with existing soil-borne inoculum

In a field experiment in 1995 in spring oilseed rape, located east of Västerås, severe attacks of clubroot were observed. Assessments of disease severity were done in the stubble after harvest.

RESULTS

Occurrence of clubroot in fields surveyed

The average degree of infestation according to the bioassay and average pH-values for the soils for each farm are given in Figure 1.

Relationship between degree of infestation and field conditions

There was a direct relationship between the level of infestation in the test and the frequency of oilseed rape crops in the field (Figure 2). The greatest level of infestation (82%) was observed on fields where oilseed rape had been grown five times during the period 1969-85. pH-values ranged between 4.9 and 7.5 with an average value of 6.05 in the 1986-87 survey.

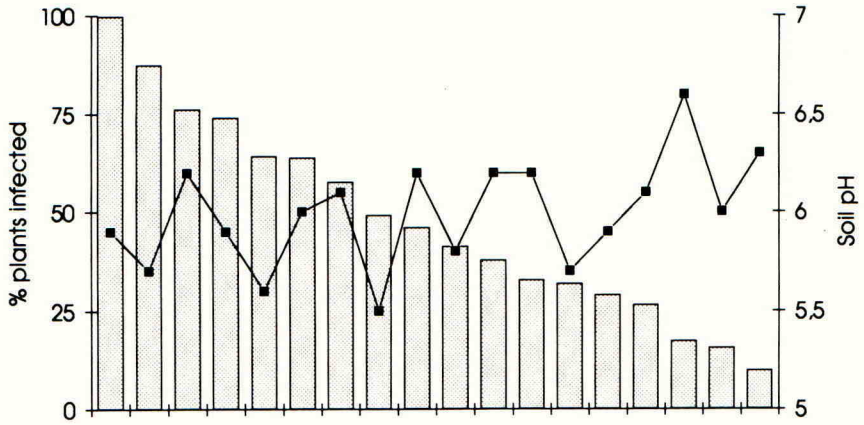

Figure 1. Average infestation (% plants infected) according to the bioassay (bars) and average soil pH for each farm investigated in the 1986-87 survey.

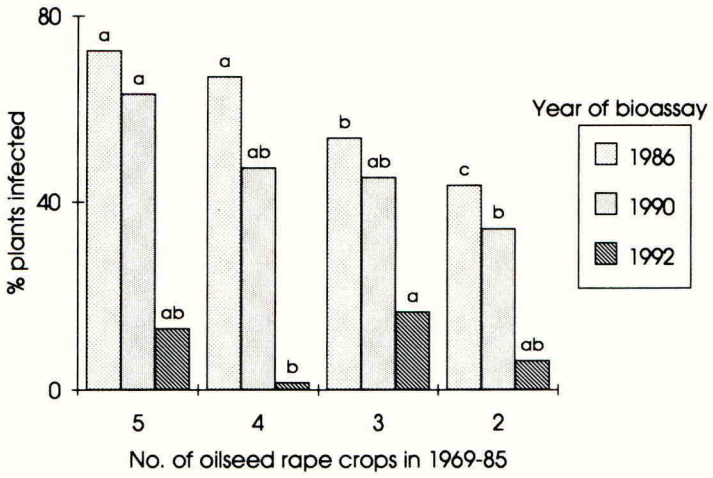

Figure 2. Relationship between the percentage of plants diseased in the bioassay and oilseed rape cropping frequency. Letters above the bars show significance according to Duncan's multiple range test ($P < 0.05$).

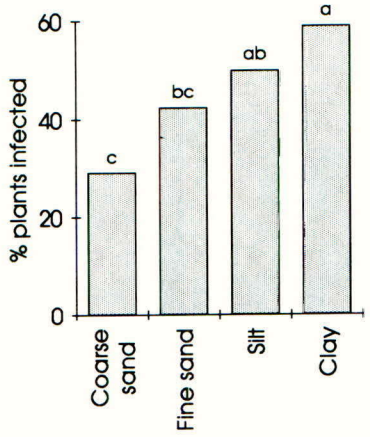

Figure 3. Relationship between the percentage of plants diseased in the bioassay and soil type. Letters above the bars show significance according to Duncan's multiple range test ($P< 0.05$).

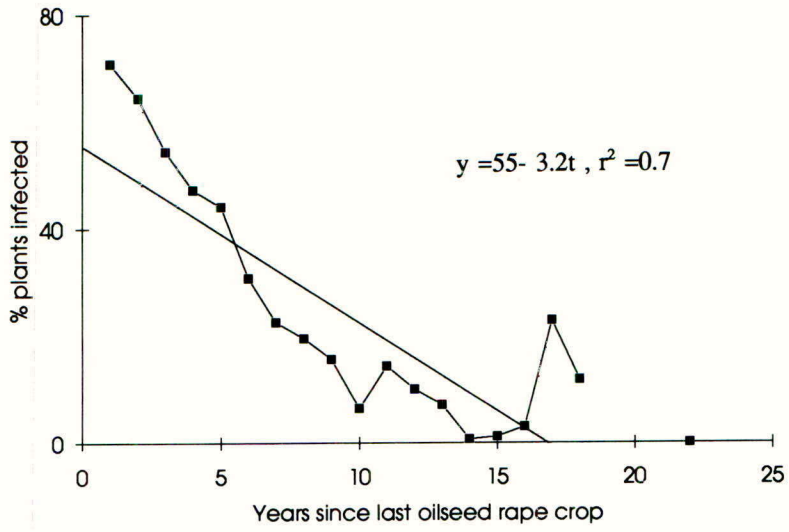

Figure 4. Percentage of plants diseased in the bioassay in relation to years since the last oilseed rape crop was grown in the field investigated.

High levels of infestation according to the bioassay were recorded for a wide range of pH-values (5.2-6.6). The average level of infestation in relation to soil type is given in Figure 3. The largest average infestation according to the bioassay was recorded at the lowest content of organic matter. Comparing the soils with respect to clay content, the disease was most severe on loams (58%), while soils without clay showed the lowest level of infestation (Wallenhammar, 1996*a*).

Variation in infestation with time

Subsequent assays of fields where no further *Brassica* crops were grown in the following years showed the relationship between degree of infestation (y) and time (t) since the most recent oilseed rape crop y= 55-3.18t (R^2= 0.70, Figure 4). This indicates that, in a severly infested soil, the level of infestation declines to below the detection level after 17 years. The half-life of the spore inoculum was estimated as 3.6 years (Wallenhammar, 1996*a*).

Field experiments with added soil-borne inoculum

The classification of plants in the field experiment of 1994 shows that clubroot has been efficiently distributed from infested plots to plots where no disease was expected. In the control treatment 53.8% of the plants were infected. A good relationship between yield (y) and disease incidence (d) y= 2956- 31.6d (R^2= 0.90) was observed and the relationship between yield (y) and degree of infestation according to bioassay (d) was y= 3009- 27d (R^2= 0.94). The relationship between disease incidence (y) and degree of infestation according to bioassay (d) was y=1.88+ 0.78d (R^2= 0.87).

Field experiment with natural soil-borne inoculum

A threshold level for soil-borne inoculum can be defined. At a low disease incidence (<20% plants infected) the yield decrease is small (about 300 kg ha^{-1}), while yield was decreased by 1.5 t ha^{-1} (50%) at the highest disease incidence (90% plants infected).

CONCLUSIONS

A high infestation level with *P. brassicae* was observed in the area surveyed (Wallenhammar 1996*b*), and 148 of the fields investigated (78%) showed infestation (Wallenhammar,1996*a*). Clubroot is a difficult plant disease to combat. The longevity of *P. brassicae* in soils is reported to be seven years by several workers (Gibbs, 1939) and the pathogen was detected by Buczacki *et al.* (1975) in soil samples after eighteen years of non-susceptible grass following an outbreak. These results demonstrate that, in a highly infested soil, the degree of infestation declined to below the detection level after 17.3 years. The half-life of the spore inoculum was estimated as 3.6 years (Wallenhammar,1996*a*). Furthermore, evidence that the pathogen may rapidly spread between fields is proposed by the high disease incidence in fields cropped with only two oilseed rape crops during 1969-85 (Figure 2) and by the 1994 field experiment where plots with no inoculum were severely contaminated after the seedbed preparation.

The economic loss to a crop is dependent on many factors influencing disease development

(e.g. soil moisture content, soil temperature, soil pH and soil type), but the most important factors determining the size of yield loss are time of infection and the inoculum density in the soil. The dissemination of the disease in oilseed rape growing districts is probably underestimated and not observed by the grower.

No chemical treatments are available against clubroot for use in field crops, and breeding of resistant cultivars is complicated because field populations of *P. brassicae* contain a mixture of pathotypes (Jones *et al.*, 1982). Therefore, the present strategy for controlling clubroot is to use advisory testing using soil bioassays to avoid growing oilseeds in contaminated soils and to decrease the amount of resting spores in the soil. Although, low levels of disease do not seem to affect yield significantly, cropping with oilseed rape has to be avoided when inoculum is detected, in order to prevent multiplication and further dissemination of the pathogen.

ACKNOWLEDGEMENT

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TRIAZOLE MIXTURES AND THE CONTROL OF WHEAT LEAF BLOTCH (*SEPTORIA TRITICI*)

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ABSTRACT

Effects of cyproconazole and flusilazole mixtures on growth and disease development of *Septoria tritici* have been examined in a series of laboratory, controlled environment and field experiments. Interactions between these mixture partners were evaluated from dose-response curves using the Shifted Multiplicative Model rather than the Colby equation, which does not attach significance to interactions. Synergy was not observed and antagonism between mixture partners was observed only in laboratory experiments. Both *in vitro* and on seedlings grown under controlled environment conditions, poorer performance of mixtures was not always caused by antagonism but, instead, reflected the phenomenon of "low dose enhancement", which is independent of any interaction between the partners. "Low dose enhancement" was not observed in field experiments over two years and the cyproconazole-flusilazole mixture performed as well as the single products. Although "low dose enhancement" was not evident in these field experiments, its existence in laboratory and controlled environment studies complicates the evaluation of synergy and antagonism between mixture partners.

INTRODUCTION

The chemical and biological diversity of fungicides that inhibit the 14α-demethylation step in sterol biosynthesis (DMIs) provide unique resources that can be combined in mixtures to improve disease control. Although several broad spectrum fungicides with new modes of action are becoming available, and may be used as a preventative measure aimed at reducing inoculum levels early in the season, DMIs will continue to play a role in late season cereal disease control. To maintain efficacy of older DMIs, mixtures with newer, more expensive azoles, can exploit differences in their disease control spectrum, mobility and curative and eradicant activity. In this way, improved disease control and, subsequently, yield potential can be achieved more cheaply than by newer azoles as single products.

Laboratory experiments have shown that both synergy and antagonism can occur between triazoles in mixtures, depending on the concentration ratio of the two components and the target pathogen (Kendall *et al.*, 1994). Although the sterol 14α-demethylase is the main target enzyme of all the DMIs, they undoubtedly have other target sites, including other steps in sterol biosynthesis. For some mixtures, complementation of this difference in modes of action may further improve disease control, enabling the use of lower dose rates.

The Septoria complex, comprising *Septoria tritici* (*Mycosphaerella graminicola*) and *Septoria nodorum* (*Leptosphaeria nodorum*), can cause significant yield losses in most

wheat-growing areas worldwide. Although resistant wheat varieties are available, emphasis over recent years has been on high-yielding, bread-making varieties, which are invariably susceptible to Septoria. Control of this disease complex, therefore, relies predominately on the use of fungicides and, in this respect, the triazoles have been particularly effective. In the present work, mixtures of cyproconazole and flusilazole, two triazoles likely to be used as major components of control measures for Septoria epidemics, were examined under laboratory, controlled environment and field conditions in order to identify whether the interactions, observed previously with other mixture combinations (Kendall et al. 1994), can be exploited to improve Septoria control.

MATERIALS AND METHODS

In vitro measurement of synergy/antagonism in *S. tritici*

Two *S. tritici* strains, *RL2* and *S27* differing in sensitivity to triazole fungicides, were grown in tubes (12mm diam) of a clear viscous medium of Czapek dox liquid medium (Oxoid CM95), plus 0.5% mycological peptone (Oxoid L40) and 0.4% purified agar (Oxoid L28), amended with all combinations of five doses of flusilazole and cyproconazole, in a three-fold dilution series. After incubation at 18°C for 10d, growth was assessed by resuspending cells and measuring the turbidity of each tube in triplicate (Ratio turbidimeter, Hach. model 18900, Camlab Instruments, Cambridge, UK.).

In vivo measurement of synergy/antagonism in *S. tritici*

Seedlings of winter wheat cv. Riband (five seedlings/pot) were grown in a controlled environment (20°C; 16h photoperiod) until the second leaf was fully expanded. Two pots were taken for uninoculated baseline measurements by immunodiagnosis (Joerger *et al*, 1992). The remainder were sprayed to run-off with a conidial suspension (2.5 x 10^6 conidia ml^{-1}) of *S. tritici* strain (*St16*). The suspension was dried and plants from three pots cut off at the stem-base and frozen for use in inoculated baseline measurements by immunodiagnosis. The remaining pots were incubated in a dew-chamber (17°C, 100% r.h. for 72h) and then allowed to dry. Three replicate pots were untreated or treated with either flusilazole (Sanction) at 80, 16, 3.2, 0.64 or 0.128μg ml^{-1} a.i., cyproconazole (Alto) at 40, 8, 1.6, 0.32, 0.064μg ml^{-1}a.i. (highest concentrations equivalent to 1/10 field rate) or combinations of the two fungicides at each concentration. After drying, the seedlings were incubated in a controlled environment (16°C, 90% r.h. 16h photoperiod) for 24 d. When symptoms appeared, seedlings were cut off at the stem-base and frozen. The *S. tritici* antigen was extracted from all inoculated and uninoculated seedlings and its concentration measured by immunodiagnosis.

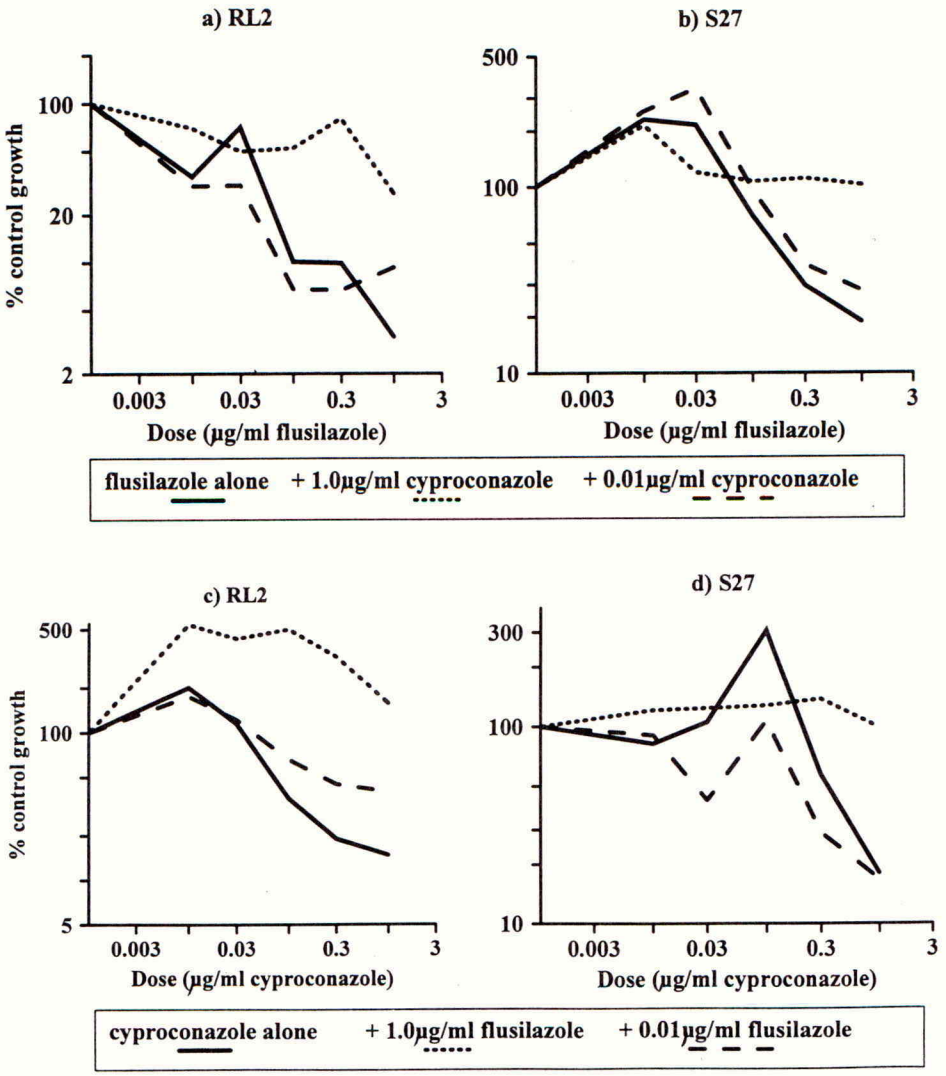
Field evaluation of triazole mixtures

In 1995 and 1996, field trials were set up to examine flusilazole (Sanction) and cyproconazole (Alto) as single active ingredients and in mixtures, for the control of *S. tritici* in winter wheat (cv. Riband). Each trial was set up as a randomised block design with three replicate plots (12 x 8m) per treatment. In both seasons, disease progress was monitored weekly on the top three leaves, both visually and by immunodiagnosis, until a predetermined spray threshold of 5 antigen units ml^{-1} of *S. tritici* was detected in leaf 3 by immunodiagnosis. In 1995, treatments were applied at growth stage (GS) 39 when this predetermined threshold was first reached (Table 2). Disease progress on the top

three leaves continued to be monitored every two weeks until harvest. In 1996, treatments were applied at GS41 (Table 2), and disease incidence monitored three weeks later and again at GS85.

RESULTS

Figure 1. Effects of mixtures of cyproconazole and flusilazole on the *in vitro* growth of *Septoria tritici* strains *RL2* (triazole sensitive) and *S27* (triazole resistant)



Measurement of synergy/antagonism in *S. tritici*

The ED_{50} values for each fungicide were calculated from the dose-response at a single concentration of the other partner. The basis of the analysis was independent and non-interactive action between the mixture partners; any deviation away from the ED_{50} value in the absence of the mixture partner indicated either synergy or antagonism (Brain & Davies, 1995). Synergy or antagonism were detected using the Shifted Multiplicative Model (SHMM) by fitting dose-response curves of one partner fungicide for each dose of the other using the equation :-

$$(1) \quad y = a + \frac{c}{1 + e^{b(\log(\text{dose}) - \log ED_{50})}}$$

where:- a = growth at the greatest fungicide concentrations
b = the response rate versus the increasing dose
c = growth in the untreated

At each fungicide dose, growth in the presence of just one mixture partner was normalised to 100. Unlike the more usual Colby equation (Colby, 1967), this analysis provides a measure of the significance of any interaction.

In vitro measurement of synergy/antagonism in *S. tritici*

The two Septoria strains each responded differently to mixtures of flusilazole and cyproconazole (Figure 1). After statistical analysis, "low dose enhancement" (Brain & Cousens, 1989) was evident in *RL2* treated with cyproconazole (Figure 1c) but not with flusilazole (Figure 1a). The addition of either flusilazole ($1.0\mu g\ ml^{-1}$) to the serial dilutions of cyproconazole, or cyproconazole ($1.0\mu g\ ml^{-1}$) to flusilazole decreased the sensitivity of strain *RL2* (Figures 1a & c), indicating antagonism. However, the addition of a low dose ($0.01\mu g\ ml^{-1}$) of either triazole to the other did not alter the sensitivity significantly or generate synergy. "Low dose enhancement" occurred in strain *S27* to both triazoles (Figures 1b & d) and, again, addition of flusilazole or cyproconazole (both at $1.0\mu g\ ml^{-1}$) to the other mixture partner caused antagonism but, there was no clear evidence for synergy.

In vivo measurement of synergy/antagonism in *S. tritici*

In this experiment, growth was measured as antigen units and analysed using equation 1. Although amounts of *S. tritici* antigen produced generally increased at all but the highest fungicide doses (Table 1), this was not caused by antagonism but, again, reflected "low dose enhancement". A similar analysis based on visual assessments produced the same conclusion (data not shown).

Field evaluation of triazole mixtures

In 1995, Septoria development was arrested midway through grain filling due to the unusually hot dry season. Treatments based on the immunodiagnostic threshold reduced Septoria antigen levels in the flag leaf and leaf 2 but not leaf 3, which was already infected when sprays were applied (Table 2). The mixture (flusilazole (0.3) / cyproconazole (0.2)) performed as well as either partner used alone.

In 1996, intense rain-splash events coinciding with flag leaf emergence moved inoculum from the base to the top of the crop. Consequently, the top three leaves were infected simultaneously with Septoria and the spray timing adopted was inaccurate for adequate protection of the top two leaves. Thus, none of the single a.i.s controlled Septoria (Table 2). However, the mixture (flusilazole (0.3) / cyproconazole (0.2)) gave better control and reduced antigen levels significantly, especially on the flag leaf. These observations correlate well with visual assessments. Unlike laboratory and controlled environment experiments, lower dose rates of cyproconazole did not enhance disease development.

Table 1. *In vivo* growth of *S. tritici*, assessed by immunodiagnosis, in wheat seedlings treated with mixtures of flusilazole and cyproconazole, expressed as a percentage of control growth.

Flusilazole (μg ml^{-1} a.i.)	Cyproconazole (μg ml^{-1} a.i.)					
	0	0.064	0.32	1.6	8.0	40.0
0	100	137.3	146.6	127.8	144.5	79.2
0.125	109.3	121.0	124.6	147.2	142.8	38.2
0.64	95.4	134.5	136.0	172.2	142.9	49.0
3.2	112.5	137.3	143.5	140.9	161.8	39.9
16.0	122.7	162.7	132.7	152.2	172.6	48.9
80.0	42.7	52.1	43.4	53.3	52.7	49.0

Table 2. Effects of fungicides, applied at GS39 (1995) or GS41 (1996) 0n amounts of *S. tritici* in wheat leaves as measured by immunodiagnosis

Treatment (l ha^{-1})	Antigen levels (Au ml^{-1})*					
	1995			1996		
	Flag leaf	Leaf 2	Leaf 3	Flag leaf	Leaf 2	Leaf 3
Untreated	122.8a	107.1a	93.2a	62.1a	38.5ab	37.0ab
Cyproconazole (0.8)$^{+}$	54.9b	74.4b	80.0a	38.3ab	50.3a	31.7b
Cyproconazole (0.6)	NT**	NT	NT	44.7ab	51.7a	30.5b
Cyproconazole (0.4)	NT	NT	NT	42.8ab	42.3ab	37.3ab
Cyproconazole (0.2)	NT	NT	NT	56.7ab	36.7ab	34.8ab
Flusilazole (0.4)$^{+}$	64.9b	87.5ab	86.1a	42.7ab	41.8ab	34.7ab
Flusilazole (0.3) + Cyproconazole (0.2)	77.4b	82.5ab	81.4a	32.8b	33.2ab	28.2b

* Figures in columns followed by the same letter are not significantly different (P = 0.05)

\+ Cyproconazole (0.8 litres ha^{-1} Alto) and Flusilazole (0.4 litres ha^{-1} Sanction) = recommended field rate

** NT = not tested

DISCUSSION

Despite the identification, *in vitro*, of synergy and antagonism between triazoles in mixtures, the same phenomenon was not observed in either controlled environment or field studies. Instead, the "low dose enhancement" frequently observed *in vitro* was highly significant in the controlled environment experiments, and could easily be confused with antagonism (or **resistance**), even though it involves no interaction between the mixture partners. However, there was no evidence that this occurred in field plots treated with low doses of cyproconazole or indeed flusilazole (data not shown). Leaves produced under controlled environment conditions are more susceptible to pathogen infection than field-grown plants. The absence of any "low dose enhancement" under field conditions may reflect differences in the physiology of the host rather than any interaction between pathogen and fungicide. Whatever the explanation, if such an effect were to occur in the field it would complicate any interpretation of fungicide performance data relating to synergy and antagonism, and the possible effects of low doses on the development of resistance.

The use of triazole mixtures contradicts Fungicide Resistance Action Committee (FRAC) recommendations that anti-resistance strategies require mixture partners with different modes of action. However, there is increasing biochemical and genetic evidence that several mechanisms can contribute to triazole-resistance, and providing that each mixture partner selects a different spectrum of these mechanisms, triazole mixtures may represent an alternative anti-resistance strategy. In both seasons, the Septoria population was found by bioassay to be triazole-sensitive (unpublished data). However, the *in vitro* studies showed that the response to triazole mixtures of triazole-sensitive and -resistant strains differ. Further study is needed to establish the extent of selection for resistance exerted by triazole mixtures.

ACKNOWLEDGEMENTS

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EM 1503: A BROAD-SPECTRUM CEREAL FUNGICIDE

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ABSTRACT

A triazole/morpholine co-formulation of fenbuconazole and tridemorph, EM 1503, was evaluated as a broad-spectrum fungicide for cereals in small plot, replicated trials at a range of UK locations between 1994 and 1996. EM 1503 (75+450 g a.i./ha) was shown to provide effective control, comparable to the standard fungicides, of *Septoria tritici, Puccinia striiformis, Puccinia recondita* and *Erysiphe graminis* on wheat and *Puccinia hordei* and *Erysiphe graminis* on barley. Useful yield increments were generated.

INTRODUCTION

EM 1503 is triazole/morpholine co-formulation containing 37.5 g fenbuconazole and 225.0 g tridemorph per litre, formulated as an emulsifiable concentrate. EM 1503 was designed to provide cost-effective broad-spectrum control of a wide range of cereal diseases.

Fenbuconazole is a member of the triazole group (Driant *et al.*, 1988). In addition to the control of foliar diseases of wheat and barley fenbuconazole also gives control of powdery mildew and scab on pome fruit; brown rot and powdery mildew on stone fruit; powdery mildew, black rot and grey mould on vines; rust on beans; and beet leaf spot on sugar beet and a wide range of diseases on field crops, rice, bananas, tree nuts, vegetables and ornamentals. Fenbuconazole is a systemic fungicide with protectant, curative and eradicant properties.

Tridemorph is a well established systemic morpholine fungicide (Kradel *et al.*, 1969; Pommer *et al.*, 1969), providing excellent control of *Erysiphe graminis* in cereals and of a wide range of diseases in other crops. Tridemorph gives some protective action as well as having eradicant properties.

EM 1503 is currently awaiting approval by the UK authorities for use on cereals at a recommended rate of 75+450 g a.i./ha. This paper reports on the results of field trials done in the UK between 1994 and 1996.

METHODS AND MATERIALS

Replicated, randomised block trials were done at a wide range of locations in England and Scotland. The treatments, as shown in the results tables, were applied as overall sprays at 200 litres/hectare using an Azo knapsack sprayer fitted with 6 Lurmark 02/F110 flat fan nozzles on a 3 metre boom operating at approximately 2.6 bar pressure. Trials received one or two applications between GS 32-59 (Zadoks *et al.*, 1974) according to disease severity. Disease levels were assessed at application and at intervals after application as specified in the results tables. Assessments were made by scoring the percentage area affected by each disease on

leaves from a randomly selected sample of 10 main tillers per plot. For all the assessment data described in this paper, treatment means with no letter in common are significantly different at the 5% probability level. Yield was assessed by combine harvesting a 2.1 m strip down the centre of each plot. Grain weight was recorded and corrected to 15% moisture content.

RESULTS

Foliar disease results are presented as the percentage control of disease in treated plots compared with the area of disease in the untreated plots. Tables 1, 2, 3 and 4 show the performance of EM 1503 on winter wheat against the foliar diseases *Septoria*, rusts and powdery mildew.

Control of *Septoria tritici* in winter wheat

Data presented in Tables 1 and 2 demonstrate the activity of EM 1503 against *S. tritici*, which is comparable to that given by standard fungicides. The 1994 trials were subject to high levels of disease severity, whereas the 1995 and 1996 trials experienced hot dry conditions and as a consequence, disease severity was correspondingly lower.

Table 1: % Control of *Septoria tritici* in winter wheat field trials in the UK: 1994

Site ref.		6565	6566	6567	6568	
Leaf assessed		1	2	2	3	
Days after treatment		59	59	44	44	
Treatments	g a.i./ha					
Untreated (% area diseased)	-	(39.8) a	(43.6) a	(49.7) a	(67.7) a	mean
EM 1503	60+360	95.4 b	87.8 b	99.2 b	84.8 bc	91.8
EM 1503	75+450	95.5 b	88.6 b	98.8 b	93.4 cd	94.1
tebuconazole	250	89.7 b	77.9 b	100.0 b	98.9 d	91.6
flusilazole+carbendazim	78+156	79.2 b	92.1 b	99.9 b	96.2 d	91.9
propiconazole+tridemorph	125+350	91.9 b	89.3 b	99.7 b	88.9 bcd	92.5
flutriafol+chlorothalonil	118+750	87.3 b	86.2 b	97.5 b	80.3 b	87.8

Table 2: % Control of *Septoria tritici* in winter wheat field trials in the UK: 1995 and 1996

Site ref.		6762	6765	6782	6841	6843	
Year		95	95	95	96	96	
Leaf assessed		1	2	2	2	2	
Days after treatment		50	48	45	45	59	
Treatments	g a.i./ha						
Untreated (% area diseased)	-	(16.1) a	(6.5) a	(13.8) a	(17.5) a	(22.5) a	mean
EM 1503	60+360	100.0 b	96.2 b	96.4 b	85.0 b	78.9 b	91.3
EM 1503	75+450	100.0 b	100.0 b	97.1 b	97.0 b	90.0 c	96.8
tebuconazole	250	99.7 b	100.0 b	100.0 b	95.4 b	91.9 c	97.4
propiconazole+tridemorph	125+350	99.8 b	100.0 b	94.2 b	97.4 b	91.9 c	96.7
epoxiconazole+tridemorph	125+375	100.0 b	100.0 b	100.0 b	98.4 b	93.0 c	98.3

Control of rusts in winter wheat

The results below demonstrate the ability of EM 1503 to achieve excellent control of the low to moderate yellow and brown rust severity epidemics in 1994 and 1995.

Table 3: % Control of yellow rust (*Puccinia striiformis*) and brown rust (*Puccinia. recondita*) in winter wheat field trials in the UK: 1994 and 1995

		Yellow rust		Brown rust				
Site ref.		6567	6567	6679	6764	6765	6761	
Year		94	94	94	95	95	95	
Leaf assessed		1	2	1	2	2	2	
Days after treatment		44	44	18	41	48	51	
Treatments	g a.i./ha							
Untreated (% area diseased)	-	(35.4) a	(8.1) a	(3.8) a	(8.8) a	(10.0) a	(5.8) a	mean
EM 1503	60+360	100.0 b	100.0 b	99.3 b	100.0 b	100.0 b	100.0 b	99.8
EM 1503	75+450	100.0 b	100.0 b	100.0 b	100.0 b	100.0 b	100.0 b	100.0
tebuconazole	250	100.0 b	100.0 b	100.0 b	100.0 b	100.0 b	100.0 b	100.0
flusilazole+ carbendazim	78+156	100.0 b	100.0 b	97.4 b	-	-	-	-
propiconazole+ tridemorph	125+350	100.0 b	100.0 b	97.4 b	100.0 b	100.0 b	100.0 b	99.4
flutriafol+ chlorothalonil	118+750	100.0 b	100.0 b	78.1 b	-	-	-	-
epoxiconazole+ tridemorph	125+375	-	-	-	100.0 b	100.0 b	100.0 b	(100.0)

Control of powdery mildew in winter wheat

Table 4: % Control of powdery mildew (*Erysiphe graminis*) in winter wheat field trials in the UK: 1994 to 1996

Site ref.		6567	6764	6783	6834	6837	6842	
Year		94	95	95	96	96	96	
Leaf assessed		3	3	3	2	2	1	
Days after treatment		44	41	45	59	61	50	
Treatments	g a.i./ha							
Untreated (% area diseased)	-	(5.8) a	(5.9) a	(2.8) a	(23.8) a	(23.8) a	(11.3) a	mean
EM 1503	60+360	84.8 b	95.4 b	89.3 b	81.1 b	80.0 b	92.9 b	87.3
EM 1503	75+450	91.3 b	96.6 b	100.0 b	95.2 b	91.6 b	97.6 b	95.4
tebuconazole	250	100.0 b	100.0 b	100.0 b	97.1 b	90.5 b	98.0 b	97.6
propiconazole+ tridemorph	125+350	65.2 b	99.6 b	89.3 b	96.0 b	91.1 b	95.6 b	89.5
flutriafol+ chlorothalonil	118+750	80.4 b	-	-	-	-	-	-
epoxiconazole+ tridemorph	125+375	-	100.0 b	100.0 b	97.6 b	92.6 b	99.6 b	(98.0)

Wheat mildew severity was low in the three years evaluated, but the results above demonstrate that EM 1503 gave good levels of control similar to the standard fungicides. The tridemorph component of EM 1503, being a specific fungicide with good activity against mildew, is a major contributor for the control of this disease.

Tables 5, 6, and 7 show the performance of EM 1503 on barley against powdery mildew (*Erysiphe graminis*), brown rust (*Puccinia recondita*), rhynchosporium (*Rhynchosporium secalis*) and net blotch (*Pyrenophora teres*).

Limited data on moderate levels of barley mildew in 1994 showed that EM 1503 provided similar good levels of control to the standard fungicides, again demonstrating that the addition of tridemorph is a valuable tool in the control of barley mildew. The barley brown rust epidemics in 1995 were severe, but the results show that the control of this disease by EM 1503 and the standard fungicides was excellent.

Control of powdery mildew in winter barley

Table 5: % Control of powdery mildew (*Erysiphe graminis*) in winter barley field trials in the UK: 1994

Site ref.		6660	6660	6672	6672	
Leaf assessed		2	3	1	2	
Days after treatment		21	21	23	23	
Treatments	g a.i./ha					
Untreated (% area diseased)	-	(2.5) a	(25.4) a	(7.0) a	(28.1) a	mean
EM 1503	60+360	93.1 b	74.3 b	87.2 b	69.7 b	81.1
EM 1503	75+450	95.0 b	74.1 b	89.0 b	69.5 b	81.9
tebuconazole	250	96.0 b	67.0 b	89.0 b	77.2 b	82.3
propiconazole+tridemorph	125+350	93.1 b	77.0 b	87.5 b	72.8 b	82.6

Control of rusts in winter barley

Table 6: % Control of brown rust (*Puccinia hordei*) in winter barley field trials in the UK: 1995

Site ref.		6769	6769	6771	6771	
Leaf assessed		1	2	1	2	
Days after treatment		48	48	50	50	
Treatments	g a.i./ha					
Untreated (% area diseased)	-	(31.5) a	(70.0) a	(14.0) a	(30.9) a	mean
EM 1503	60+360	88.2 b	86.7 b	85.3 b	89.5 b	87.4
EM 1503	75+450	90.2 b	93.4 b	87.8 b	94.3 b	91.4
tebuconazole	250	96.0 b	97.8 b	93.9 b	97.0 b	96.2
propiconazole+tridemorph	125+350	93.1 b	96.0 b	86.4 b	91.6 b	91.8
epoxiconazole+tridemorph	125+375	98.0 b	98.4 b	90.7 b	95.4 b	95.6

Control of *Rhynchosporium* and net blotch in winter barley

Diseases such as net blotch and *Rhynchosporium* in barley continue to prove difficult to control as demonstrated by the results from 1994 to 1996. Data from trials with low levels of disease showed that both these diseases were not well controlled by EM 1503, although results were not significantly different from other treatments.

Table 7: % Control of rhynchosporium (*Rhynchosporium secalis*) and net blotch (*Pyrenophora teres*) in winter barley field trials in the UK: 1994 to 1996

		Rhynchosporium				Net blotch			
Site ref.		6661	6661	6770		6770	6771	6836	
Year		94	94	95		95	95	96	
Leaf assessed		1	2	2		2	1	1	
Days after treatment		21	21	26		26	50	32	
Treatments	g a.i./ha								
Untreated (% area diseased)	-	(2.4) a	(4.6) a	(1.2) a	mean	(11.2) a	(13.8) a	(5.8)	mean
EM 1503	60+360	52.6 b	31.1 ab	89.4 b	57.7	54.6 b	42.0 b	56.5 b	51.0
EM 1503	75+450	77.3 b	32.2 ab	100.0 b	69.8	58.8 b	52.2 bc	56.0 b	55.7
tebuconazole	250	49.5 b	22.4 ab	89.4 b	53.8	74.7 b	64.5 c	69.8 bc	69.7
propiconazole+ tridemorph	125+350	78.4 b	44.8 bc	85.1 b	69.4	74.9 b	62.5 c	73.3 bc	70.2
epoxiconazole+ tridemorph	125+375	-	-	89.4 b	-	68.7 bc	68.9 c	83.6 c	73.7

Crop yield results

Yield data from both wheat and barley in the 1994 and 1995 trials is summarised in Table 8. In 1994, wheat disease severity was high and this is reflected in the results which show that EM 1503 produced yield increases of 12 to 14%. In 1995, EM 1503 on barley gave rise to 6 to 7% yield increases on trials where the main disease was brown rust.

Table 8: Mean yield responses in winter wheat and winter barley field trials in the UK: 1994 and 1995

		Grain yield as % of untreated			
Year Crop.		94 wheat	95 wheat	94 barley	95 barley
Treatments	g a.i./ha				
Untreated (tonnes/ha)	-	(8.80)	(9.63)	(6.58)	(7.44)
EM 1503	60+360	111.9	102.2	104.9	105.9
EM 1503	75+450	113.8	103.5	103.0	106.7
tebuconazole	250	112.6	103.2	105.4	107.0
propiconazole+tridemorph	125+350	111.3	102.4	103.5	107.5
flutriafol+chlorothalonil	118+750	111.1	-	-	-
epoxiconazole+tridemorph	125+375	-	103.7	-	110.1
(numbers of sites)		(5)	(8)	(2)	(4)

DISCUSSION AND CONCLUSIONS

EM 1503 (75+450 g a.i./ha) demonstrated effective control of the important foliar diseases of wheat. *Septoria tritici*, the disease which generally determines the recommended dose rate of fungicides in wheat, was well controlled up to 60 days after treatment. Limited data on yellow rust *(Puccinia striiformis)* indicated good control up to 44 days after treatment and there was also good control of brown rust *(Puccinia recondita)*, equivalent to the commercial standard fungicides, for up to 51 days after treatment. Control of powdery mildew (*Erysiphe graminis*) in the trials described was acceptable. The wheat yield results from EM 1503 reflected the disease severity with overall increments over the untreated of 12 to 14% in 1994, as a result of relatively severe *Septoria* epidemics and 2 to 4% in 1995 where disease severity was small.

EM 1503 (75+450 g a.i./ha) gave excellent control of severe epidemics of brown rust on barley up to 50 days after treatment and gave comparable powdery mildew control to the standard fungicides. Limited data from three sites showed that net blotch was not well controlled by EM 1503, but this control, was however, not significantly different from the other treatments. The results for *Rhynchosporium* were similar. EM 1503 gave yield increments in barley, 3 to 5% in 1994 and 6 to 7% in 1995, which were comparable with the standard products, with the exception of the epoxiconazole+tridemorph treatment in 1995 (10% increment). Early senescence due to drought inhibited the realisation of the full yield potential of these crops.

Although there was rarely a significant difference, the higher rate of EM 1503 (75+450g a.i./ha) consistently outperformed the lower rate. EM 1503 was shown to provide a spectrum of control which was comparable with a range of widely used commercial standard products.

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FOLIAR APPLICATION OF PHOSPHONATE FORMULATIONS FOR THE CONTROL OF POTATO TUBER BLIGHT

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ABSTRACT

In the field, foliar sprays of partially neutralised phosphonic acid substantially reduced tuber infection by *Phytophthora infestans* (potato late blight), compared with other fungicide programmes, despite the development of some foliage infection particularly on older leaves. Trials in which tubers from foliar-sprayed plants were lifted and inoculated with *P. infestans* demonstrated that phosphonic acid directly reduces the susceptibility of tubers to infection. Application of either 2 or 4 kg/ha markedly decreased the proportion of tubers which developed blight. A spray programme in which application of phosphonic acid is combined with a compatible foliar protectant fungicide would appear to offer the possibility of controlling both phases of the disease and could have a major impact in reducing overwinter survival of the pathogen in tubers.

INTRODUCTION

Fosetyl-aluminium ('Aliette'), first described in 1977, is unique amongst fungicides in being both acropetally and basipetally mobile (Williams *et al.*, 1977) and is widely used to control diseases caused by oomycete fungi. *In vivo*, fosetyl-aluminium is converted to the fungicidally-active phosphonic acid (phosphorous acid, H_3PO_3) (Luttringer & Cormis, 1985; Fenn & Coffey, 1989). Wicks *et al.* (1990) reported on the use of partially neutralised phosphonic acid, developed as a fungicide in Australia, against a range of plant diseases and referred to control of potato late blight caused by *Phytophthora infestans*. Fosetyl-aluminium has been considered inactive against *P. infestans* (Bertrand *et al.*, 1977; Williams *et al.*, 1977) and has not been developed commercially for potato blight control in temperate climates, although some workers have reported activity (Samoucha & Cohen, 1986). It appeared possible that phosphonic acid might be more effective than fosetyl-aluminium on potato and so a study of its activity against late blight was commenced. In the light of the basipetal mobility of phosphonates, this concentrated particularly on effects on tuber infection. The trials described here represent part of this investigation, which will be reported in full elsewhere. In all except the first trial, tubers from fungicide-sprayed plants were lifted and inoculated with the pathogen, allowing tuber susceptibility to be assessed without interference from effects on foliar infection.

MATERIALS AND METHODS

All trials were carried out at the Agriculture & Food Science Centre, Newforge Lane, Belfast. For foliar application, phosphonic acid (Prolabo, >98% pure) was dissolved in water at the required rate and partially neutralised to pH 6.4 with potassium hydroxide.

1992: trial 1

Tubers of the maincrop potato cv. Up-to-date were planted on 18 May 1992 in fully randomised blocks with five replicate plots per treatment. Each plot (2.8 x 3.0 m^2) contained four rows of ten tubers. Pairs of rows of unsprayed plants adjacent to each treated plot served as an infection source. Plants in these rows were inoculated (8 July) with a phenylamide-resistant isolate of *P. infestans*. The plots were misted daily from 8 July for 2-3 h at dawn and dusk to encourage spread of blight.

Two spray programmes using commercially-available fungicides were compared with phosphonic acid. These were mancozeb (1275 g/ha as 'Dithane DF') applied at 10-day intervals (22 June - 20 August) and metalaxyl + mancozeb (150 + 1350 g/ha as 'Fubol 75WP') at 14-day intervals (22 June - 3 August) followed by fentin hydroxide (266 g/ha as 'Du-ter 50', 13, 24 August). Phosphonic acid was used at 2 kg/ha and 10-day intervals (22 June - 20 August, 7 applications). Treatments were applied as foliar sprays in *c.* 560 litres water/ha with a Cooper Pegler knapsack sprayer.

Foliage late blight was assessed twice weekly during July and August. Plots were desiccated on 28 August and lifted on 5 October. After lifting, tubers were graded and the number and weight in each class and the numbers and weight of tubers with blight (>35 mm) from each plot recorded.

1992: trial 2

Tubers of the first early potato cv. Dundrod were planted on 15 May 1992 in fully randomised blocks with five replicate plots per treatment. Each plot contained two rows of five tubers. Treatments were unsprayed, chlorothalonil (1275 g/ha as 'Bravo 500'), phosphonic acid (2 kg/ha), chlorothalonil + phosphonic acid (1500 g + 2 kg) and mancozeb (1350 g/ha). Treatments were applied as foliar sprays in *c.* 560 litres/ha using a Hozelock Killaspray 8 sprayer on 9 July.

On each of 1, 7 and 12 days after treatment, ten leaflets were sampled in pairs from five different plants in each plot. One leaflet of each pair was placed adaxial side up and the other abaxial side up within humid boxes. Each leaflet was inoculated with two 20 µl drops of a sporangial/zoospore suspension of *P. infestans* isolates containing phenylamide-resistant strains (10^5 sporangia/ml). Leaflets were incubated in daylight at 15°C and infection assessed after 7 days. On 28 July (19 days after treatment), 10 tubers were lifted from each plot, placed in humid boxes and sprayed with a sporangial/zoospore suspension of phenylamide-resistant *P. infestans*. Tubers were incubated at 15°C in darkness and infection assessed after *c.* 3 weeks.

1994 trial

Potato tubers cv. Dundrod were planted on 6 May 1994 in four fully randomised blocks of seven treatments. Each plot contained two rows of seven tubers. Treatments were unsprayed, fosetyl-aluminium (3.2 and 6.4 kg/ha as 'Aliette'), phosphonic acid (2 and 4 kg/ha), and dipotassium phosphonate (2 and 4 kg phosphonic acid equivalent/ha, supplied by Rhône-Poulenc). Treatments were applied as a single foliar spray in *c.* 560 litres/ha using a Hozelock Killaspray 8 sprayer on 19 July. In addition, mancozeb was applied to all plots at 10-day intervals from mid-June onwards to prevent foliage infection. On 12 August (sample 1: 24 days

after treatment) and 26 August (sample 2: 36 days after treatment), 10 tubers were lifted from each plot and inoculated, incubated and assessed.

1995 trial

Potato tubers cvs Dundrod and Bintje were planted on 27 April 1995 in a split-plot design with four replicate blocks containing cultivars as main plots and fungicide treatments as sub-plots. Sub-plots contained two rows of seven tubers. Phosphonic acid was applied as follows:

4 kg/ha, 1 application, 27 July
2 kg/ha, 2 applications, 17 and 27 July
1 kg/ha, 4 applications, 10, 21 31 July and 10 August
2 kg/ha, 6 applications at 10-day intervals (22 June - 10 August)

An unsprayed control was included. Mancozeb was applied to all plots at 10-day intervals from mid-June onwards to prevent foliage infection. On 16 and 30 August, 20 tubers were lifted from each sub-plot and inoculated, incubated and assessed.

RESULTS

1992: trial 1

Phosphonic acid applied every 10 days appeared to have a similar effect to mancozeb in preventing phenylamide-resistant foliage blight and was more effective than metalaxyl + mancozeb at longer intervals (Table 1). However, accurate assessment of infection on phosphonate-treated plots was difficult as, atypically, lesions developed on older leaves which then abscissed, resulting in plants with healthy upper leaves, but few lower leaves. Treatment had no effect on yield, but a highly significant effect on tuber blight, which was very much less in tubers from phosphonic acid-treated plots than in those from other treatments.

Table 1. 1992, trial 1: foliage and tuber blight assessments.

Treatment	Foliage blight (%)			Yield (kg per plot)	Tuber blight (%)
	7 Aug	14 Aug	28 Aug		
mancozeb	4.7	19.9	75.8	36.8	19.0
metalaxyl + mancozeb	29.0	75.3	96.8	30.3	17.6
phosphonic acid	7.8	18.5	67.5	39.8	1.9
LSD (P<0.05)	11.30	12.80	10.21	ns	5.18

1992: trial 2

Phosphonic acid conferred substantial protection from infection to both surfaces of leaflets sampled one day after treatment, but after 7 days protection was negligible (Table 2). In contrast, the non-systemic fungicides chlorothalonil and mancozeb protected the adaxial, but

not the abaxial surfaces for at least 7 days. As expected, the non-systemic fungicides had no effect on the susceptibility of tubers to blight and *c.* 60% developed symptoms after inoculation, but the two treatments containing phosphonic acid greatly reduced infection.

Table 2. 1992, trial 2: susceptibility of inoculated potato leaflets and tubers to blight.

Treatment	Leaflets infected (%)						Tubers infected (%)
	Abaxial inoculation			Adaxial inoculation			
	day 1	day 7	day 12	day 1	day 7	day 12	
untreated	100	100	100	100	100	100	68
mancozeb	100	96	100	16	8	76	58
chlorothalonil	92	92	100	8	0	4	66
phosphonic acid	12	100	100	8	100	100	6
chlorothalonil + phosphonic acid	4	92	100	0	0	0	10
LSD (P<0.05)	11.5	ns	ns	18.2	6.6	17.0	19.3

1994 trial

A high percentage of tubers of both samples from untreated plants developed blight after inoculation (Table 3). Neither rate of fosetyl-aluminium significantly reduced blight compared with the untreated control for either sample. The higher rate of both phosphonic acid and dipotassium phosphonate significantly reduced blight in both samples. The lower rate of phosphonic acid significantly reduced blight in the first sample only, while the lower rate of dipotassium phosphonate significantly reduced blight in the second sample only.

Table 3. 1994 trial: susceptibility of inoculated potato tubers to blight

Treatment	kg a.i./ha	Tuber blight (%)	
		Sample 1	Sample 2
untreated control	0.0	80	80
fosetyl-aluminium	3.2	83	63
fosetyl-aluminium	6.4	63	58
phosphonic acid	2.0	40	60
phosphonic acid	4.0	10	28
dipotassium phosphonate	2.0	73	48
dipotassium phosphonate	4.0	35	25
LSD (P<0.05)		25.1	26.3

1995 trial

Phosphonic acid reduced the susceptibility of tubers of both cultivars to blight (Table 4). Six sprays applied throughout the season at 10-day intervals had the greatest effect. Of the three

programmes in which a total of 4 kg of phosphonic acid was applied as one, two or four sprays, the single 4 kg application proved most effective.

Table 4. 1995 Trial: susceptibility of inoculated potato tubers to blight

Treatment	Tuber blight (%)[a]			
	Sample 1		Sample 2	
	Dundrod	Bintje	Dundrod	Bintje
untreated control	90	95	90	89
phosphonic acid 1 x 4 kg	36	65	65	59
phosphonic acid 2 x 2 kg	78	80	79	80
phosphonic acid 4 x 1 kg	69	75	81	81
phosphonic acid 6 x 2 kg	23	28	46	33
LSD (P<0.05)	17.9		20.2	

DISCUSSION

Phosphonic acid substantially reduced foliar infection by *P. infestans* in trials in 1991 (Cooke & Little, unpublished) and 1992. Lesions tended to develop on lower leaves, although the upper remained healthy. On this basis, it might have been predicted that tubers would be severely infected, since there was a potential inoculum source near the soil surface. In fact, very few tubers from phosphonic acid-treated plots developed blight and the percentage was much less than in tubers from treatments with a similar level of foliar infection. This suggested that some factor other than reduction of inoculum might be influencing tuber infection.

In further trials, foliar sprays with phosphonic acid reduced the proportion of tubers which developed symptoms after inoculation, demonstrating that the tubers themselves were protected from blight. This may be due to stimulation of host-plant defences, which have been implicated in activity of fosetyl-aluminium (e.g. Bompeix *et al.*, 1981), but it seems more likely that a direct fungicidal effect of phosphonic acid is involved as a result of movement of chemical into the tubers via the xylem or phloem. Fenn & Coffey (1983) found a close correspondence between the *in vitro* and *in vivo* activity of phosphonic acid against a number of *Phytophthora* and *Pythium* spp., although they reported that *P. infestans* was not very sensitive.

There are few reports of foliar-applied fungicides protecting potato tubers from blight. Metalaxyl is known to be active in this way (Stewart & McCalmont, 1982), but not against phenylamide-resistant strains of *P. infestans* (Cooke, 1986). Filippov *et al.* (1995) reported that 'Efal-M' (phosphorous acid, aluminium triphosphate, fosetyl-aluminium and cuprous sulphate) and 'Alufit' (phosphorous acid, aluminium triphosphate and cuprous sulphate) increased resistance of potato tubers to *P. infestans* when plants were sprayed with sub-fungicidal concentrations.

Much remains to be elucidated regarding the metabolism and movement of phosphonate formulations within the potato. In the trials reported fosetyl-aluminium did not reduce tuber blight significantly, but elsewhere it has proved effective (Mercer, R T, personal

communication). The timing of applications may also be critical, since in some trials a single application of 2 kg/ha was effective, whereas in others consistent reductions were only obtained with much greater doses, which might not be economic. A spray programme with a suitable non-systemic fungicide to enhance protection of the foliage plus phosphonic acid to protect tubers would seem likely to optimise disease control and minimise the risk of resistance. Unfortunately, phosphonic acid does not currently have approval within the EU for application to potatoes as a fungicide. The ability of phosphite compounds to protect potato tubers by the application of foliar sprays is the subject of a UK Patent Application (Cooke *et al.*, 1996).

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INFLUENCE OF POTATO TUBER TREATMENTS WITH FLUDIOXONIL ON DEVELOPMENT OF FUNGAL DISEASES

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ABSTRACT

Fludioxonil 100 FS at the rate 50 ml / t (5 g a.i. / t) potatoes provides adequate protection against *Fusarium* dry rots at a level comparable with that of thiabendazole 450 FS at a rate of 100 ml / t (45 g a.i. / t). Fludioxonil, however, also decreases the incidence of *Rhizoctonia infection* level in the following crop, whereas thiabendazole has no effect. Pre-planting application of fludioxonil 100 FS to potato tubers at a rate of 100 ml/t (10 g a.i. / t) decreased the susceptibility of potato leaves to *Phytophthora infestans* up to the budding stage of plants under artificial inoculation conditions and delayed appearance of the symptoms of disease under field conditions.

INTRODUCTION

Phenylpyrroles are a relatively new group of compounds that have been developed by Ciba for use as agricultural fungicides. Their development was based on the antimycotic substance pyrrolnitrin produced by various soil inhabiting bacteria, e.g. *Pseudomonas pyrocinia* (Kempf *et al.*, 1993), *Pseudomonas fluorescens* (Hammer *et al.*, 1995), *Myxococcus fulvus* (Gerth *et al.*, 1982). Pyrrolnitrin shows activity against a number of phytopathogenic organisms (Leadbitter *et al.*, 1994). However the practical use of this compound as an agricultural fungicide is limited because of its low photostability.

Pyrrolnitrin was used as the basis for a wide ranging synthetic chemistry project in Ciba which led to the development of fenpiclonil and fludioxonil as fungicides (Leadbitter *et al.*, 1994). They show activity against a wide range of fungi in the basidiomycete, ascomycete and deuteromycete groups. In Russia, fludioxonil is registered as a potato tuber treatment, under the trade name Maxim 100 FS (Flowable for Seed treatment), for control of *Fusarium spp.*, *Helminthosporium solani*, *Phoma exigua* and *Rhizoctonia solani*. It is recommended that the product is applied by Ultra Low Volume treatment at a rate of 50-100 ml / tonne (5-10 g a.i. / t) potatoes.

This paper reports work that has been done with this product to evaluate its activity against several pathogens on potato tubers.

PRE-STORAGE TREATMENT AGAINST STORAGE ROTS: FARM TRIALS

Methods

10 tonnes of tubers from two farms (Moscow region and Bryansk region) were treated with the following products, using commercial ULV equipment: fludioxonil, 5 g a.i. / tonne (as Maxim 100 FS); thiabendazole (as Tecto 450 FS). They were compared with an untreated control. Treatments were applied after harvest and prior to storage. The potatoes were stored under normal commercial conditions (2-3 ^{0}C) and sampled after 6 months. Three samples, of 100 tubers, were assessed for incidence (% tubers affected) of storage rots (especially those caused by *Fusarium spp.* or *Phytophthora infestans*).

Results

Under commercial storage conditions after 6 months of storage the decay caused by *Fusarium sambucinum* and *F. solani* had occurred at both farms in the untreated control (Moscow, 20 % tubers infected; Bryansk, 12 % tubers infected). Both fludioxonil and thiabendazole gave comparable levels of control of these diseases (Table 1). The incidence of other pathogens on treated or untreated tubers was not great.

Table 1. Effect of the pre-storage treatment of potato tubers on incidence (% tubers affected) of storage rots after 6 months.

Region	Storage rots	% tubers affected			
		Untreated control	Fludioxonil, 5 g a.i. / t	Thiabendazol, 45 g a.i. / t	LSD (p=0.05)
Bryansk	*Fusarium spp.*	12.0	1.5	2.4	3.2
	Phytophthora	0.7	0.3	0	0.7
	Completely rotten	1.7	0	0	-
Moscow	*Fusarium spp.*	20.0	8.7	7.9	5.0
	Phytophthora	1.7	1.4	1.4	0.6
	Completely rotten	1.7	1.9	0.4	1.0

PRE-STORAGE AND PRE-PLANTING TREATMENT AGAINST *RHIZOCTONIA SOLANI*: FIELD TRIALS

Methods

Tuber treatments with fludioxonil and thiabendazole were applied prior to storage in 1994 and prior to planting in 1995. In 1994 rate of application - 5 g a.i. /t for

fludioxonil, and 45 g a.i. / t for thiabendazole; in 1995 the rates were 10 g a.i. / t for fludioxonil and 45 g a.i. /t for thiabendazole. They were compared with an untreated control. Tubers were planted into experimental field on 25 May 1995, arranged in a randomised complete block design with four replications. Each plot consisted of four rows of potatoes 15 m long. During the planting process the soil in experimental plots was inoculated with *Rhizoctonia solani* grown on wheat grains (2 grains per tuber). The initial incidence of *Rhizoctonia solani* on seed tubers with was less than 1%.

Stems of the ten potato plants in each plot were examined for presence of *Rhizoctonia solani* symptoms at the 6-7 leaf stage of development. The stems were classified into six categories based on the percentage of the surface area with disease. The following scale was used:

Table 2. Scale for *Rhizoctonia solani* symptoms evaluation.

Category	1	2	3	4	5	6
% surface area affected	0	<5	5-10	10-25	25-50	>50
No. stems in category	n_1	n_2	n_3	n_4	n_5	n_6

A surface lesion index (SLI) was calculated:

$$SLI = \frac{(n_2 \cdot 2.5) + (n_3 \cdot 7.5) + (n_4 \cdot 17.5) + (n_5 \cdot 37.5) + (n_6 \cdot 75.0)}{n_1 + n_2 + n_3 + n_4 + n_5 + n_6}$$

Results

The trials showed that where there was soil-borne infection, a pre-storage treatment with fludioxonil gave some control of *Rhizoctonia* on stems (Table 3). Pre-storage tuber treatment with thiabendazole was not effective. Where pre-planting treatments were made, fludioxonil and thiabendazole were equally effective, and gave similar control to a pre-storage treatment with fludioxonil.

Table 3. Effect of the pre-storage and pre-planting treatment of potato tubers with fludioxonil or thiabendazole on *Rhizoctonia solani* (% surface area affected on the stems)

Time of treatment	Untreated control	Fludioxonil, 5 g a.i. / t	Thiabendazol, 45 g a.i. / t	LSD (p=0.05)
Pre-storage	81.3	52.4	87.4	16.2
Pre-planting	81.3	57.8	59.8	20.4

ACTIVITY AGAINST FOLIAR *PHYTOPHTHORA* INFECTIONS USING PRE-PLANTING TUBER TREATMENTS: FILED AND LABORATORY TRIALS

Methods

Immediately prior to planting potato tubers were treated with fludioxonil (10 g a.i. / t) and thiabendazole (45 g a.i. / t). Tubers were planted in experimental fields and compared with an untreated control. Field plots were arranged in a randomised block design and replicated four times, each plot consisted of four rows of potatoes 15 m long.

Starting at the 5-7 leaf stage of development, on 8 regularly spaced occasions per season 10 leaves from each plot were cut off. In laboratory conditions these detached leaves were artificially inoculated with sporangia of *Phytophthora infestans*. They were transferred to moist filter papers in moisture chambers. After 4-5 days the number of late blight lesions on each leaflet was noted. Further details of the method are given in Filippov & Kuznetzova (1995).

Results

Data indicate that during the first stages of crop development (pre-budding, up to 50-70 days after planting) the application of fludioxonil as a seed treatment (10 g a.i. / t) reduced the susceptibility of the leaves to *P. infestans* (Table 4). Thiabendazole had no effect on the susceptibility of the plants to the pathogen. In the same field plots the first symptoms of *P. infestans* infection were noted earlier in the untreated and thiabendazole-treated plots than in the fludioxonil-treated plots (8 d and 15 d difference in 1994 and 1995 respectively).

Similar effects were observed when pre-planting applications of living cells of *Pseudomonas fluorescens* (A-33 strain) to the tubers had been made. This agent also reduced the susceptibility of the leaves towards *P. infestans* prior to budding but then tended to increase the susceptibility after budding (Filippov & Kuznetzova, 1995).

Table 4. The effect of pre-planting treatment of potato tubers with fludioxonil 100 FS on susceptibility of potato leaves to *Phytophthora infestans* infection

Year	Period between planting and inoculation, days	The mean number of lesions per inoculated leaf in % compared to untreated control
1994	33	62*
	40	62.4*
	45	37.3*
	53	52.0*
	70	80.5
	76	62.9*
	82	82.6
	87	98.5
1995	33	56.2*
	39	48.4*
	43	65.6*
	50	72.4*
	54	81.8
	59	81.9
	66	112.0
	74	100.8

(*) = Significantly less than the untreated control (P=0.05).

CONCLUSIONS

The phenylpyrrole group of fungicides is known to have activity against tuber-borne diseases of potatoes when applied pre-planting or pre-storage. The data presented demonstrate that under Russian conditions, fludioxonil, a phenylpyrrole fungicide, gives good control of *R. solani* when applied as a pre-storage or pre-planting application and that it is more effective than thiabendazole at the pre-storage timing. Fludioxonil also gives good activity against *Fusarium* spp. equal to the standard thiabendazole.

The application of fludioxonil as a tuber treatment prior to planting appeared to decrease the susceptibility of leaves of the plant to *P. infestans* during the first weeks after plant emergence. Appearance of the first symptoms of late blight in the field were also delayed. As this fungicide is considered to be non-systemic, these effects were unexpected. It is not clear how these effects are being caused and further studies are needed to understand them.

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CONTROL OF SILVER SCURF AND BLACK SCURF IN POTATOES WITH A PENCYCURON/TOLYLFLUANID SEED TUBER TREATMENT

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ABSTRACT

Tolylfluanid has been used as a fungicide for fruit since the early 1970 s, and following *in vitro* tests a series of trials was carried out in the UK to test its effectiveness against potato tuber diseases. Applied as a seed tuber treatment with pencycuron a mean of 90% control of silver scurf was obtained, comparing well with a current standard fungicide. In this combined formulation, the performance of pencycuron against black scurf was not affected and 97% control was observed. There was some evidence in 2 trials of control of black dot by pencycuron/tolylfluanid. From observations on plant growth and yield, the co-formulation would appear to be very safe to the crop.

INTRODUCTION

The increasing popularity of washed pre-packed potatoes has led to a particular demand for tubers with a high quality of skin finish. Skin blemishes can be caused by many bacterial and fungal pathogens and whilst some, e.g. common scab (*Streptomyces scabies*) can only be controlled practically by cultural techniques, others such as black scurf (*Rhizoctonia solani*) and silver scurf (*Helminthosporium solani*) can be successfully controlled by means of chemical treatment.

Pencycuron is a highly specific active ingredient for control of *R. solani* (Adam & Malcom, 1988; Rollett *et al.,* 1987) which was introduced in 1988 by Bayer UK as Monceren DS, a 12.5% dry powder tuber treatment for the control of tuber-borne inoculum. In order to broaden the range of application methods, a flowable formulation of pencycuron was made available in 1991.

Since resistance to thiabendazole has been observed in some isolates of *H. solani* (Hide *et al.*, 1988), imazalil has become the principal fungicide used on seed tubers to control silver scurf. In order to extend the spectrum of activity for Monceren, a formulation containing imazalil (Monceren IM) was subsequently developed. It has been observed that imazalil can sometimes delay crop emergence, though this is not normally reflected in yield loss.

Tolylfluanid is a broad spectrum fungicide used widely in Europe for the control of *Botrytis* and other diseases of fruit and vines (Wackers & Berge, 1972) and recent results from *in vitro* tests have suggested that it may be effective against *H. solani* (G Hide, personal communication). The tests also indicated activity against the potato pathogens *Polyscytalum pustulans* and *Colletotrichum coccodes*, causative agents of skin spot and black dot

respectively. As a consequence, a series of field trials was carried out on potatoes to evaluate tolylfluanid applied alone and in co-formulation with pencycuron. This paper reports on 14 trials carried out in the UK between 1993 and 1995.

MATERIALS AND METHODS

The treatments, which are listed in Table 1, were all applied as dry powder (DS) formulations with the exception of two trials in 1993 when a flowable formulation (FS) of pencycuron was used in conjunction with a water dispersible granule (WG) formulation of tolylfluanid, which was also applied alone in water (300 ml/100 kg tubers).

Table 1. Formulations included in trials and rates of application.

Treatment	Rate a.i. formulation	Application rate per 100 kg seed g (or ml)	g a.i.
pencycuron	12.5% DS	200	25
pencycuron + imazalil	12.5 + 0.6% DS	200	25 + 1.2
tolylfluanid	50% WG	40	20
*pencycuron + tolylfluanid	7.5 + 10% DS	200	15 + 20
°pencycuron	250 g/litre FS	60 (ml)	15
+ tolylfluanid	+ 50% WG	50	25

* This formulation was also included in trials at 400 g/100 kg tubers
° Applied in trials FA/38/93 and NP/01/93
DS = Dry powder, FS = Flowable concentrate, WG = Water dispersible granule

Treatments were applied by adding the appropriate dose to pre-weighed tubers (normally 10 kg) in a large polythene bag, then rolling the bag a standard 5 times in one direction and then in the other. Batches of tubers planted in the trials were infected with silver scurf and/or black scurf and the varieties used were Vanessa, Maris Peer or Fianna. The trials were planted using a modified Johnson, semi-automatic, manned, 2-row planter and tubers were spaced at 25 cm. Trials had four replicates in randomised block designs and plot sizes were 0.75 - 0.9 m (one row) x 10 m (row length). Trials were done throughout England (Tables 2 & 3).

Assessments

1 Early crop growth and development was assessed by visually estimating total above ground biomass of plants in each of the treated plots relative to the untreated control. The assessments were done shortly after crop emergence and at intervals until plots appeared even.

2 Crop stand was assessed by counting the total number of plants per plot at full emergence.

3 Plots were harvested during September-October and yields recorded.

4 Tuber skin diseases (black scurf, silver scurf and black dot) were assessed after a period of storage at *c.* 8°C for 3 - 5 months. The percent surface area affected was recorded from a sample of 100 washed tubers per plot. The similar symptoms of silver scurf and black dot were distinguished by using a low-power binocular microscope. In addition, observations on the extent of common scab were also recorded. Crop stand, yield and disease control data were statistically analysed using analysis of variance. An angular transformation was applied to per cent data before analysis.

RESULTS

At least one assessment of early crop growth was made for each trial and Fig. 1a includes data obtained between 36 and 64 days after planting. During this period a delay was observed with pencycuron/imazalil which was largely outgrown at a later stage. Some reduction in yield was noted for this treatment (Fig. 1c) although it was statistically significant in only one trial. Crops were particularly affected by drought in 1995, and would have experienced more difficulty in compensating for early delays in development. Crop tolerance to tolylfluanid and pencycuron + tolylfluanid, even applied at double rate, was clearly very good.

Silver scurf developed in 11 trials and was decreased significantly in 10 trials (Table 2). Black scurf occurred in 8 trials and was decreased significantly in 6 trials (Table 3). The results clearly demonstrated the specificity of imazalil or tolylfluanid compared with pencycuron in controlling silver scurf or black scurf, respectively, together with the merits of a combined formulation. Black dot occurred in 6 trials, although significant disease control was obtained only at the 2 sites (Table 3) which had no previous history of potato growing. The principal source of inoculum in situations where potatoes had been grown previously was presumed to be from soil, and treatments were not effective. In both FA/38/93 and FA/30/94, incidence of black dot on the tuber was low and although pencycuron + tolylfluanid reduced disease at both sites, results with pencycuron and pencycuron + imazalil were inconsistent.

Lesions caused by common scab were assessed in trials FA/24/95 and FA/25/95, but from these assessments together with observations in other trials, no differences could be attributed to treatments.

DISCUSSION

Fundamental to the interpretation of trials and indeed the outcome of commercial applications against tuber diseases is an appreciation of the aetiology of the pathogens involved and the potential for interactions in mixed populations. Both black scurf and black dot are transmitted by soil-borne as well as tuber-borne inoculum. Black scurf was known to be present on the Vanessa and Maris Peer seed tubers used in trials and the results of the 6 trials reported (Table 3) were not confounded by soil-borne inoculum. Clearly the activity of pencycuron against black scurf was not adversely affected by mixing with either imazalil or tolylfluanid.

Table 2. % reduction in silver scurf infection on harvested tubers

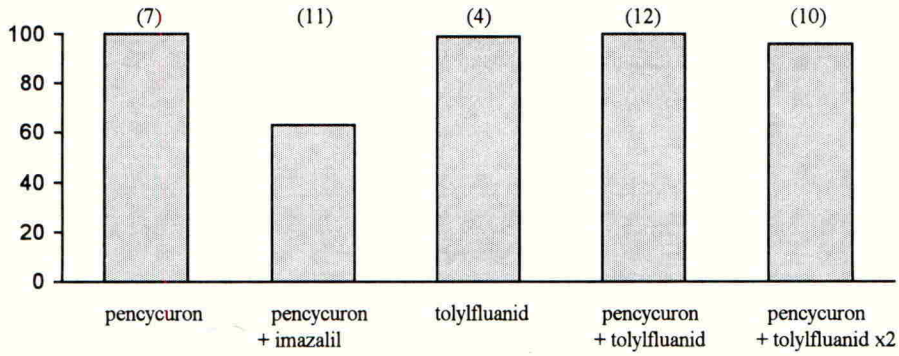
Treatments	Trial No Cultivar Location	FA/38 1993 Van'sa Suffolk	NP/01 1993 Van'sa Notts	FA/30 1994 Van'sa Suffolk	FA/24 1995 M Peer Suffolk	FA/25 1995 Fianna Suffolk	NR/17 1995 M Peer Humber	WR/17 1995 M Peer Worcs	MR/19 1995 Fianna Lincs	NR/16 1995 Fianna Humber	WR/16 1995 Fianna Worcs	Mean (No of sites)
Untreated (% area affected)		(6.4)a	(1.8)a	(19.6)a	(9.4)a	(7.9)a	(9.9)a	(2.2)a	(5.1)a	(3.5)a	(3.6)a	(6.9)(10)
pencycuron		11a	0a	39b	-	-	35a	10a	-	-	-	19 (5)
pencycuron + imazalil		47b	53b	80c	94b	78b	-	-	98b	92b	81b	78 (8)
tolylfluanid		-	-	82c	97b	93bc	-	-	-	-	-	91 (3)
pencycuron + tolylfluanid		73c	70b	87c	97b	91bc	98b	95b	99b	92b	95b	90 (10)
pencycuron + tolylfluanid (x2)		-	-	-	98b	94c	99b	94b	100b	97b	92b	96 (7)

Table 3. % reduction in black scurf and black dot on harvested tubers

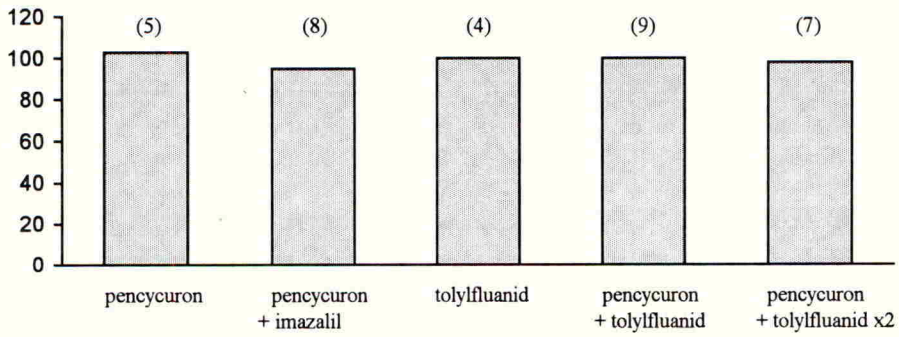
Treatments	Trial no Cultivar Location	black scurf FA/38 1993 Van'sa Suffolk	FA/31 1994 Van'sa Notts	FA/24 1995 M Peer Suffolk	ER/19 1995 M Peer Norfolk	NR/17 1995 M Peer Humber	WR/17 1995 M Peer Worcs	Mean (No of sites)	black dot FA/38 1993 Van'sa Suffolk	FA/30 1994 Van'sa Suffolk
Untreated (% area affected)		(0.2)a	(0.1)a	(1.0)a	(1.3)a	(0.6)a	(0.8)a	(0.7)(6)	(0.5)a	(0.1)a
pencycuron		100b	89bc	-	90bc	100b	99c	96 (5)	61b	0a
pencycuron + imazalil		100b	98c	98b	-	-	-	99 (3)	36ab	0a
tolylfluanid		-	52ab	25a	-	-	-	39 (2)	-	54ab
pencycuron + tolylfluanid		99b	100c	100b	85b	100b	98c	97 (6)	73b	82b
pencycuron + tolylfluanid (x2)		-	-	98b	97c	100b	84b	95 (4)	-	-

Treatments having the same suffix letter are not signicantly different (LSD, P≤ 0.05)

a. Visual estimate of aerial crop growth at 36-64 days after planting



b. Number of plants at full emergence



c. Final crop yield - whole plot harvested

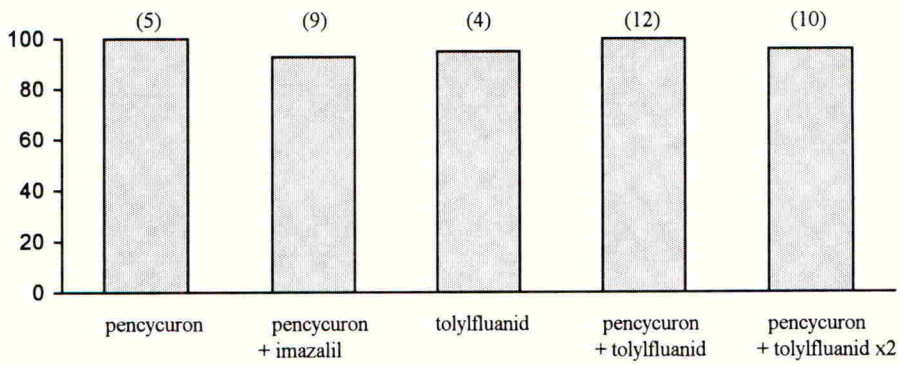


Fig 1. Mean results relative to untreated (%) for: a. visual assessments of early crop growth, b. plant count at full emergence and c. final total yield of clean tubers. The numbers of trials are given in parentheses.

As silver scurf is largely tuber-borne, the results were not complicated by other sources of inoculum. The disease which developed to different levels on untreated tubers at the different sites, was well controlled by treatments containing either tolylfluanid or imazalil although tolyfluanid was consistently more effective. In one trial in Kent, using infected tubers (cv. Fianna) silver scurf failed to develop whilst black dot was observed in all treatments, indicating the presence of soil-borne inoculum. Treatments gave no significant control of black dot at this site, and it would appear that *C. coccodes* had infected tubers where silver scurf had been controlled, also competing with *H. solani* on untreated tubers. *In vitro* tests indicated useful activity with tolylfluanid against *C. coccodes* and this was substantiated by significant results at two sites, though clearly further observations are desirable.

For control of silver scurf, a heavy reliance has been placed on imazalil in recent years; the different chemistry of tolylfluanid, together with excellent crop compatibility and efficacy, should make it a valuable addition to the limited range of treatments currently available.

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UPTAKE AND REDISTRIBUTION OF PROPAMOCARB HYDROCHLORIDE IN POTATO AND GRAPEVINE

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ABSTRACT

Propamocarb hydrochloride was rapidly taken up by potato and grapevine foliage and potato stems. Within 24h of application, uptake of propamocarb hydrochloride by potato foliage was 20 times greater than for vine foliage. Redistribution within potato tissues was rapid, extensive and acropetal. Within 3 days after application to potato stem bases, all tissues contained propamocarb hydrochloride. All new foliage produced within the 11 day duration of the experiment contained propamocarb hydrochloride. Autoradiographs of whole potato plants indicated that propamocarb hydrochloride was at a greater concentration within the stem than foliage. It is postulated that these reserves not only protect stems against infection but are also a source of product for newly developing growth. No similar evidence of redistribution was found in grapevine foliage and the significance of this for disease control is discussed.

INTRODUCTION

Propamocarb hydrochloride is a carbamate fungicide active against oomycete fungi. In the past it has enjoyed extensive usage in a wide variety of horticultural applications and is currently proving to be very effective for the control of late blight of potato (*Phytophthora infestans*). Any independent studies on the uptake and mobility of propamocarb hydrochloride appear not to have been published in the public domain. Although 'in-house' evidence indicated xylem mobility there was a need to possess more detailed information on the uptake and redistribution of propamocarb hydrochloride in potato to optimise disease control strategies. Because propamocarb hydrochloride is less active than might be expected against vine downy mildew (*Plasmopara viticola*), parallel studies in potato and vine were made to investigate this difference and improve activity against this disease.

MATERIALS AND METHODS

Active ingredients

'Previcur®N' (propamocarb hydrochloride 722g/l SL) was diluted with de-ionised water to a concentration of 0.69% v/v product. This equates to the concentration of propamocarb hydrochloride in a commercial field application of 'Tattoo®' (248g/l propamocarb hydrochloride + 301.6g/l mancozeb SC) or 'Tattoo®C (375g/l propamocarb hydrochloride + 375g/l chlorothalonil). Application solutions for quantitative uptake studies were spiked with sufficient ^{14}C propamocarb hydrochloride (specific activity = 224μCuries/mg) to achieve

approximately 20,000 degradations/min/µl. Because of the high specific activity of the radiolabelled compound, the contribution of mass from this source was discounted. For autoradiograph studies, potato stem bases were treated with undiluted radiolabel.

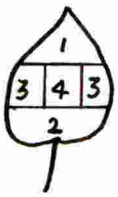
Plants

Potato plants, cultivar Home Guard, were grown from excised eyes planted in John Innes potting compost. At a temperature of 20-25°C and a 16h photoperiod, plants were ready for use about 9 days after emergence. Grapevine seedlings were raised from seed taken from vines of the cultivar Trollinger. Seedlings at the 2-3 leaf stage were transferred to the conditions detailed above and used at about the 3-4 fully expanded leaf stage.

Quantitative uptake and redistribution studies

Ten 0.2µl droplets of the application solutions were applied to the upper surface of terminal potato leaflets or vine leaves using a Burkard Pax 100 microapplicator and syringe fitted with a PTFE coated cannula. For both species, the last fully expanded leaf was used. Droplets were deposited in a close group with approximately 2mm between individual droplets to avoid coalescence. Each treatment was replicated fourfold. Accurate determination of the applied dose was achieved by depositing single 0.2µl droplets of the application solutions on small (c. 5x5mm) Parafilm targets. Three droplets were applied to targets before and another 3 after application to the plants. The targets were placed individually in 10ml of scintillation cocktail (naphthalene 200g, 2,5-diphenyloxazole 10g, 1,4-dioxan 2l) in 20ml glass vials. Following application, treated plants were returned to the previously defined environment.

Sampling of surface residues 1, 4, 8 and 11 days after application was by removal using the cellulose acetate (CA) stripping technique described by Silcox & Holloway (1986). CA strips were placed individually in 10ml of scintillation cocktail and radioactivity measured using an Intertechnique SL30 Scintillation Spectrometer. Surface recoveries were expressed as percentages of the applied dose. To estimate internal residues, surface stripped leaves were dried flat between layers of blotting paper sandwiched between sheets of plate glass. Leaves were dissected as shown in Fig.1 and the fractions submitted for combustion analysis using a Packard 307 sample oxidiser fitted with an Oximate 80 robotic arm. Carbosorb E and Permaflur scintillation cocktail were used to absorb $^{14}CO_2$ and a Beckmann LS5000TD scintillation counter used to quantify the radiolabel. Accurate determination of the applied dose was achieved by depositing single 0.2µl droplets of the application solutions onto potato or vine foliage and excising the treated area (*c.* 5x5mm). Tissue pieces were kept in a deep freezer and analysed with the dried samples. Three droplets were applied before and after application to the experimental plants. Internal residues were expressed as a percentage of the applied dose and together with the surface residues used to prepare a mass balance summary.



1 distal portion
2 proximal portion
3 lateral portions (combined)
4 application (central) zone

Figure 1, Details of dissection of potato leaflets for combustion analysis

Autoradiograph studies

For autoradiograph studies of foliar uptake and redistribution, the application, surface recovery and drying of specimens was done exactly as detailed above. To investigate the fate of applications to potato stems, a single 1µl droplet of undiluted radiolabelled compound (approximately 1µCurie) was applied to the stem base just above soil level. At sampling, 3, 7 and 10 days after treatment, surface residues were removed using CA and whole plants dried in the same manner as for foliage. There were two replicates of each treatment. After pressing and drying, samples were held firmly in place against a plate glass sheet using thin plastic film of the catering variety. X ray plates were tightly pressed against the samples using plate glass sheets securely taped together. To provide padding and apply even pressure of the X ray plates against lumpy tissue, such as potato stems, a layer of 'bubble wrap' was interposed between the glass sheets and X ray plates. Depending on anticipated levels of radiolabel in the tissue, X ray plates were exposed for periods of up to 10 weeks before developing.

RESULTS

Quantitative uptake and redistribution studies

One day after application, 23% of the applied radiolabel was recovered from the surface of potato leaflets (Table 1). By 4 days after application and until the end of the experiment, 7 days later, 5% or less of the applied radiolabel was recovered from leaflet surfaces. Combustion analysis of tissue samples confirmed that the vast majority of the applied radiolabel was present within the leaflet tissues. Movement from the site of application occurred rapidly, 5% of the radiolabel being recovered from the leaflet tip 24h after application. The amount of radiolabel recovered increased steadily throughout the duration of the experiment and 11 days after application 17% of the applied label was found in the leaflet tip and over 7% in the leaflet margins. Unaccounted losses of label were small and within acceptable limits for mass balance studies.

Table 1, Mass balance summary for radiolabel from ^{14}C propamocarb hydrochloride applied to potato and vine leaves.

	PERCENTAGE RECOVERY					
DAYS AFTER APPLICATION			LEAF PORTION			
POTATO	SURFACE	CENTRAL	DISTAL	PROXIMAL	LATERAL	'LOSS'
1	22.5	61.5	5.2	1.3	2.8	6.8
4	5.4	78.6	6.2	0.9	4.0	4.8
8	1.2	69.0	15.1	0.6	4.1	10.0
11	3.8	64.8	17.1	0.5	7.6	6.2
VINE						
1	91.0	2.5	0.3	0.4	0.3	5.6
4	78.9	10.1	0.4	0.3	0.4	10.0
8	74.0	10.2	0.4	0.3	0.4	14.7
11	63.5	21.4	0.4	0.4	0.7	13.6

Surface and tissue recoveries from grapevine leaves were distinctly different from those of potato leaflets (Table 1). After 1 day, 91% of the radiolabel was recovered from the leaf surface and 10 days later 64% was still present on the surface. Radiolabel was only recovered in significant amounts from tissue directly underlying the application zone. No evidence of movement of radiolabel away from this zone was found. Unaccounted losses were about 10% greater than those in the potato experiment and may indicate that another loss process was involved.

Figure 2. Autoradiograph of a potato plant 10 days after application of 1μCurie of ^{14}C propamocarb hydrochloride to the stem base.

Autoradiograph studies

Autoradiographs of potato leaflet and vine leaf tissues confirmed the findings of the mass balance studies. Figure 2 shows an autoradiograph of a whole potato plant 10 days after application of ^{14}C propamocarb hydrochloride to the stem base. All tissues, both those present at the time of application and all foliage produced subsequently, acropetal to the application zone, show the presence of radiolabel. Subjective analysis of the autoradiograph seems to indicate a higher concentration of radiolabel in the stem than in the leaves.

DISCUSSION

Metabolism studies carried out by AgrEvo on a wide variety of plant species have shown that only minimal amounts of propamocarb hydrochloride are metabolised in plant tissues. The assumption that radiolabel recovered in these studies is still in the form of the parent compound is, therefore, believed to be valid.

The mass balance study indicates that the uptake of propamocarb hydrochloride into potato leaf tissue is rapid and considerable. Both this study and the autoradiographic evidence show that propamocarb hydrochloride is rapidly redistributed acropetally from the point of uptake. Redistribution throughout the plant occurs when propamocarb hydrochloride is applied to potato stems. Separate studies by Bardsley & Thompson (1995) have demonstrated that late blight control products based on propamocarb hydrochloride possess excellent rainfastness. The rapid and considerable uptake of propamocarb hydrochloride into potato foliage probably contributes to the observed rainfastness by removing it from the influence of loss processes such as wash off by rain. Rapid and significant redistribution after uptake also means that propamocarb hydrochloride is able to protect tissues developing after application. On a local basis this would enable immature leaves to be fully protected by deposits of fungicide landing on them when not yet fully expanded. Over greater distances, propamocarb hydrochloride deposited on stems would be redistributed throughout the whole plant. This would not only enhance fungicide levels in foliage directly intercepting spray droplets but also protect new foliage developing within the normal late blight spray interval of around 10 days. It is proposed that the apparently high concentration of propamocarb hydrochloride in stems serves to control stem blight and acts as a reservoir to protect newly developing tissues. Under field conditions, the magnitude of this effect will depend on how much spray is deposited on stems.

Although the uptake of propamocarb hydrochloride into grapevine foliage is substantially less than into potato foliage, a more significant difference is the absence of redistribution away from the tissues underlying the application zone. The activity of existing propamocarb hydrochloride containing products against vine downy mildew is not sufficient to merit commercialisation for control of this disease and it is possible that the lack of redistribution is a major contributor to this shortfall in activity. It is very unlikely that the lack of redistribution can be overcome since redistribution is almost certainly a result of the an interaction between the physico-chemical properties of the fungicide molecule and the host physiology. Redistribution will probably have to be achieved on the leaf surface by the use of suitable spreaders, possibly in combination with uptake enhancing adjuvants.

The consistently greater unaccounted losses of radiolabel from grapevine samples compared with potato merit consideration. Propamocarb hydrochloride has a vapour pressure of 0.8mPascals at 25°C which is relatively high for foliar applied fungicides. Propamocarb base, which is formed from propamocarb hydrochloride in an alkaline environment, possesses considerably greater volatility; 0.73Pascals at 25°C. Because uptake into grapevine is slower and less extensive than for potato, more residues of active ingredient will remain on a grapevine leaf than on a potato leaf surface. This potentially allows losses from volatility to be quantitatively greater on vine than potato, especially if conversion of some or all of the propamocarb hydrochloride residues to propamocarb base occurs.

These experiments have provided useful data to help optimise the use of propamocarb hydrochloride for potato late blight control and helped resolve the issue of why propamocarb hydrochloride has not controlled vine downy mildew satisfactorily in the field. The differences in uptake and redistribution of propamocarb hydrochloride in potato and vine also demonstrates that the development of 'model systems' must be approached with caution if cross species extrapolation of results is planned, even within monocotyledons or dicotyledons.

ACKNOWLEDGEMENTS

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