POSTER SESSION 7B

INTEGRATED CROP MANAGEMENT – EXPERIMENTAL RESULTS

Session Organiser	Dr D H K Davies *SAC, Edinburgh, UK*
Poster Papers	7B-1 to 7B-7

Intercropping for pest control: the role of predators

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ABSTRACT

Intercropping with white clover has shown potential as a means of insect pest management in cabbages, possibly due to increased predatory activity. In order to clarify the effect of surface-active predators such as carabid beetles, cabbages intercropped with alternate rows of white clover, and monocropped cabbages, were either enclosed in barriers to exclude predators or were left unenclosed and accessible to predators. Predation was found to play a significant part in reducing numbers of cabbage root fly eggs and plant mortality in both intercropped and monocropped cabbages. There was also evidence that the intercropped plots acted as refuges for at least one of the predatory carabid beetles which were nocturnally active in the monocropped plots. Nevertheless, in terms of pest control, intercropping was superior. Even when predators were excluded, mortality of intercropped cabbages was still slightly less than that of monocropped cabbages to which predators had access. Consequently, the benefits of intercropping for pest control would be lost with more separation of the clover and cabbages.

INTRODUCTION

There is increasing demand for alternatives to chemical pest control and, as conventional monocultures tend to favour insect pests, one alternative may be to modify the environment in the crop to the pests' disadvantage. Intercropping (mixing two or more crops together in the same field) is one way to do this. One reason that intercropping can be effective at suppressing pests seems to be that the mixture of plant species physically interferes with pests' ability to find or react to their host plant, but another reason may be that mixed crops harbour more natural enemies which prey on the pests (Altieri & Letourneau, 1982). Experiments have shown that intercropping has potential as a means of pest control in temperate crops, particularly vegetables. In the Netherlands, undersowing with clover has given significant control of some brassica pests, with improvements in crop quality (Theunissen *et al.*, 1992). By contrast in Scotland, the same system has tended to give significantly smaller cabbages and unacceptable yields, even though pests, in particular cabbage root fly (*Delia radicum*), have been effectively suppressed (McKinlay *et al.*, 1996). Separating the clover and cabbages into alternating rows has given acceptable yields while maintaining effective suppression of cabbage root fly (Armstrong *et al.*, 1996), but this is a difficult system to apply on a field scale compared to undersowing. In the field, it would be easier to manage more widely separated strips of clover with several rows of cabbages between, but too much separation between the two plant species would reduce the physical effect of intercropping on pests. Nevertheless, such an arrangement might be effective if predators played an important part in reducing the numbers of pests. Recent work has shown that some carabid beetles (Carabidae), including species known to prey on cabbage root fly eggs and larvae, make short-term nocturnal forays from dense vegetation such as clover into open crops (Chapman & Armstrong, 1996).

Consequently provision of clover strips as refuges in an otherwise conventional monoculture might generate enough predatory activity to give acceptable pest control.

The aim of this project was to determine the importance of predation in suppressing pests in cabbages intercropped with clover (using the system of alternate rows of cabbage and clover) and in adjacent monocropped cabbages. The target pest was cabbage root fly and special attention was given to carabid beetles as predators.

MATERIALS AND METHODS

Experiments were carried out in 1997 and 1998 near Aberdeen. In both years there were eight plots of cabbages (var. Primo) intercropped with white clover (var. Gwenda) and eight plots of monocropped cabbages which were hoed each week. The plots were 5m x 5m and were arranged in two blocks of eight, with intercropped and monocropped plots alternating. In the intercropped plots, the clover was already established in strips approximately 30cm wide with approximately 40cm width of bare soil between when the cabbages were transplanted. In 1997, clover from the previous year was retained in one block, but the other block of the experiment was cultivated in the spring and the clover was sown at the beginning of April. In 1998, the clover from the previous year was retained in all intercropped plots. The cabbages were planted into the soil between the clover strips in the first week of June in 1997 and in the second week of May in 1998. The soil in the cabbage rows was weeded each week and the clover was trimmed to prevent it from encroaching on to the cabbages.

The experiment was based on the use of barriers around individual cabbage plants to exclude surface-active predators but not cabbage root fly. Cabbage seedlings were planted into pots 17.5 cm (1997) or 12.5 cm (1998) in diameter, which were filled to the top with soil from the site. These pots were then buried in the experimental plots, either with the rim raised above the surrounding soil to form an overhanging one-way barrier about 2.5 cm high, or with the rim flush with the surrounding soil to allow predators access. In 1997, two barrier and two control pots were set in each plot among 50 conventionally transplanted cabbage seedlings (which are not considered in this paper). In 1998, 15 barrier and 15 control pots were used in each plot, and these accounted for all of the cabbages used in the experiment.

The effectiveness of the barriers at excluding predators was tested using *Drosophila melanogaster* pupae as bait. In each plot, five pupae glued to 1 cm^2 pieces of card were placed around one cabbage seedling in a barrier pot and one in a control pot. Baits were set at 08:00 and the number of pupae which had been eaten during the day was recorded at 20:00. More bait was then set at 20:00, and the number of pupae eaten during the night was recorded at 08:00 the following morning. The procedure was repeated after one week and total consumption of pupae in each plot recorded.

The effect of predation on cabbage root fly was measured by recording numbers of cabbage root fly eggs and, in 1998, cabbage mortality. Cabbage root fly eggs were extracted by flotation in saturated brine from soil taken from the base of cabbage plants. In 1997, the two barrier and control plants in each plot were used for soil samples, and soil was collected each week from 16 June until 30 August. In 1998, one barrier and one control plant in each plot was used for soil sampling. Four collections from the same plants were made on 23 May, 30

May, 6 June and 28 June. In 1998, the numbers of cabbages killed by cabbage root fly were recorded on 8 July. Twenty plants (two rows) were assessed in each plot, and plants used for soil sampling were not included. Root damage and the presence of larvae or pupae confirmed the cause of death.

The activity of carabid beetles was measured in 1997 using three pitfall traps in each plot, which were changed each week. The traps were placed in one of the cabbage rows. In August 1997, to determine whether beetles moved between the intercropped and monocropped treatments, 1 m^2 of clover at the edge of an intercropped plot and an adjoining 1 m^2 of monocropped plot were enclosed by a plastic barrier 15 cm in height, buried to a depth of 10 cm. Two such enclosures were set up between each pair of intercropped and monocropped plots. One week later, the monocropped and intercropped halves of each enclosed area were separated by another barrier, thus trapping carabids in the half that they were in when the barrier was set up. The central barrier was set up in one enclosure in each plot at midday, and in the other at midnight. Pitfall trapping was then carried out for one week in each half of the enclosed areas.

RESULTS

In 1997, plant cover in the newly sown clover strips increased from 80% to 100% during the four weeks after the cabbages were transplanted, whereas in the one year old clover, plant cover was 100% throughout (Fig. 1). The newly sown clover strips were initially dominated by weeds, particularly spurrey (*Spergula arvensis*) but when the cabbages were planted clover was dominant, as it was in the one year old strips. In 1998, plant cover in all intercropped plots was similar to the one year old clover in 1997. Both years, weed cover between the clover strips and in the monocropped plots never exceeded 40% and was usually maintained below 30%. For the purposes of this paper, no further distinction is made between recently established and one year old clover.

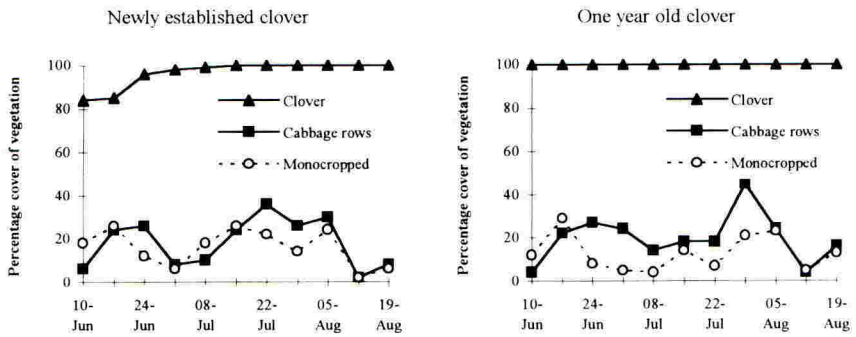

Figure 1. Plant cover in intercropped and monocropped cabbages, 1997.

In 1997, the bait cards set out during the day were destroyed by birds. However, barriers significantly reduced the proportion of *Drosophila* bait eaten during the night in both 1997 and

1998 and in both intercropped and monocropped plots (Table 1). In 1998, when the plots were protected from birds by nets, most predation on *Drosophila* bait was found to occur at night. There was no significant effect of intercropping on number of pupae eaten in 1997, but in 1998 significantly more pupae were eaten in the intercropped control than in the monocropped control pots.

Table 1. Mean arcsine-transformed proportions of *Drosophila* bait eaten in intercropped and monocropped cabbages (± SEM, * $P < 0.05$, n=8).

		Barrier	No barrier	
1997 - night				
	Intercropped	0.15 ± 0.04	0.64 ± 0.11	*
	Monocropped	0.18 ± 0.06	0.54 ± 0.12	*
		n.s.	n.s.	
1997 - day				
	Intercropped	0	0.09 ± 0.04	*
	Monocropped	0	0.04 ± 0.03	*
			n.s.	
1998 - night				
	Intercropped	0.15 ± 0.04	0.60 ± 0.11	*
	Monocropped	0.14 ± 0.03	0.30 ± 0.08	*
		n.s.	*	

Table 2. Mean numbers of cabbage root fly eggs collected from cabbage plants with and without predator exclusion barriers (1997 and 1998) and mean arcsine-transformed proportions of cabbage plants killed by cabbage root fly (1998) in intercropped and monocropped plots (*$P < 0.05$).

		Eggs 1997	Eggs 1998	Mortality 1998
Intercropped				
	Barrier	8.06	8.00	0.27
	No barrier	2.12	2.25	0.06
Monocropped				
	Barrier	7.79	8.00	0.58
	No barrier	1.56	2.60	0.29
SED		2.55	1.43	0.06
Effect of barrier (n=16)		*	*	*
Effect of intercropping (n=16)		n.s.	n.s.	*

Significantly more cabbage root fly eggs were collected from plants in barrier pots than from plants in control pots in 1997 and 1998, but there was no significant effect of intercropping (Table 2). In 1998, a significantly higher proportion of enclosed than unenclosed plants was

killed by cabbage root fly (Table 2). The proportion of plants killed was also significantly higher in the monocropped plots than in the intercropped plots. The treatment with the highest mortality was monocropped plants with barriers; more than half of these plants were killed. Slightly more unenclosed monocropped plants were killed than enclosed intercropped plants.

In 1997, fifteen species of carabid occurred in numbers averaging one or more individuals per pitfall trap over the eight weeks of collecting. Six of these species were collected in significantly higher numbers in intercropped plots, but nine species were collected in significantly higher numbers in monocropped plots. Species which were collected in higher numbers in the intercropped plots included *Pterostichus melanarius*, *Loricera pilicornis* and *Agonum dorsale*. Species which were collected in higher numbers in monocropped plots included *Bembidion tetracolum*, *B. lampros* and *Trechus quadristriatus*.

After the daytime and night-time barriers were set up, only *T. quadristriatus* was collected in sufficient numbers in the enclosed areas to compare treatments (Table 3). Significantly more individuals of *T. quadristriatus* were collected in the monocropped plots which were enclosed during the night than in those enclosed during the day, but time of enclosure had no effect on catches in intercropped plots.

Table 3. Mean numbers of *T. quadristriatus* collected in intercropped and monocropped cabbages enclosed during the day or during the night (± SEM, * $P < 0.05$, n=8).

	Day-enclosed	Night-enclosed	
Intercropped	1.00 ± 0.42	1.63 ± 0.53	n.s.
Monocropped	0.75 ± 0.41	4.10 ± 1.11	*

DISCUSSION

Use of *Drosophila* baits indicated that the barriers were effective at reducing predation around the cabbage plants. The plants were not enclosed from flying insects, therefore these results also show the importance of predation by invertebrates which move around on the soil surface, such as carabid beetles. The barriers were not effective against slugs which were active in all plots. Consequently, although it is possible that slugs may have eaten some of the baits, this effect would be independent of the effect of barriers.

The experiment with *Drosophila* baits provided strong evidence that predation was the reason for the significant differences in numbers of cabbage root fly eggs and plant survival between enclosed and unenclosed plants, in both monocropped and intercropped cabbages. Comparison of numbers of cabbage root fly eggs alone suggests that intercropping should have given little advantage over monocropping for the control of this pest. However, the difference in plant survival in 1998 showed that intercropping was the more effective of the two systems. In terms of plant survival, intercropping with predators excluded was comparable to monocropping with predation.

Carabid beetles are not the only cabbage root fly predators. However, *T. quadristriatus* is a known predator of eggs and larvae, and based on the results obtained for this species the amount of predation in the monocropped plots may have been influenced by the proximity of the intercropped plots. Pitfall trapping after setting up day and night time barriers showed that *T. quadristriatus* was less numerous in the monocropped plots during the day than during the night, at least up to a distance of 1m from the intercropped plots. This was one of the species which was collected in higher numbers in the monocropped plots by unenclosed pitfall trapping and it seems, therefore, that individuals were moving out from the intercropped plots during the night.

Provision of refuges for nocturnally active species may well increase the amount of predation in otherwise conventional monocultures, but in terms of crop protection it seems that intercropping is still more effective with the two plant species close together throughout the crop. To apply intercropping in Scotland it may, therefore, be necessary to experiment with less aggressive alternatives to clover.

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Spatial recovery of two species of Carabidae following cumulative pesticide applications in winter wheat

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ABSTRACT

The cumulative effects of insecticide applications on carabid populations over a two year cropping period were assessed between 1993 and 1996. Experiments were undertaken in paired winter wheat fields which shared a common boundary. Winter and summer sprays of deltamethrin were applied to one field and resident invertebrates in both fields were sampled using a matrix of pitfall traps within the field and either side of the boundary.

Analyses concentrated on the carabid species *Trechus quadristriatus* and *Pterostichus cupreus*. Analyses of recovery rates and crop reinvasion indicated that although pesticides lowered the number of beetles caught in traps for up to 6 weeks following sprays, the cumulative effect of applications did not have long term deleterious consequences. The significance of these results regarding the effects of cumulative insecticide applications on invertebrates and requirements for statutory testing procedures are discussed.

INTRODUCTION

The perceived threat from insect mediated crop losses within modern agricultural production results in large-scale insecticide inputs, often over consecutive seasons. Although the implications of single insecticide applications on populations of beneficial species are relatively well studied (Jepson, 1989; Asteraki *et al.*, 1992) the long term, cumulative effect of repeat applications over more than one year are less well understood.

Reinvasion of the crop by beneficial arthropods has previously been studied by Jepson & Thacker (1990), who focused in particular on family groupings of Staphylinidae, Carabidae and Linyphiidae and their relative rates and sources of reinvasion into insecticide treated fields. They concluded that univoltine carabid species, which complete their life cycle within the field and its immediate boundaries, might be at greater risk from continued exposure to insecticides due to their limited potential for dispersal. However, there is great variation in dispersal rate within invertebrate family groupings (Thiele, 1977; Mitchell, 1963) and a more detailed analysis of potential detrimental effects on individual insect species is required if

accurate assessments of risk are to be made. The current study investigated the impact of continuous cropping over two seasons with regular insecticide inputs, on the numbers of resident non-target arthropods. Preliminary analysis of data collected over a two-year study of cumulative insecticide applications on population numbers suggested that grouping large numbers of different species could mask high levels of variation that occur at the species level (Walters *et al.*, 1997).

This paper will present the analysis of the spatial recovery of two common carabid species (*Trechus quadristriatus* (Schrank) and *Pterostichus cupreus*) over two cropping seasons with common insecticide regimes, to determine relative recovery rates and variation in reinvasion source.

MATERIALS AND METHODS

A series of four, two-year experiments have been completed at two sites (ADAS Drayton and ADAS Boxworth), between 1993 and 1996. At each site a pair of fields sharing a common boundary were selected and each field was drilled with winter wheat for two consecutive seasons.

A grid of 16 pitfall traps was established in each field (20m between traps) with a further 5 pitfall traps positioned adjacent to each side of the boundary (Fig. 1). Invertebrate trapping started in September of the first growing season and was conducted over 7 day periods every third week until April. Thereafter traps were operated continuously and emptied at 7 day intervals.

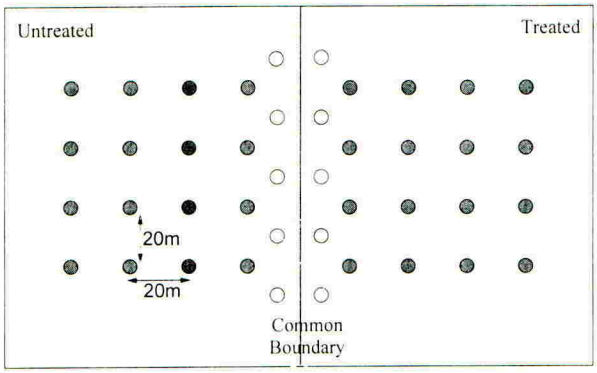

Figure 1. Pitfall trap layout in treated and untreated fields.
● Field trap ○ Boundary trap

One field was left unsprayed with insecticide to provide control data while the second field was subjected to a single autumn application and a summer application at GS61 (Zadoks *et al.*, 1974) of deltamethrin, for each of the two cropping seasons. Initial analysis of the data indicated that *Trechus quadristriatus* and *Pterostichus cupreus* were particularly abundant at

ADAS Drayton (1994-96) and ADAS Boxworth (1993-95) and therefore these sites were chosen for more detailed analysis. Data from the trap catches at both sites were used to determine the source and rate of reinvasion for each carabid species by comparing recovery time with the distance between the trap and both the common boundary and other external boundaries.

Within and between-field variation was defined as the ratio of the standard error of the mean of the pre-treatment trap catches in control and treated fields. Recovery time was then measured as the time taken for the difference between the mean numbers of each carabid species caught in the sprayed and unsprayed fields to be within this value (Jepson & Thacker, 1990). Recovery time for each trap position in the sprayed area was regressed against the distance of that trap from the common boundary and also its distance from the nearest two external boundaries. Large trap catches of *Trechus quadristriatus* at Drayton (1994-96) allowed further analysis to investigate the effects of numbers of pesticide applications, and hence consecutive sprays, on the recovery of carabids in sprayed fields.

RESULTS

Total numbers of beetles trapped over the two year sampling period indicated that catches declined following application of insecticide, remained low for several weeks but then recovered to reach similar numbers in both sprayed and unsprayed fields (Fig. 2).

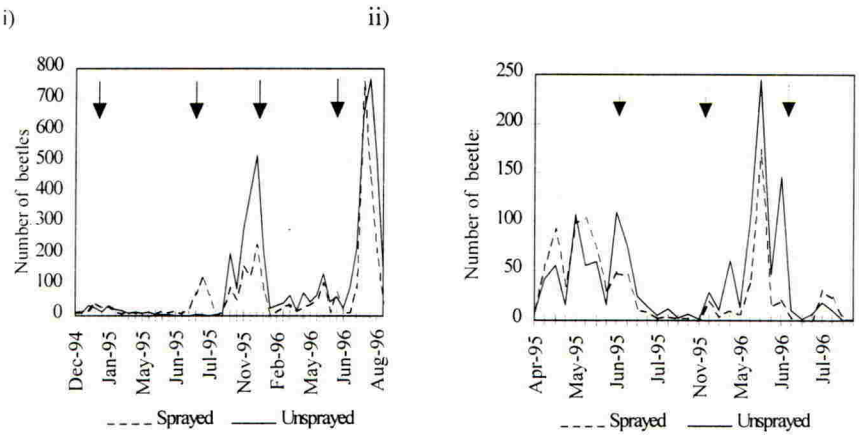

Figure 2. Total number of i) *T. quadristriatus* and ii) *P. cupreus* trapped during the two year sampling period (↓ = timing of insecticide application).

A positive linear relationship between recovery time and the distance of trap from the control field was recorded for both species (*P. cupreus*, $R^2 = 0.9$; *T. quadristriatus*, $R^2 = 0.6$; Table 1) at Boxworth. No significant linear relationships were observed when regressing the recovery time against the distance from each trap to its nearest external boundary.

Table 1. Linear regression equations for each species for both sites in successive years (box93 =ADAS Boxworth 1993-95; dra94 =ADAS Drayton 94-96).

Species	Site	Recovery period	Regression equation	R^2	P value
P. cupreus	box93	1993	y = 0.52x + 9.8	0.97	<0.001
		1994	y = 0.14x + 12.6	0.50	<0.1
P. cupreus	dra94	1994	y = 0.42x + 7.0	0.95	<0.001
		1995	y = 0.04x + 11.2	0.13	NS
T. quadristriatus	box93	1993	y = 0.14x + 9.74	0.56	NS
		1994	y = 0.07x + 12.54	0.51	NS
T. quadristriatus	dra94	1994	y = 0.07x + 8.4	0.12	NS
		1995	y = 0.21x + 26.6	0.10	NS

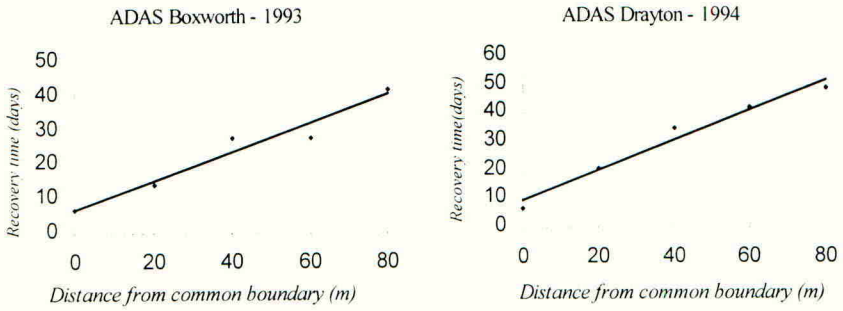

Figure 3. Recovery time and distance from common boundary in first year of sampling for *Pterostichus cupreus*.

At both sites *P. cupreus* showed a significant linear relationship between recovery time and distance from the common boundary in the first season (P<0.001, Fig. 3). This relationship is not statistically significant in the second year of sampling (P<0.1). For *T. quadristriatus*, although not statistically significant, the relationship with the common boundary explained considerably more of the variation (56%) than that with the external boundaries at both sites (1%).

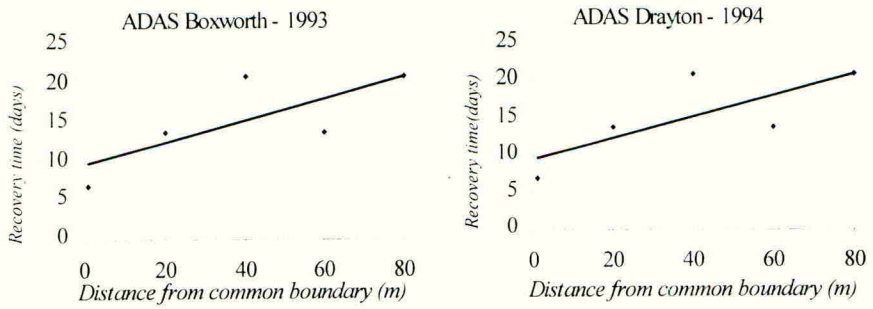

Figure 4. Recovery time and distance from common boundary in first year of sampling for *Trechus quadristriatus*.

The rate of recovery (the slope of the linear regression line) varied between the two species with *P. cupreus* showing a slower recovery rate at both sites than *T. quadristriatus* (Table 1). Rate of recovery for both species was increased in the second cropping period following 4 consecutive sprays. At a distance of 20m into the sprayed area recovery of *P. cupreus* took approximately 14 days while *T. quadristriatus* took only 7 days.

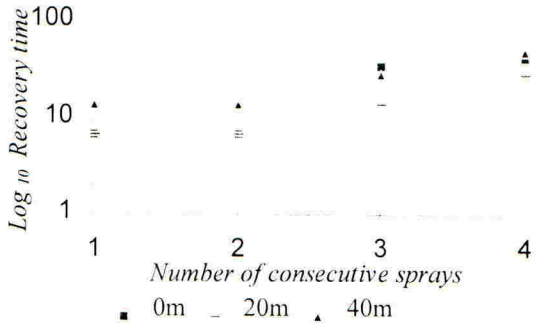

Figure 5. Recovery time for *T. quadristriatus* at ADAS Drayton (1994-96)with cumulative spray applications.

Analysis of the impact of cumulative sprays on recovery time for *T. quadristriatus* indicated that recovery time was significantly dependent on the number of sprays applied (Fig. 5). The greater the number of spray applications, the greater the recovery period. Following the first and second spray applications the traps closest to the common boundary showed the fastest rate of recovery. However, after the third and fourth sprays recovery was not linked to distance from the common boundary.

DISCUSSION

Jepson & Thacker (1990) studied the relative recovery levels and sources of reinvasion for populations of Staphyliniidae, Carabidae and Linyphiidae following exposure to a single application of dimethoate. Carabids were shown to recolonise from within the field, and to have a slower dispersal rate than the two other arthropod families. The experimentation and analyses completed in this study have highlighted the variation occurring between different species within the carabid family and the impact of cumulative insecticide applications.

P. cupreus showed a reduced potential for recovery when compared to *T. quadristriatus*, which contrasts with previous studies of the ground dispersal of the species in untreated conditions (Thiele, 1977; Mitchell 1973). However, *T. quadristriatus* is known to be a strong flyer and can thus increase its potential dispersal rate by flying as well as walking (Asteraki *et al.*, 1992; Jepson, 1989). Furthermore the dispersal of *P. cupreus* over ground would increase the likelihood of its direct contact with insecticide residues which may compound its

dispersal ability. Analysis of potential recovery sources suggested that reinvasion occurred from the adjacent unsprayed field rather than from other external boundaries.
The unexpected increase in recovery rate following the second insecticide application is explained by the method of calculating recovery time, which compares numbers in the sprayed and unsprayed areas. A reduction in numbers in the control field observed following the first insecticide application, led to a reduced time period for numbers in control and treated fields to equate following the second application.

The linear relationship between recovery time for *T. quadristriatus* and number of sprays applied indicated reduced recovery potential for this species over the two year period. Therefore, in areas with limited refuges for beneficial species, under current spraying regimes, potential for recovery of these species may be reduced.
Recovery potential is an important factor when making assessments of risk for pesticide registration. This work confirmed the study of Walters *et al.* (1997), highlighting the need for analysis at the species level to avoid masking the potential impact of insecticides on non-target arthropods when grouping large numbers of species with differing biologies in full field investigations.

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A feasibility study of the use of Integrated Crop Management for outdoor ornamentals

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ABSTRACT

The potential for use of Integrated Crop Management (ICM) systems in the hardy nursery stock sector is evaluated utilising an approach developed by the British Agrochemical Association. A series of audit questions covering all aspects of ICM was prepared. This detailed audit was then piloted in the industry, and scored for performance in reaching ICM standards. Particular attention was paid to the environmental impact of crop protection practices, overall scores were calculated, and growers were advised where improvements could be made towards achieving ICM in their own business.

INTRODUCTION

For some years the aim of crop production has been for increasing intensification, with increases in mechanisation and crop protection enabling high yields and high quality produce to be achieved resulting in good economic return. The horticulture industry has followed this trend, and consumers are particularly sensitive to the quality and appearance of their purchases. However, this increasing intensification has had detrimental effects on the environment both in farming and horticulture.

In arable farming there are many concerns with respect to pesticide use and their effect on agro-ecosystems. This has lead to the development of Integrated Crop Management (ICM) systems, which aim to balance the requirements of running a profitable business with responsibility and sensitivity to the environment. (BAA 1996). In the fruit and vegetable sector of the horticulture industry, the big supermarket chains have lead the way by producing integrated crop management protocols for quality assurance in individual crops (e.g. Nature's Choice programme of Tesco PLC). However, such an approach has not been considered for the production of non-food crops in the hardy nursery sector. This sector of the industry faces greater problems than either the vegetable sector or arable farming when it comes to crop protection. There is a lack of approved products as the sector only represents a very small proportion of the overall potential market for agrochemicals. In general the hardy nursery stock sector is making great use of broad acting compounds that deal with many problems, the opportunity to choose target specific chemicals which might be less harmful to the environment is simply not present. Many growers are not aware of the details of ICM, nor how they could benefit from using the concepts of it to assist them to reduce their reliance on chemicals whilst improving the overall performance of their business. This study aimed to examine the concepts of ICM and customise them for the hardy nursery stock industry.

MATERIALS AND METHODS

A detailed study was made of the ICM training pack and associated literature produced by the British Agrochemical Association (BAA 1996). This approach considers arable farm management under nine headings, which are: Site, Organisational Management, Waste and Pollution Management, Crop Rotation, Energy, Soil Management and Crop Nutrition, Wildlife and Landscape Management, Crop Protection, and finally Monitoring and Auditing. A wide variety of other related literature was examined, including the monthly ADAS Notes (Anon, 1995, 1996, 1997 and 1998). In addition some preliminary site visits were made, in order to ascertain the concerns of the growers, and in the case of garden centres, the perceived pressure from consumers. The next stage of the process was to design an audit questionnaire that covered all aspects of ICM with respect to the hardy nursery stock industry. The resulting questionnaire, organised under the nine headings listed above, had a around 250 questions. As examples, the audit questions for Waste and Pollution Management and Site are in Tables 1 and 2. The audit questionnaire was then given ethics approval by the University of Hertfordshire Natural Sciences Ethics Committee (protocol number 5/11/97U) and used as an interviewer administered questionnaire at several hardy nursery stock enterprises. Interestingly, all growers wished to remain anonymous.

Table 1. Final audit questions for Waste and Pollution Management.

	Yes	*No*	*Why/Comments*
Is a programme for waste disposal in practice?			
Are all COSHH forms filled in?			
Is pesticide store locked and frost proof.?			
Are powders stored above liquids?			
Are pesticide containers disposed of properly?			
Are unused pesticides disposed of properly?			
Are pesticides disposed of by approved contractors?			
Are calculations carried out to make sure that the exact amount of pesticide needed is mixed and sprayed?			
Are checks made on conditions before spraying?			
Are operators trained & certified?			
Are sprayers calibrated?			
Are checks made when spraying near watercourses?			
Is fuel stored on site?			
Are wastes recycled wherever possible?			
Are items re-used wherever possible?			
Is cardboard/ paper recycled?			
Are plastic based pots and containers re-used?			
Is woody waste chipped/ shredded to be used as mulch?			
Are plant residues composted?			
Is care taken when burning?			
Are staff aware of emergency procedures?			

Table 2. Final audit questions for Site.

	Yes	*No*	*Why/Comments*
Total area of nursery?			
Crops Grown-Trees, Shrubs, Ornamentals, Herbaceous etc. (List).			
Are nursery 'windbreaks' in good order?			
Are hedges present?			
Are hedges cut?			
Are they cut to specific heights for wildlife purposes?			
Is woodland maintained as a wind break?			
Are eyesores concealed by trees/ shrubs?			
Are fences in place to keep site secure?			
Is fencing in place to prevent damage from rabbits etc.?			
Are barriers place to prevent erosion?			
Is an area of shade provided?			
Are overhead irrigation systems checked for alignment and efficiency?			
Are all irrigation systems checked for leakage/ blockages?			
Are nozzles cleaned and checked regularly for blockages?			
Are buildings repaired and maintained?			
Are pipes insulated against freezing up?			
Are nettings/coverings/ tunnels checked and repaired if necessary?			
Are access roads regularly checked and repaired if necessary?			
Are there any public rights of way?			
Are wildlife-rich areas identified?			
Are pollution vulnerable areas identified?			
Are areas identified as those in need of improvement?			
Are previous problems identified and considered?			
Is ease of access considered?			
Is location to domestic residences considered?			
Is location to market considered?			

RESULTS

The questionnaires took about one and a half hours to administer at each enterprise. As an example, the results of the survey from two growers for Wildlife and Landscape Management are shown in Table 3. In addition to the survey, the individual results were also scored to give a simple 'eco performance' rating. This was somewhat subjective, with 'eco-friendly' answers receiving +1, 'eco-unfriendly' answers receiving -1 and neutral answers receiving zero. The results for this eco-rating appear in Table 4. Recommendations were then drawn up for the grower for each section, and finally flow charts were prepared for each ICM topic (Fig. 1), as an aid to growers to improve their performance section by section.

Table 3. Results for questions on Wildlife and Landscape.

Question	Site 1		Site 2	
	Y	N	Y	N
Is a conservation plan in place?				
Are tree and hedge boundaries checked and improved/ replanted if necessary?				
Are trees and hedges pruned before spring nesting starts?				
Are buildings kept in good repair/repaired when necessary?				
Are waterbodies monitored?	N/R		N/R	
Is care taken when spraying near water?	N/R		N/R	
Is care taken when spraying and applying fertilisers/ herbicides to avoid wildlife habitats?				
Are wildlife havens identified and mapped?				
Are footpaths kept in good condition?	N/R			
Are hedge and tree management plans in place?				
Is the view from off-site considered and maintained?				
Are indicator species monitored?				
Are records kept?				
Are staff trained and involved in maintaining wildlife aspect?				
Are uncultivated strips left?				
Are local people informed of spraying?	N/R			
Are MAFF publications read?				

Response Y =Yes

N/R Not Relevant N = No

Table 4. Summary of plus and minus 'eco-scores' from sites 1 and 2.

	Waste and pollution	Crop rotation	Crop protection	Site	Soil and crop nutrition
Site 1	13+ 7-	3+ 2-	35+ 20-	23+ 1-	18+ 2-
Site 2	11+ 8-	7+ 2-	39+ 12-	18+ 7-	17+ 10-

	Energy	Wildlife and landscape	Monitoring and auditing	Organisational management
Site 1	7+ 6-	7+ 6-	27+ 10-	16+ 1-
Site 2	6+ 10-	6+ 9-	19+ 19-	14+ 3-

+ = 'eco-friendly' response

- = 'eco-unfriendly' response

The apparent imbalance between questions is due to a) 'eco-neutral' answers and b) not relevant responses.

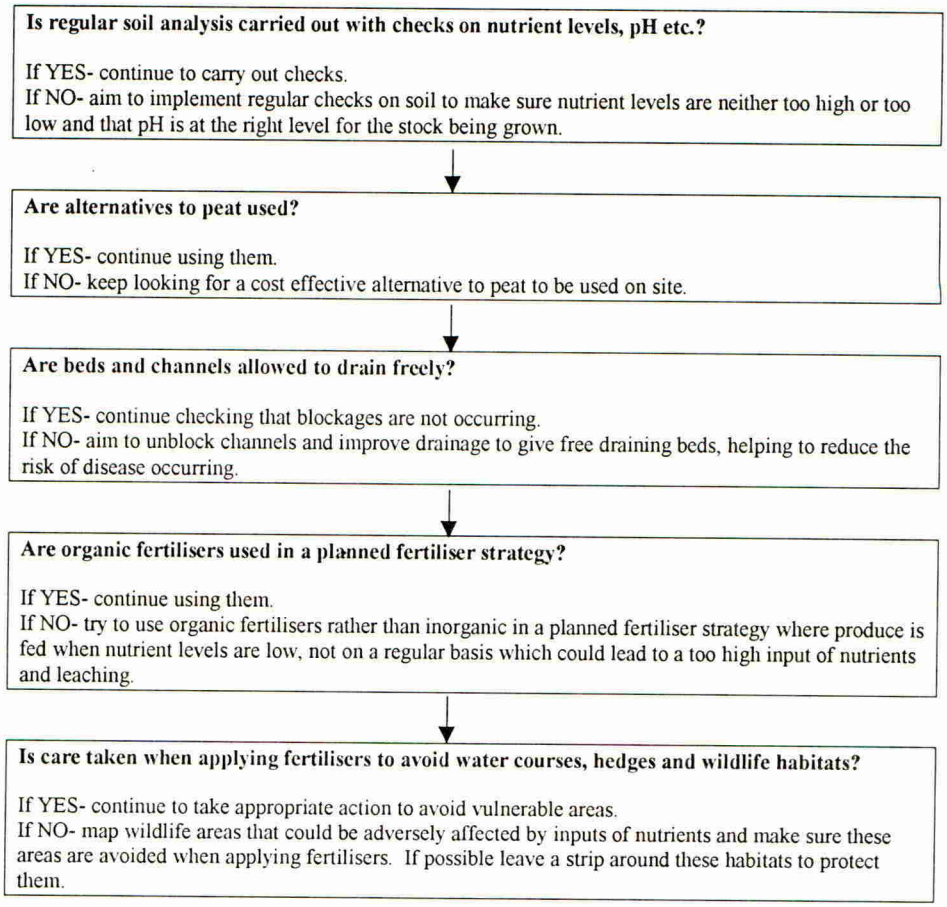

Figure 1. Flow diagram for Soil Management and Crop Nutrition.

DISCUSSION

Nearly all the growers who were approached expressed interest in participating in the survey. Interestingly, all growers who did participate in the project expressed great surprise at the range and scope of an ICM programme. Apparently they were unaware of the literature that has been aimed at arable farmers. The growers who participated were very ready to give honest answers to the survey questions, even when those answers did not necessarily give them credit (e.g. a grower who admitted that he did not fill in COSHH forms, or another who admitted that the chemical store was not properly organised). The

ready to give honest answers to the survey questions, even when those answers did not necessarily give them credit (e.g. a grower who admitted that he did not fill in COSHH forms, or another who admitted that the chemical store was not properly organised). The enterprises recorded in the results section were of very different size, both in terms of numbers of employees and numbers of hectares and range of crops produced. However they were both doing well in some sections of the survey and less well in others, though the sections that each one excelled in did not appear to be linked to the size of the enterprise. One or two worrying features were recorded, particularly the grower who was applying large quantities of manure to a site by a river, and then subsequently ploughing it. He seemed to have no knowledge of possible run-off problems. Also, though there is a very limited range of pesticides available, it was surprising that one grower had no sprayer washings to dispose of, as he did not wash the sprayer out unless he thought that there might be a problem of 'incompatibility'. As a general rule, he backed sprayed over the part of the crop that he had already sprayed until the sprayer was empty. The next compound was then put into the sprayer. In one case no staff meetings or staff training took place.

At this stage the 'eco-ratings' are both subjective and crude, and little reliance should be placed on them. However they are interesting in that they do show that hardy nursery stock growers, like arable farmers, could benefit from taking a more holistic approach to crop management.

CONCLUSION

It would be feasible to customise ICM from arable farming for the hardy nursery stock industry, and the resulting audit could be used to enable growers not only to run more environmentally conscious enterprises but also to improve their business management .

ACKNOWLEGEMENTS

We would like to thank the growers who gave up their time to be interviewed, and M. E. Smith for technical assistance.

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The impact on non-target arthropods of integrated compared to conventional farming: results from the LINK Integrated Farming Systems project

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ABSTRACT

In the recently completed LINK Integrated Farming Systems project integrated and conventional farming were compared using split or quartered fields at six experimental sites across the UK and through a five-course rotation of cereals and break crops. Non-target arthropods were monitored as the main bio-indicator within the study. Numbers and diversity of non-target arthropods (Carabidae and Linyphiidae) differed most between the sites, years and crops and to the least extent between the two farming systems. Spring non-cereal crops and especially potatoes were the least favoured by Carabidae and Linyphiidae.

INTRODUCTION

The cereal ecosystem is highly complex with up to 550 different species of arthropods having been recorded (Potts, 1991). Many of these species are beneficial because they predate on cereal pests or are an essential dietary component for farmland birds. Many farming practices are known to effect arthropods such as type and timing of soil, pesticide usage, crop type and rotation, all of which are components of integrated farming. By measuring the abundance and diversity of non-target arthropods some indication may be gained of the environmental impact of a farming system (Büchs *et al.*, 1997). Furthermore if integrated pest management is to be a component of integrated farming it is essential that predatory arthropods are encouraged to ensure a sufficient level of pest control by natural enemies is achieved. Two of the most abundant and widespread arthropod taxa in arable crops are the Carabidae (ground beetles) and Araneae (spiders), of the latter 90% are from the Linyphiidae (money spiders) in terms of abundance and species composition (Sunderland *et al.*, 1986). These taxa include many

predatory species important as bio-control agents and because they are also relatively easily sampled and identified are often the most frequently used bio-indicators in agro-ecosystem research. In 1992 the LINK Integrated Farming Systems Project was started to develop and research integrated compared to conventional farming (Ogilvy *et al.*, 1994). Non-target arthropods were monitored as one of the main environmental indicators.

METHODS AND MATERIALS

The LINK IFS project was set-up at six sites located in the main arable production areas of the UK. At each site there were a minimum of seven pairs of plots in which integrated and conventional farming systems were compared. Each plot was a minimum of 2.5 ha and had a minimum width of 72 m. The five-course rotation of cereals and break crops with rotational set-aside at some sites, was chosen according to local practice, details in Ogilvy *et al.* (1994). A range of husbandry measures were implemented in the integrated plots to reduce the need for agrochemical treatment; integrated methods adopted are described in Ogilvy *et al.* (1994). In each field, there were two transects of five pitfall traps, spaced at 10 m intervals, extending into the field starting at 30 m from a common boundary. The pitfall traps were partly filled with water and detergent and were operated for 5-day periods, at monthly intervals, throughout each crop's growing period. Sampling during the baseline year was used to determine the species most often found at the six sites and 19 carabid and eight Linyphiid taxa were then identified in all further sampling.

Data was analysed for the month of June when most husbandry inputs had been completed and all fields were sampled at each site. Numbers and number of taxa for Carabidae and for Linyphiidae within each site, except for Pathhead, were analysed using a model comparing the effect of system, phase (of rotation) by system, year by system and system by phase by year. All data were $\log_{10}$ transformed. Only means are presented from Pathhead because replicate fields were not sampled and the data could not be analysed using the above model.

RESULTS

Carabidae

The type of farming system, crop or year had no statistically significant effect on numbers of Carabidae at four of the sites. At High Mowthorpe there was a significant three-way interaction ($F_{16,30}=2.6$, $P<0.05$) effect for abundance because of differences between years and lower numbers in potatoes, wheat after potatoes and the integrated spring beans compared to conventional winter oilseed rape. There were some consistent trends found across the sites. Spring non-cereal crops were often the least favoured, especially potatoes (Fig. 1). Six times fewer Carabidae were caught each year in spring linseed at Boxworth and four times fewer in spring beans at High Mowthorpe compared to winter oilseed rape. At Pathhead half as many Carabidae were captured in spring compared to winter oilseed rape. There was considerable variation between the sites. The mean across all phases was approximately three times higher in both systems for Manydown and Lower Hope compared to the more northerly sites of Pathhead, Sacrewell and High Mowthorpe. There was little evidence that the measures applied in the integrated compared to the conventional system were encouraging carabids

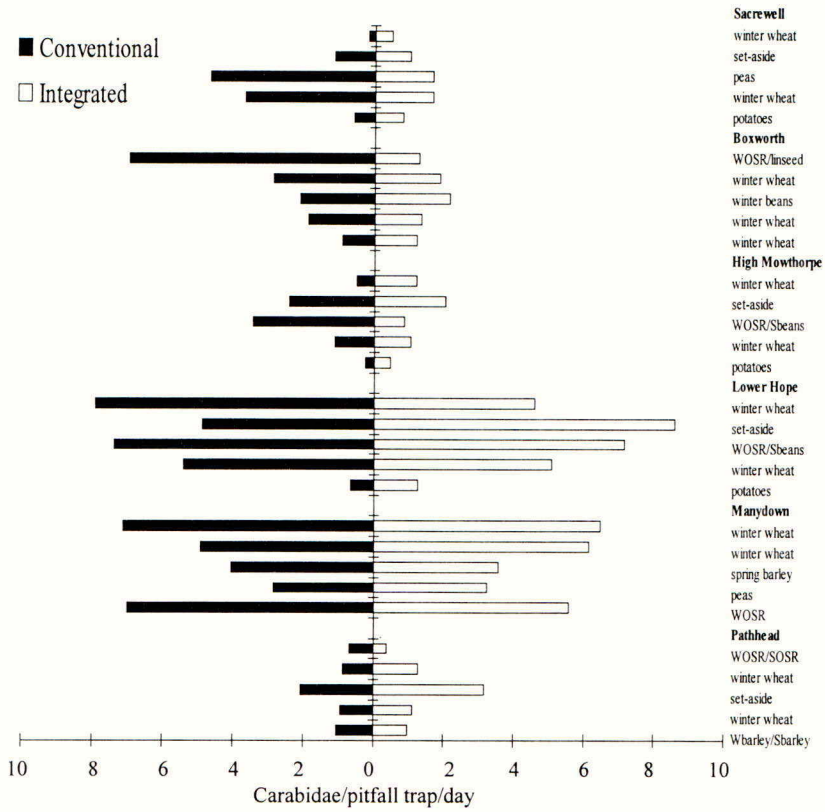

Figure 1. Mean number of Carabidae within each phase and system during June.

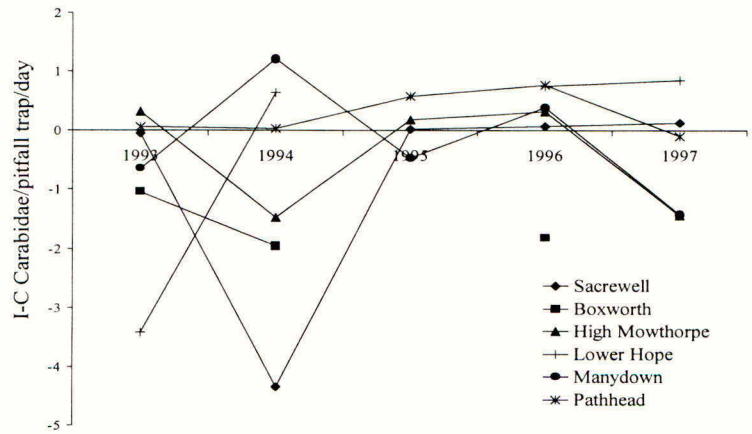

Figure 2. Mean difference between the integrated (I) and conventional (C) systems for number of Carabidae during June.

over the duration of the project (Fig. 2). The same results were found for the number of carabid taxa verifying these conclusions, although diversity was highest at Boxworth.

Linyphiidae

Total numbers of Linyphiidae were very low at Sacrewell, High Mowthorpe and Pathhead (Fig. 3). At Sacrewell there was a significant three-way interaction ($F_{13,19}$=4.7, P<0.001) for abundance caused by the occasional high catch within individual years and crops. There was a significant phase by system effect for abundance at Lower Hope ($F_{4,10}$=3.4, P=0.05) because fewer Linyphiidae were captured in the integrated spring beans compared to conventional winter oilseed rape whilst more were caught in the integrated wheat after spring beans (Fig. 3). There was also a year by system ($F_{3,10}$=6.6, P<0.01) effect for abundance because more Linyphiidae were captured in the integrated plots during 1997 (Fig. 4). Figure 3. Mean number of Linyphiidae within each phase and system during June. The same results were found for diversity at Lower Hope. At Boxworth there was a significant three-way interaction ($F_{11,21}$=3.2, P<0.01) for diversity primarily because this was low in the integrated linseed crop. No other significant effects were found for abundance or diversity.

DISCUSSION

The largest differences in the abundance and diversity of Carabidae and Linyphiidae were found between sites and years, then crops and to the least extent between the farming systems. The main differences detected between the farming systems were usually because the spring crop grown in the integrated system was less favourable to the arthropods. Similarly, the type of farming system had little effect on non-target arthropods in the LIFE (Winstone *et al.*, 1996), SCARAB (Frampton & Cilgi, 1996) and Nagele projects (Booij & Noorlander, 1992). In contrast, non-target arthropods were enhanced by the integrated approach of the Lautenbach (El Titi & Ipach, 1989) and Intex (Büchs *et al.*, 1997) projects in Germany.

Considerable variation was found between the sites with especially low numbers of all arthropods at Sacrewell, High Mowthorpe and Pathhead. At Sacrewell and High Mowthorpe arthropods were probably lower because of the husbandry practices associated with growing potatoes. There was no indication that invertebrate numbers and diversity increased in the integrated compared to conventional crops over time. Pest control by natural enemies is, therefore, likely to be very low at the sites with few beneficial invertebrates. Even at Manydown, which had the highest invertebrate numbers, a bolt-on study revealed that aphid control by polyphagous arthropods was only detectable in those fields where predators were most numerous (Holland & Thomas, 1997). This indicated that further measures are needed if arthropods are to be encouraged within arable fields.

Insecticide usage was relatively infrequent within both systems, with the exception of potatoes, and was unlikely to have many long-term effects as use of broad-spectrum organophosphate products was avoided in both systems. The intensity of weed control may also influence arthropods because many species favour weedier crops (Speight & Lawton, 1976). Herbicide use was reduced but this was to lower costs and the risk of leaching rather than preserve individual species for arthropods. Differences occasionally occurred as a result of poor weed control and greater tolerance of, for example *Poa anua* at Pathhead, and this

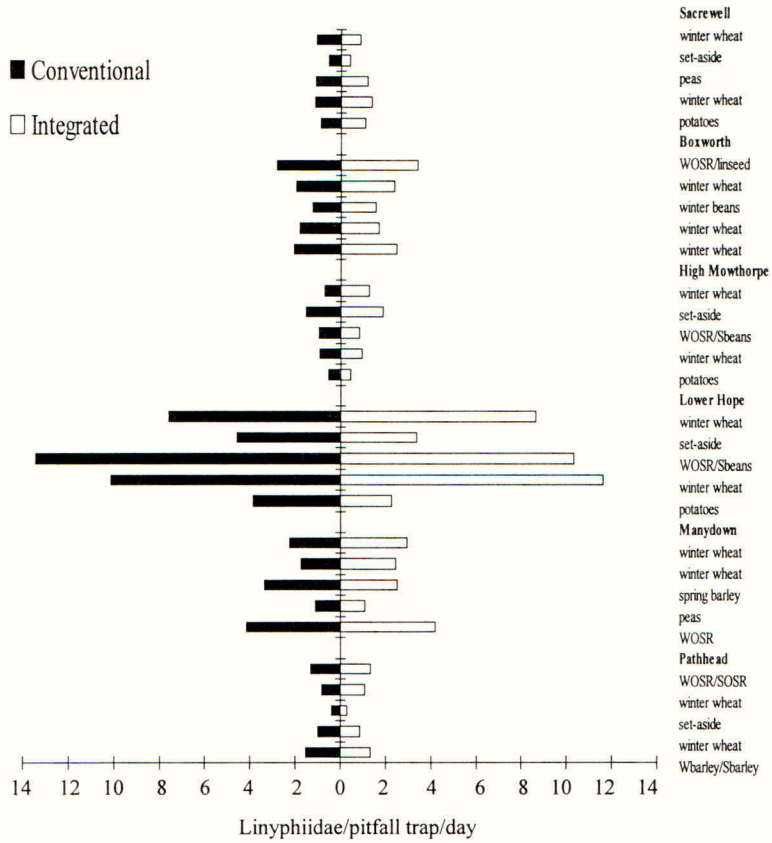

Figure 3. Mean number of Linyphiidae within each phase and system during June.

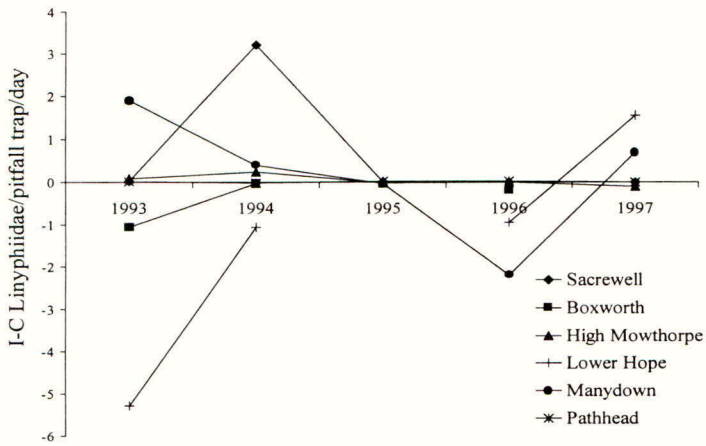

Figure 4. Mean difference between the integrated (I) and conventional (C) systems for number of Linyphiidae during June.

resulted in a two-fold increase in arthropods (Richards *et al.*, 1997). Speight & Lawton (1976) also captured more carabids in areas where *Poa annua* was abundant. Only the main results are presented here and further analysis may reveal short-term affects of individual practices.

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The control of diseases of winter wheat using integrated farming techniques

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ABSTRACT

A farming systems experiment compared conventional and integrated disease control strategies, using data derived from replicated small plot experiments. The integrated approach achieved reductions in both fungicide costs and active ingredients applied but, although yields were lower, profitability was maintained, and increased at lower price levels.

INTRODUCTION

The Focus on Farming Practice Project was established by CWS Agriculture in 1989 to evaluate three systems of organic farming. In 1993 CWS joined with crop protection specialists Profarma and crop nutrition specialist Hydro Agri UK Ltd to establish a further trial to investigate the economic performance, technical feasibility and environmental impact of an integrated farming system (Leake, 1995). Extending over 60 ha, the trial is the largest of its type in the UK and is scheduled to continue until the year 2000.

Integrated farming seeks to use a range of techniques, principally biological, cultural and mechanical, to suppress pests, diseases and weeds and build fertility. The effectiveness of these techniques is variable and they are unable in isolation to deliver the high yields associated with conventional agriculture today. Hence both crop protection products and fertiliser inputs are required to protect and enhance yields. However, the use of these products is judicious, with pesticides used only when the economic injury level is attained and doses modified towards suppression rather than necessarily elimination of an antagonist. It is often assumed that reducing inputs is an inherent objective of integrated farming but this is not the case; input reductions are a consequence of the approach but only where the system itself has been effective in inhibiting yield threatening factors. Over the period of the trials all inputs have been used at sub-label rates but the difficulty faced by the farmer is ascertaining what rate is appropriate to achieve the desired level of suppression. Applications in excess of need will reduce profitability and may in some circumstances disrupt the biological control mechanisms the system is seeking to encourage. Applications below that required to control the antagonist sufficiently to obtain the optimum economic response are likely to reduce yield, and may in some circumstances require a repeat treatment to be made.

In response to this need for appropriate sub-label doses Profarma have developed a range of trials known as "Profarma Select Agronomy". These trials are carried out in replicated plots using different varieties and are sited in various locations around the UK in order to evaluate the interactions between different cropping sequences, soil types and climatic conditions. In 1998 over 10,000 individual plots were harvested and the yields measured to ascertain the

effect of different fungicide molecules, rates and sequences and their interaction. This information can then be used on a site-specific basis to provide farmers with a disease control programme which is appropriate to their needs. Evidence published recently suggests that many UK wheat crops receive inappropriate fungicide applications (Stevens *et al.*, 1997), particularly in relation to the level of resistance afforded by the variety of wheat grown.

Further interactions may also occur in response to plant population and nitrogen (N) supply, and soil type can have a profound effect on both the level and recovery of mineral and applied N (White *et al.*, 1997), which in turn can effect disease pressure.

MATERIALS AND METHODS

Small Plot Experiments

Each winter wheat variety to be evaluated was sown in a randomised block with three replicates. All other factors are identical including sowing rates, dates, herbicide, plant growth regulators and fertilisers. Each of the treatments was yielded.

Field Scale Experiments

To provide a direct comparison between conventional and integrated farming techniques, seven fields have been divided in two with three of the fields growing wheat crops on each side each year. Hence all crops grown are first wheats and the rotation for both systems is the same. The conventional system grows feed varieties with drilling targeted between mid September and early October. Seedbed preparations are either ploughing or heavy discing followed by lighter cultivations. Autumn herbicide and Barley Yellow Dwarf Virus (BYDV) treatments are routinely applied, as is the nitrogen programme. Fungicide treatment is a prophylactic low dose regime typically being 2–3 applications with the fungicides used and doses selected reflecting the disease pressure and variety present.

The integrated system also grows feed wheats but the varieties are selected for disease resistance and standing power rather than yield alone. Cultivations are always minimal and range from direct drilled crops sown at low seed rates in early September to minimally cultivated stale seed beds direct drilled at higher seed rates in mid October. Disease levels are monitored through the spring and nitrogen applications are made in three splits based on residual N levels and fine tuned using the Hydro Agri PrecisioN programme and the N tester. Mineral N levels are generally lower and more stable than the conventional system because less mineralisation occurs under reduced tillage.

RESULTS

Small Plot Experiments

The results of the trials carried out on the four main wheat varieties usually grown in the field scale experiment are given in Table 1.

Table 1. Small plot grain yield response results (all doses in litres/ha, a.i. in g/litre).

Treatment	a.i (g/litre)	Growth stage and dose 31	39	Yield increase over control (t/ha)
cv Riband				
1. epoxiconazole	125	0.30	0.40	0.40
2. epoxiconazole	125	0.33	0.66	1.20
3. tebuconazole + triadimenol	250 : 125	0.40	0.70	1.20
4. cyproconazole + prochloraz	80 : 300	0.33	0.66	1.00
				LSD 0.30
cv Hunter				
1. cyproconazole + prochloraz	48 : 320	0.625	cyproconazole + prochloraz 80 : 300	0.50
2. tebuconazole	250	0.50	0.50	0.50
3. epoxiconazole	125	0.50	0.50	0.60
4. propiconazole + tebuconazole	250 : 250	0.25	0.25	0.00
				LSD 0.50
cv Hussar				
1. cyproconazole + prochloraz	48 : 320	0.625	cyproconazole + prochloraz 80 : 300	0.50
2. tebuconazole	250	0.50	0.50	0.00
3. epoxiconazole	125	0.50	0.50	0.60
4. propiconazole + tebuconazole	250 : 250	0.25	0.25	0.00
				LSD 0.50
cv Reaper				
1. cyproconazole + prochloraz + quinoxyfen	48 : 320	1.0 0.1	propiconazole + tebaconazole 250 : 250	1.00
2. cyprodinil	750	0.67	propiconazole + tebaconazole 250 : 250	1.84
3. cyprodinil + epoxiconazole	750 125	0.40 0.50	propiconazole + tebaconazole 250 : 250	2.24
4. cyproconazole + prochloraz + expoxiconazole, keresoxim-methyl, fenpropimorph	48 : 320 125 : 125 : 150	0.625 0.25	cyproconazole + prochloraz 80 : 300 + expoxiconazole, keresoxim-methyl, fenpropimorph 125 : 125 : 150	3.06
				LSD 0.82

(epoxiconazole, Opus, BASF; tebuconazole + triadimanol, Silvacur, Bayer; cyproconazole + prochloraz, Sportak Delta + Tiptor, AgrEvo; tebaconazole, Folicur, Bayer; propiconazole + tebuconazole, Cogito, Ciba Agric; quinoxyfen, Fortress, DowElanco; cyprodinil, Unix, Novartis; expoxiconazole + keresoxim-methyl + fenpropimorph, Mantra, BASF).

Field Experiment Results

Table 2. Comparison of field pairs; conventional (C) and integrated (I) systems.

	Pair A 1995		Pair B 1997		Pair C 1997	
	C	I	C	I	C	I
cv	Riband	Hussar	Reaper	Reaper	Hunter	Hunter
Sowing date	21 Sep 94	21 Oct 94	21 Sep 96	09 Oct 96	30 Sep 96	30 Sep 96
Sowing rate	190 kg/ha	220 kg/ha	160 kg/ha	110 kg/ha	160 kg/ha	180 kg/ha
N applied	186 kg/ha	209 kg/ha	198 kg/ha	131 kg/ha	198 kg/ha	159 kg/ha
Fungicide cost	£69.72	£17.42	£70.27	£39.90	£76.26	£43.44
Fungicide a.i.	2.003 kg/ha	0.167 kg/ha	0.778 kg/ha	0.438 kg/ha	0.665	0.450
Yield £/ha	9.39	8.22	8.16	6.45	5.62	5.88
Margin @ £80/t	210.59	234.99	154.06	93.15	(36.69)	58.47
Margin @ £120/t	586.19	563.29	479.42	351.27	187.99	293.63
Margin @ £100/t	398.39	399.39	316.24	222.21	75.65	176.05

The data from three field pairs, one from 1995 and two from 1997 were selected to give a contrasting range of varieties, sowing dates and N application rates to demonstrate the effect of these upon fungicide use in the conventional and integrated systems (Table 2). Pair A were selected since the cv Riband is known for its high yielding capacity but is disease susceptible, while Hussar is resistant but with lower yield potential (Anon. 1998). Sowing dates were also a month apart, but N usage and sowing rate were higher in the integrated system.

Pair B were selected because the varieties were identical but the sowing date of the integrated crop was two weeks earlier than the conventional and the seed rate 50 kg/ha lower. Pair C consisted of Hunter sown in the field pair on the same day. Seed rate was slightly higher on the integrated plot but N rate 39 kg/ha lower.

Across all plot pairs fungicide costs have been reduced by between 43% and 75% while active ingredient reductions ranges from 32% to 92%. Yields, however, for the integrated systems vary from 21% lower to 4% higher, averaging 11% lower across all sites. The reduction in yield has implications for profitability but only where grain prices are high. At £120/tonne this results in a loss of income of around 4% while at £100/tonne the profitability of the systems is equal. Where the price falls to £80/tonne the integrated approach is 15% more profitable (Figure 1).

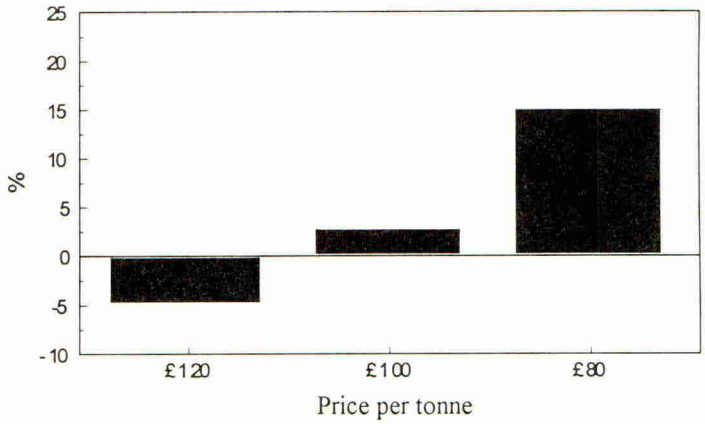

Figure 1 Percentage profitability integrated over conventional systems at different output prices.

DISCUSSION

The small plot data demonstrates the very different way that varieties respond to fungicide inputs. All applications, regardless of molecule or dose rate, produced a significant yield response on Riband while Hunter and Hussar respond to one treatment with additional yield being half that achieved by Riband.

The trial carried out using multi low dose mixed fungicide chemistry on Reaper indicated that although this variety has good NIAB disease ratings and is capable of high yields even at low inputs, strong positive responses were achieved using the mixed chemistry approach. This was particularly so where the new strobilurin chemistry was used in two sub-label rate applications.

In the field the complexity of disease management strategies reflects variety × sowing date and fungicide rate and the interaction with nitrogen. In the most complex conventional programme Riband was grown with 11 different active ingredients applied at four separate growth stages while the equivalent integrated crop comparison Hussar was grown with two active ingredients applied at sub-label rates on a single occasion. The rationale for using such a range of strategies can only come from replicated trials work such as the Profarma Select Agronomy programme. Despite the detail, this in itself is not a complete guide to the farmer because sowing rates and dates may be outside the ranges in which Profarma Select operates, and N application rates in the integrated system depend heavily on soil mineral N levels which vary according to season. However, the Select data provides a useful guide to the dose reductions which can be used effectively and these, varied in conjunction with the other factors mentioned along with crop inspection, provide the backbone of the integrated strategy.

The present strength of Sterling is depressing feed wheat values to well below the £80 floor used in this comparison. Where crop values are low, efficient input use becomes a more economically viable strategy than yield maximisation, but it remains important to protect most of the yield potential and so it is the precise use of inputs which becomes the most efficient means of achieving these objectives. Furthermore, because the integrated approach seeks to use a range of strategies to minimise antagonists, and views each aspect of crop as an integral part of the system, interactions between factors can be utilised to minimise costs.

The development of the strobilurin based fungicide chemistry will provide an additional dimension to integrated disease control by enabling treatments to be made to cereal crops and protection afforded prior to the onset of visible disease symptoms.

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Measures of sustainability in New Zealand apple orchards: investigating biodiversity in managed ecosystems

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ABSTRACT

Species diversity was compared in apples produced under conventional, integrated, and biological fruit production systems. The conventional system is based on broad-spectrum pesticides, while the integrated system uses minimal applications of selective pesticides. The biological system uses mating disruption of codling moth, *Bacillus thuringiensis* for leafrollers, copper/lime sulphur/sulphur/slaked lime for diseases, and matting or mulches for weed control. Low beneficial arthropod biodiversity was characteristic of the conventional production system. Natural enemy biodiversity was generally as high or higher in the Integrated system compared with the biological system. Biological measures of sustainability are discussed.

INTRODUCTION

Pest management in New Zealand apple orchards has been based since the 1960s on the use of broad-spectrum insecticides and selective acaricides. While successful at meeting quarantine requirements of overseas markets, the use of broad-spectrum pesticides is undesirable for a number of reasons. These include insecticide resistance (Suckling, 1996), destruction of natural enemies, and the potential for adverse trade and domestic implications in response to increasing consumer concerns (Christie, 1993). Integrated fruit production is an alternative production system undergoing rapid adoption in New Zealand, with the aim of reduced environmental and human health impact (Walker *et al.*, 1997a). A system compatible with the organic marketing label "BIO-GRO" has also been under development (Wearing *et al.*, 1996).

The New Zealand apple pest and beneficial arthropod fauna consists of both endemic and cosmopolitan species (Charles, 1998), but includes only a small subset of the fauna associated with *Malus* sp. elsewhere. Codling moth, along with four native and one introduced leafroller species are the key pests of apples in New Zealand. A number of natural enemies of the mostly cosmopolitan pests have been introduced this century (Charles, 1998). Very few examples are known of native species attacking apple pests.

New Zealand's Resource Management Act (1991) has highlighted the need to develop the basis for measuring the sustainability of orchard management practices, which can be determined in a number of ways (Wearing, 1997). For example, agrochemical inputs can be compared using a pesticide rating system (Walker *et al.*, 1997b). The ecological impact of management practices on the pests and beneficial organisms is also important (Wearing,

1997). Such characterisations must be accompanied by an evaluation of the level of pest management achieved (e.g. Walker *et al.*, 1997a, Suckling *et al.*, 1998) as well as economic sustainability (e.g. Greer, 1997). Particular studies have investigated the impact of specific pesticides on important natural enemies (Shaw and Walker, 1996, Bradley *et al.*, 1997, Shaw *et al.*, 1997). Here, we report only on trends in the biodiversity of arthropods under conventional, integrated and biological fruit production.

METHODS AND MATERIALS

Production systems

Three production systems were applied to unreplicated mature research orchard blocks in Canterbury (lat. 43° S) and Otago (lat. 45° S). Results here are confined to cv. 'Fuji'. Blocks ranged from 0.3 ha to (Canterbury) to 3 ha (Otago). Disease control in the Biological Fruit Production (BFP) system was based on the use of slaked lime, baking soda, pre-bloom oil, pre-bloom lime sulphur (Otago only), copper and sulphur. Pest management relied on natural enemies, mating disruption of codling moth with sex pheromone (Isomate C+), and in Canterbury, four applications of *Bacillus thuringiensis* against leafrollers. Weed control was achieved by either matting (Otago), or mulching (Otago and Canterbury). Integrated Fruit Production (IFP) used minimal applications of selective materials, such as prebloom oil with or without buprofezin (Applaud 25 W), two or three applications of the highly selective tebufenozide (Mimic 70W) for tortricids, minimal use of non-miticidal fungicides (based on infection periods of *Venturia inaequalis* Cke.Wint.), and non-residual herbicides on a 1 m wide strip. Conventional Fruit Production (CFP) used the existing standard export spray programme for each region (pre-bloom oil and chlorpyrifos (Lorsban 40 EC), three (Otago) or five (Canterbury) post-bloom organophosphate applications, carbaryl for thinning, one propargite spray (Otago), conventional fungicides (including metiram) based on infection periods, and a 2 m herbicide strip. All pesticide applications were made at 2000 litres/ha. More details of the three systems are reported in Suckling *et al.* (1998).

Beating Trays

Beating trays constructed from 1 mm thick white aluminium sheet (500 × 500 mm), with turned-up 12 mm edges were used in Canterbury and Otago. Sampling involved tapping a branch once using a rubber-covered metal pipe, with the tray underneath. Any arthropods landing on the tray were collected. Samples were collected on nine occasions in 1995/96, and on five occasions in 1996/97 and 1997/98 (20 samples per block in 1995/96, and 15 samples subsequently).

Pitfall Traps

Pitfall traps consisted of 600 ml 'Lily' plastic containers buried flush with the ground, with 40 ml of Gaults solution to preserve invertebrates. Pitfall trap samples (12 samples × 3 treatments) were collected monthly (with beating tray samples) on five occasions in Canterbury and Otago in 1996-97 and again in Otago in 1997–98. The pitfall traps were operated in the centre of the alleyway in each block for 72 hours.

Statistics

It was not possible to replicate research plots in each region, and regional faunal and plot size differences prevent them from being true replicates. Estimates of abundance or diversity, compared by t-test, are best considered as a guide to these regimes, and inferential extrapolation is not recommended (Hurlbert, 1984). The Shannon-Wiener index (Magurran, 1988) provided estimates of diversity based on "recognisable taxonomic units" (Suckling *et al.*, 1996), using all arthopods, or predators alone.

RESULTS

Diversity

Beating tray sampling indicated that the biodiversity (Shannon-Wiener index) in Canterbury and Otago was greatest in every season in the BFP and IFP systems (Fig. 1, for Canterbury, Otago results not shown), although differences between the three treatments varied slightly over time. When the analysis from pitfall and beating tray samples was confined to predatory insects and spiders, few natural enemies were present in the CFP system (Fig. 2), and similar or higher levels of their biodiversity were present in the IFP compared with BFP systems in both regions.

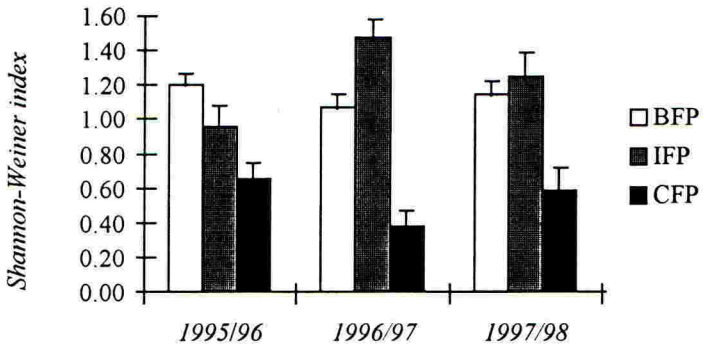

Figure 1. Arthropod diversity per tree assessed by beating tray sampling in Canterbury, New Zealand apple orchards under Biological (BFP), Integrated (IFP) and Conventional (CFP) fruit production. Bars are one standard error.

Biodiversity of both the total fauna and natural enemies was generally higher in the pitfall samples which sampled the orchard understorey fauna. There were significant treatment differences in the diversity of natural enemies between treatments and in some cases important natural enemies were involved. For example, the mean number of adult lycosid spiders (three-clawed hunting spiders) in the Canterbury BFP block was significantly higher than in the CFP block on all five sampling dates ($P < 0.01$). In the IFP block, the mean number of adult lycosid spiders was significantly higher ($P < 0.01$) than in the CFP block on three of the five sampling dates. Other understorey natural enemies found more often in

the IFP and BFP than in the CFP included a number of species of linyphiids (sheet web spiders), salticids (jumping spiders) and the phalangiid *Phalangium opilio* (harvestman).

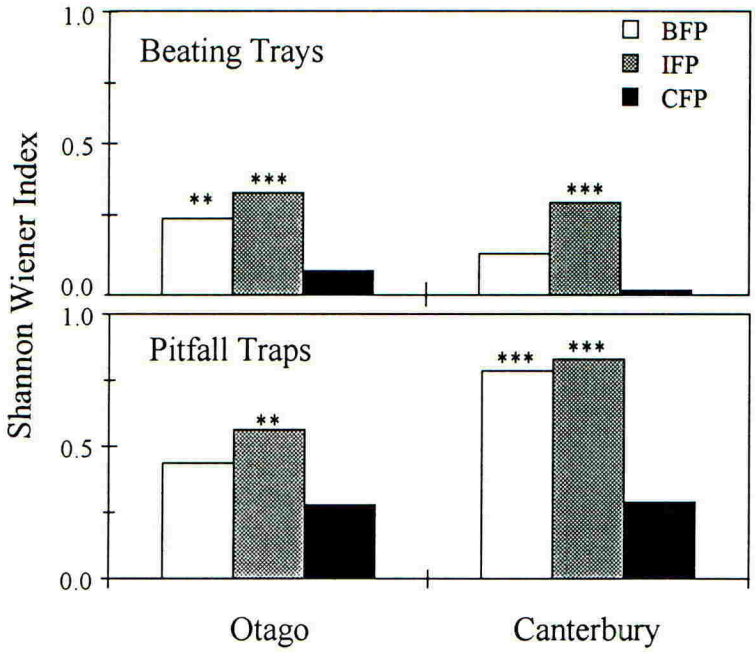

Figure 2. Diversity of predators sampled using beating tray or pitfall traps in apple orchards under three management regimes at two New Zealand locations in 1996/97. Asterixes indicate level of significance from CFP (** $P < 0.01$; *** $P < 0.001$).

DISCUSSION

Biodiversity in managed ecosystems results from an interaction between the local fauna and management practices. The impact of organophosphate applications, with a broad-spectrum effect on the fauna, was readily detectable both in the arboreal and understorey components of the CFP orchard. The differences between the other treatments tested here (or variations which might be developed in the future) may be more subtle and consequently difficult to detect.

Biological measures of sustainability should be linked to other measures of ecological and economic sustainability, and this will require the development of indicators of sustainability for other components of the ecosystem (Wearing, 1997). Biological indicators of ecological sustainability must respond to the orchard management system in a consistent manner, be widespread throughout orchards, easy to detect and record quantitatively, and without obvious bias. Regional differences must also be taken into account. Suckling *et al.* (1998) discussed three broad approaches for measuring sustainability using biological

indicators, viz. the use of single indicator species, groups of species, or the biological diversity and community structure across a wide range of organisms present in the system.

Single species indicators could be natural enemies, but should be shown to be important in the regulation of pest species, thereby contributing to economically sustainable production. However, there are several complications with using single species as key indicators, including the potentially selective influence of some pesticides on their abundance. Some natural enemies are not ubiquitous, or numerous enough to be readily detectable.

A second alternative would use representative groups of pests or natural enemies to form a more integrated measure of sustainability. This is analogous to the biotic indices used in water quality assessment (Cook, 1976). It would be possible to use those natural enemies known or likely to regulate pests.

Ecological diversity or community structure may be the lowest quality indicator of environmental sustainability in managed ecosystems, particularly where the understanding of functional ecological relationships is poor. More unsubstantiated assumptions about the importance of particular groups or diversity are likely to be made with this approach, and bias from the sampling regime (Suckling *et al.*, 1996) cannot be ignored. For example, the beating tray was not a good method for monitoring certain taxa, such as very small or mobile species. Small insects were difficult to see on the tray, but included important parasitic Hymenoptera.

The high cost incurred in determining orchard faunal biodiversity would not be warranted on a routine basis. However, if this approach is to provide an estimate of the broad environmental impact of a pest management regime, then it will be necessary to determine this parameter at more than one location in each region. It might be sufficient to consider the biological diversity and abundance of natural enemies sampled with a single technique. Evidence in favour of this approach comes from the comparison of predator diversity from pitfall traps in the three regimes, which is in general agreement with results from the wider fauna on the foliage (Fig. 2). The use of sticky traps, where the identification and analysis focused on beneficials likely to have a role in pest regulation, may be a practical alternative for the development of a sustainability index for managed ecosystems (Bradley *et al.*, unpublished). However, further research is needed with this approach.

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Efficiency of biofertilization management of rice and soybean in the Nile delta with application of pesticides

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ABSTRACT

Trails on the impact of pesticides use on biofertilization of soybean and rice in Egypt are reviewed. Considerable variation was found in response of each of nodulation of soybean, nodule development and symbiotic nitrogen-fixation (assessed as N-content in seed yield) to application of certain herbicides and insecticides. Some of them showed stimulatory effects and some were inhibitory. A different pattern of response was detected with application of certain herbicides, insecticides and fungicides in rice fields inoculated with nitrogen-fixing cyanobacteria, with grain yield, grain N-content and agronomic chemical fertilizer N-use efficiency responding in a similar manner towards application of the chemicals.

INTRODUCTION

A large area of arable land in Egypt is now subjected to fertilization schedules containing biofertilizer preparations. Biological nitrogen (N)-fixation by free-living cyanobacteria in the rice ecosystem, and the symbiotic association between the nodule forming rhizobia micropartners and legume crops are sufficiently efficient to compensate for a considerable portion of nitrogen requirements.

Pesticides may affect biofertilization by interacting with soil microbes, potentially changing pesticide and microbial behaviour (Bollag & Liu, 1990). Such interactions are not easily detected in the laboratory, so field trials are a preferred method to check whether or not a biofertilizer preparation can be successfully used in practical situations where specific pesticides are used. They also help biofertilizers producers to select the tolerant strains capable of withstanding the stress created with application of a given pesticide and, simultaneously, benefit crop growth and performance.

This paper reviews trials in Egypt evaluating the efficiency of biofertilizers in the presence of pesticides.

MATERIALS AND METHODS

A series of nine wire-proof greenhouse and field experiments with rice and soybeans were conducted from 1983 to 1996, in the fertile clay-loamy alluvial soil of the experimental farm of the Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt. Biofertilizers including the soybean symbiotic micropartner *Bradyrhizobium japonicum* and N_2-fixing cyanobacteria of rice were applied in the presence of a range of herbicides, fungicides or insecticides. Application of the herbicides was within the first seven days after soybean sowing or rice transplanting. Application of the insecticides and fungicides coincided with the start of the infestation or infection symptoms. Some of the pesticides were used as soil application, in which 100 kg fine

soil was used as a carrier to facilitate equal distribution of the pesticide over the treated sub-plots. In the case of foliar spray application, 480 litre of water was used to make an emulsion of the pesticide. The agrochemicals tested are listed in Tables 1 and 2. They contain the insecticides: Cypermethrin (5% EC, CCN-52), Methomyl (20% EC, Lannate), Tetrachlorovinphos (70% EC, Gardona), Bactospeine (200 Anagasta units/mg, *Bacillus thuringiensis*), Dicofol (18.5% EC, Kalthane), Omethoate (80% EC, Filomate), Methamidophos (60% EC, Thamaron), Diazinon (14% EC), Carbofuran (5% EC), Carbofuran (10% EC, Endosulfan), Dursban (15% Gr), Phenylpyrazole (25% Gr., Fipronil), Fenitrothion (3% EC, Cyfen), the fungicides: Kitazin-17 (17% Gr.), Isoprothiolane (40% EC, Fuji-1), Tricyclazole (75% WP, Beam), Prochloraz (40% EC, Sportake), and the herbicides: Metolachlor (25% EC, Dual), Afalon (50% Gr., Linuron), Oxadiazone (25% Gr., Ronstar), Trifuralin (48% EC, Treflan), Alachlor (48% EC, Lasso), Pendimethalin (50% EC, Stomp), Butachlor (60% EC, Machete), Thiobencarb (50% EC, Saturn). Fertilizer-N application for soybean was performed as two doses, a starter dose of one-third of the fertilizer at 20 days from sowing and the other two-thirds 25-30 days thereafter. Fertilizer-N for rice was applied in two equal doses, at 20 days from transplanting and at the late tillering stage. The targeted pests and parameters used for evaluation of nodulation, crops growth and performance are presented in the Tables 1 and 2. Only data collected from the plots which were inoculated with the *Rhizobia* or the cyanobacteria and treated with the manufacturers', entomologists', plant pathologists' and agronomists' recommended amounts of fertilizer-N or pesticides are presented in this review paper. Details about performance of different doses of the pesticides and N amounts and their effects on population densities, intensity of infestation and severity of the different pests over the experimental programme, and on crop growth and performance, are discussed in the corresponding published articles (Tables 1 and 2).

RESULTS AND DISCUSSION

Effect of pesticides on nodulation and N_2- fixation in soybean

The data presented in Table 1 show the marked differences in the effect of application of the pesticides on nodulation, N_2-fixation and soybean crop performance. Some of the pesticides were found to be enhancers while others seemed to be inhibitors. Some of the chemicals increased nodule formation, indicating greater rhizobia activity within the roots, and enhanced establishment of nodule structures. Nodule growth responded variably to application of the same pesticide. Some of the pesticides increased seed yield and N-content, although the number and weight of nodules did not change significantly or even showed reduced number than the untreated counterparts (Yanni and Salem 1987). It seems, therefore, that each of nodulation, nodule development and N_2-fixation has its own response towards a given pesticide. It is not then an easy task to assess whether or not a pesticide will interfere with N_2-fixation through merely a test tube or a greenhouse experiment. Field data are needed to construct the most suitable package of recommendations containing rhizobial legume inocula of tolerant strains towards different pesticides. Nodulation, nodule development and N_2-fixation by these strains must not be seriously affected by the presence of pesticides intended to be used for the crop. The effects of the interactions between application of more than one pesticide on nodulation and N_2-fixation must also be taken into account in developing biofertilization recommendations for soybean.

Table 1. Response of nodulated soybean to application of insecticides and herbicides.

Reference	Pesticides	Targeted pest (s)	Dose/ha	Nodulation		Seed yield	Seed N-content
				no./plant	mg/plant		
Yanni &	Control	*Spodoptera littoralis*	-	44	117	-	63 mg/plant
El-Dahan	Cypermethrin		1.4 litre	51	110	-	54 mg/plant*
(1983)	Methomyl		3.0 litre	55*	152*	-	67 mg/plant
	Tetrachlorovinphos		4.8 litre	51	159*	-	61 mg/plant
	Bactospeine		6.0 kg	53*	114	-	52 mg/plant*
Yanni &	Hand weed	*Echinochloa grungall*	-	25	180	5.17 g/plant	245 mg/plant
Salem	Metolachlor	*Echinochloa colonum*	1.8 litre	27	173	5.48 g/plant	252 mg/plant
(1987)	Metolachlor + linuron	*Portulaca oleraceae*	1.44 litre + 1.8 kg	19	207	7.51 g/plant	275 mg/plant
	Oxadiazone	*Amaranthus caudatus*	3.6 kg	21	113**	5.44 g/plant	232 mg/plant
	Trifluralin		12.4 litre	21	203	7.39 g/plant	312 mg/plant**
	Alachlor		4.8 litre	31	243**	6.29 g/plant	286 mg/plant
	Pendimethalin + linuron		4.08 + 1.8 kg	28	257	3.89 g/plant	183 mg/plant*
	Control	*Tetranychus*	-	20	370	2.77 t/ha	147 kg N/ha
Yanni *et al.*	Dicofol	*cucurbitacearum*	2.4 litre	18**	250**	3.63 t/ha**	172 kg N/ha**
(1987)	Omethoate		1.8 litre	27**	460**	3.10 t/ha**	139 kg N/ha**
	Methamidophos		1.8 litre	5**	60**	3.65 t/ha **	182 kg N/ha **

*, **: statistically significant at the 95 or 99% confidence level, respectively.

Table 2. Response of rice inoculated with cyanobacteria to application of herbicides, insecticides and fungicides.

Reference	Pesticides	Targeted pest (s)	Dose/ha	Grain yield t/ha	Grain-N kg N/ha	N-use efficiency #
Yanni *et al.* (1988)	Control	*Cyperus difformis*	-	8.40	63.2	175
	Handweed	*Ammania baciffera*	-	11.92 **	101.6 **	248**
	Thiobencarb		4.8 litre	14.26 **	98.7 **	297**
	Butachlor		1.8 litre	12.01 **	100.7 **	250**
	Oxadiazone		4.8 litre	13.58 **	106.1 **	283**
Yanni & Abdallah (1990)	Control	*Chilo agamemnon*	-	7.89	83.9	117
	Carbofuran (10% Gr.)	*Hydrellia prostornalis*	10.0 kg	8.65 **	94.2 **	131 **
Yanni & Osman (1990)	Control	*Pyricularia oryzae*	-	6.79	52.8	189
	Kitazin-17		30.0 kg	8.17 **	60.9 **	227 **
	Isoprothiolane		30.0 kg	6.95	51.0	193
	Tricyclazole		0.6 kg	8.34 **	63.4 **	232**
Yanni (1992)	Control	*Chilo agamemnon*	-	8.3	89.2	58
	Diazinon		15.0 kg	8.2	87.9	60
	Carbofuran (5% Gr.)		15.0 kg	7.8 **	74.0 **	51 **
	Carbofuran (10% Gr.)		15.0 kg	8.6 **	93.1 **	65**
Yanni & Sehly (1995)	Control	*Pyricularia oryzae*	-	8.27	68.5	57
	Prochloraz	*Helminthosporium oryzae*	1.0 litre	9.37 **	81.2 **	65 **
		Alternaria sp. &Sclerotium oryzae				
Yanni *et al.* (1996)	Control	*Chilo agamemnon*	-	8.93	72.4	62
	Chlorpyrifos		12.0 kg	9.12	76.3 **	63
	Phenylpyrazole		24.0 kg	9.34 **	79.7 **	65**
	Fenitrothion		28.8 kg	9.62 **	83.1 **	67**

** : statistically significant on the 99% confidence level. # N-use efficiency (the agronomic N-use efficiency): kg grain yield/kg fertilizer-N.

Effect of pesticides on rice inoculated with N_2-fixing cyanobacteria

Unlike for soybean, the data from the trials on rice grain yield and N-content and chemical fertilizer N-use efficiency (kg grain yield/kg fertilizer-N) (Table 2) indicate more or less the same pattern of response towards different pesticides. However, it must be taken into account that N_2-fixation in rice fields is mostly associated with the activity of free living cyanobacteria in flood water in direct contact with the pesticide. Their sensitivity or tolerance towards certain pesticides has a direct effect on their activity as N_2-fixers. The response of the microorganisms to such chemicals is less complex than in case of inoculation of soybean with its symbiotic micropartner. However, the issue of cyanobacteria growth responses to pesticides in inoculated rice fields and their consequent efficiency in N_2-fixation, in addition to any synergistic effect of the application of more than one pesticide to the same field are as true for rice as for soybean. Only field testing can verify whether or not a package of input recommendations including pesticides will interfere with N_2-fixation in a rice ecosystem.

Although the positive or negative effects of biofertilization with N_2-fixing microorganisms in the presence of pesticides are interpreted mainly by indirect evidence, it may give a clearer understanding of the precautions which must be taken into account with biofertilization technology in situations where pesticides are used.

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POSTER SESSION 7C

INNOVATIVE METHODS OF PEST AND DISEASE MANAGEMENT

Session Organisers

Dr C Prior
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and

Dr H F Evans
Forest Research Agency, Farnham, UK

Poster Papers 7C-1 to 7C-9

Effect of a *Beauveria bassiana*-based mycoinsecticide on beneficial insects under field conditions

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ABSTRACT

The impact of *Beauveria bassiana* Strain GHA on *Eretmocerus* sp. and the whitefly predator complex in cotton was assessed in field trials. In one trial three applications of fungus at recommended rates were superimposed on a field release of *Eretmocerus* sp. wasps in commercial melon. Impact on the parasite population was minimal. In the second, an above-label rate of *Beauveria* applied to cotton significantly controlled the whitefly population (80% reduction), yet the impact on the predaceous insect complex was minimal, even though other data have shown several species to be susceptible in the laboratory. Numerous abiotic and biotic factors may help protect non-target insects from mycoinsectides.

INTRODUCTION

A major concern in the development of mycoinsecticides, such *as Beauveria bassiana, B. brongniartii, Metarhizium anisopliae*, and *Paecilomyces fumosoroseus*, is their compatibility with other natural enemies, especially insect predators and parasites. As a result, regulatory agencies require data about the effects of a candidate fungus on non-target insects. These data are usually generated by laboratory bioassays involving direct topical application of the fungus to the insects. Such methods can lead to exaggerated adverse effects, especially with deuteromycete fungi, because many are somewhat non-specific in a laboratory setting. For example, adult *Eretmocerus* sp. are very susceptible to infection by several *B. bassiana* isolates (Jones & Poprawski, unpublished data). In nature, however, the impact of these fungi may be minimized by a complex of ecological and behavioral barriers that prevent ready infection of the non-target insects.

As a result Mycotech, the U S Department of Agriculture, and private cooperators set out to evaluate the effects of Mycotech's *B. bassiana* Strain GHA on the natural enemy complex of the silverleaf whitefly, *Bemisia argentifolii* in commercial field situations. Herein we report observations from two studies. In one study, *B. bassiana* sprays were superimposed upon release of the parasitic hymenopteran *Eretmocerus* nr. *californicus* in commercial melon

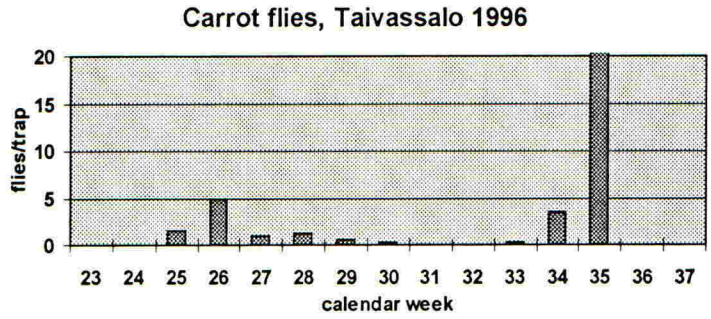

Figure 4. Weekly amount of the catches of carrot flies from yellow sticky traps in Taivassalo, southern Finland (1996).

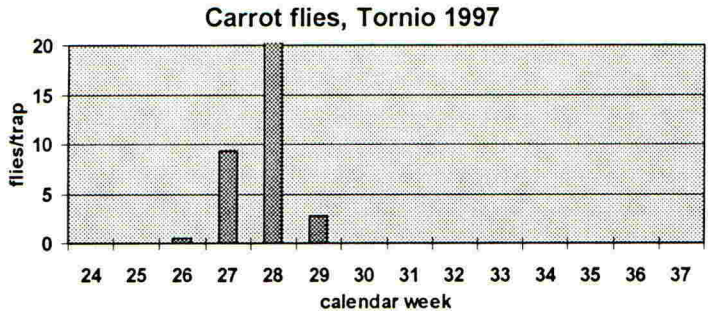

Figure 5. Weekly amount of the catches of carrot flies from yellow sticky traps in Tornio, northern Finland (1997).

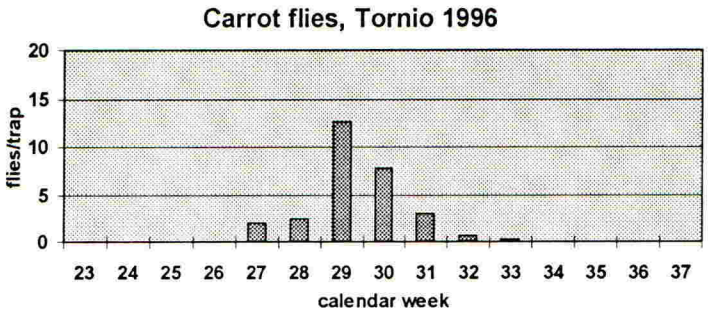

Figure 6. Weekly amount of the catches of carrot flies from yellow sticky traps in Tornio, northern Finland (1996).

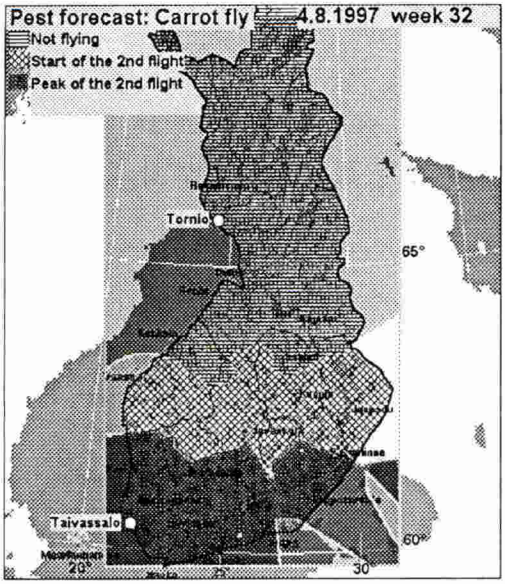

Figure 7. Forecast for the activity of carrot fly in calendar week 32 (1997).

DISCUSSION

Preliminary validation of the forecast showed that the activity of the carrot fly was predicted well. Differences between the two types of the carrot fly are small, and in practise the same ETS values can be used for both: 255 DD_5 for the start and 355 DD_5 for the peak of the first flight. For the second flight, the corresponding ETS values are 800 DD_5 and 860 DD_5. The relationship between accumulated temperature and the activity of the overwintered generation proved to be very similar in Finland and in southern Ontario (Stevenson 1989). In contrast, start of the second period of activity required a much lower heat accumulation in Finland (800 DD_5) than in Ontario (1142 DD_5). Adaption of *P. rosae* to the short growing season in Finland may be one reason for the observed difference in activity.

Experiences of the use of GIS as a "forecasting tool" were very positive. GIS also has the potential to produce more complicated simulation models for forecasting pest activity. Likewise the AGRONET (the internet service run by MTT) proved to be a rapid and relatively easy way to deliver "on-line" information to farmers and extension services. Forecasts of the activity of the univoltine type (northern Finland) of the carrot fly were precise enough to allow farmers to be informed about the need to initiate monitoring and control at the level of the individual field. Some carrot farmers have started to co-operate and hired together an assistant for monitoring. Options for control include applications of pesticides alpha-cypermethrin, deltamethrin, dimethoate, malathion, mevinphos or cypermethrin. Pyrethrin is the only allowed pesticide in organic farming.

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Manipulating the behaviour of beneficial insects in cereal crops to enhance control of aphids

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ABSTRACT

The release of a synthesised analogue of the aphid alarm pheromone, (E)-β-farnesene from dispensers sited in winter wheat crops significantly affected the activity of carabid beetles, aphid numbers on plants and levels of aphid parasitism. The potential uses of this approach for the management of aphids is discussed.

INTRODUCTION

The use of semiochemicals as part of an integrated approach for the control of aphid pests has been suggested by Leszczynski *et al.* (1995) and Losel *et al.* (1996). Predators and parasitoids have been shown to use prey-derived semiochemicals as long range orientation cues (Bowers & Borden, 1992; Grasswitz & Paine, 1992), and (E)-β-farnesene, the aphid alarm pheromone, has been shown to elicit long range orientation in polyphagous predators (Kielty *et al.*, 1996). Many potential semiochemicals of prey and plant origin have been screened in the laboratory and field for their potential use as attractants for predatory and parasitic insects, the full results of which will be published elsewhere. This paper illustrates some of the results obtained with manipulating the activity of carabid beetles within crops, and of levels of aphid parasitism by hymenopteran parasitoids, by an analogue of the aphid alarm pheromone, (E)-β-farnesene.

MATERIALS AND METHODS

(E)-β-farnesene (EBF) was prepared in the laboratory in a hydration reaction from nerolidol according to the method of Dawson *et al.* (1982). NMR confirmed the EBF as being 70% pure, the remainder being unreacted nerolidol and trace amounts of other β-farnesene isomers. The EBF was stored at 4°C. Field experiments were carried out from the end of May, 1996 and May 1997 for seven weeks in a crop of winter wheat (cv. Riband - 1996; cv. Avalon - 1997) at SAC, Edinburgh.

In 1996, the crop was split into 50-m^2 plots, at the centre of which was a 10 m^2 experimental plot. Various treatments were used but only the results for the following are presented: Control plots (95% methanol) (n = 3); 1% EBF dispensed at crop canopy height (n = 3); 1% EBF dispensed at ground height (n = 3).

Wick dispensers contained 1% EBF in methanol and were placed at the centre of the 10 m^2 experimental plots, and were replenished weekly. Once a week from the beginning of May,

five pitfall traps sited at random within the experimental plots were uncovered overnight and the contents collected and identified the following day.

Ten wheat plants were taken at random once a week from the experimental plots and the numbers of aphids present determined.

In May 1997, wick dispensers containing 7% EBF in methanol were placed at crop canopy height 10 m from the crop edge and every 20 m until 70 m from the edge of the crop (n = 4) along a transect from the crop edge. Control plots (n = 2) consisted of dispensers of 95% methanol alone 10 m and 30 m from the crop edge.

Ten plants, 1 m apart, were labelled along a diagonal across the position of the dispenser. At weekly intervals the numbers of aphids were recorded from each marked plant. Aphid 'mummies' were recorded as being aphids parasitised by hymenopteran parasitoids.

RESULTS

1996

The activity of carabid beetles measured by the mean numbers caught in pitfall traps is shown in Fig. 1. With 1% EBF released at canopy level there was a significant increase in beetle numbers compared to the untreated ($P < 0.05$, analysis of variance). EBF released at ground level did not demonstrate a significant increase in beetle numbers.

Aphid (*Sitobion avenae*) numbers on plants from plots with no EBF release had significantly greater numbers of aphids compared to both EBF treatments (Fig. 2, $P < 0.05$).

1997

Analysis of variance of the data indicated no effect of distance from the dispenser on the numbers of aphids or parasitised aphids (*S. avenae*) on individual wheat plants, and there were no differences between each replicate for either treatment. Consequently the data from each replicate were combined to obtain the mean number of aphids/10 plants/replicate for the EBF and control treatments on each sampling date.

There were significantly fewer aphids on plants around the EBF dispensers compared with the control dispensers (Fig. 3, $P < 0.001$). Aphid counts remained relatively high (> 20 per replicate) in the control treatment after 4 weeks, whereas the level of aphid infestation was consistently 40-50% less in the EBF treatment (Fig. 3).

There was a significant difference in the level of aphid parasitism by parasitoids between the two treatments (Fig. 4, $P < 0.001$). Over 60% parasitism was recorded in the EBF treatments, whereas in the control treatments parasitism peaked at 2.5% on week 6 (Fig. 4).

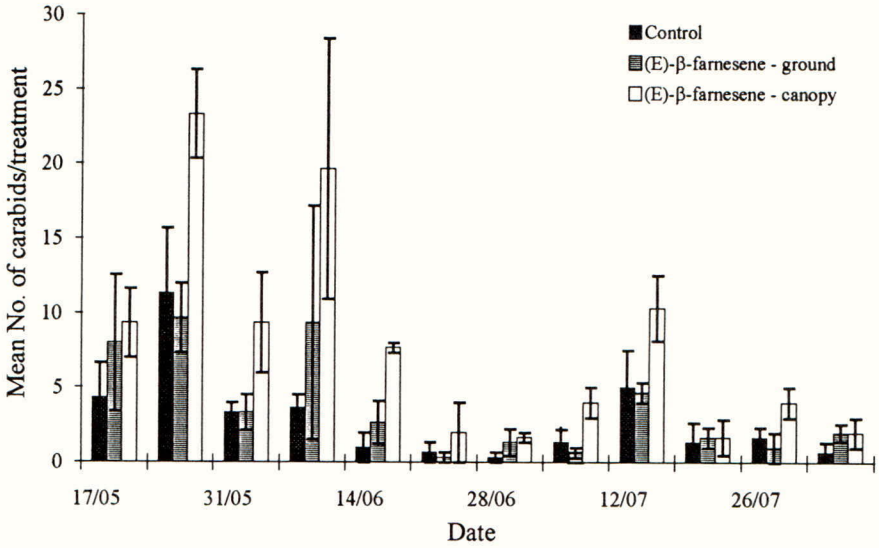

Figure 1. Mean number (± SE) of carabid beetles/treatment caught overnight in five pitfall traps within the experimental area of winter wheat in 1996.

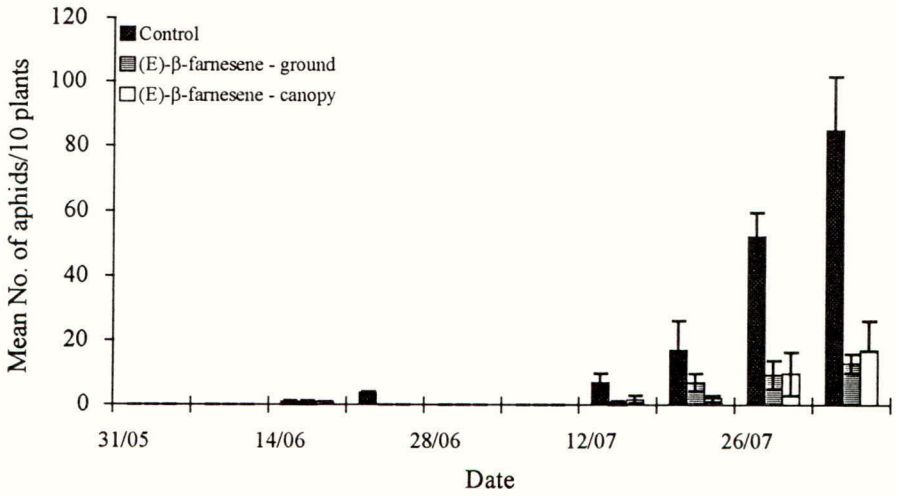

Figure 2. Mean number (± SE) of aphids/10 plants/replicate within the experimental area of winter wheat in 1996.

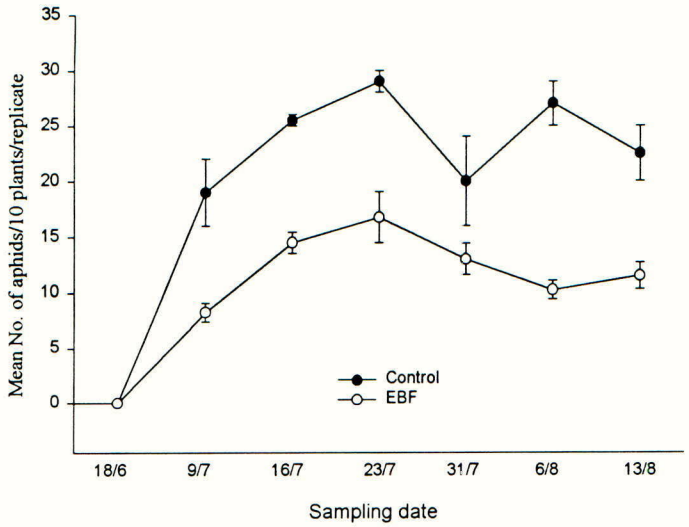

Figure 3. Mean No. of *S. avenae* (± SE) from 10 plants in areas of a winter wheat crop with control dispensers (–●–, n = 2) and EBF dispensers (–O–, n = 4).

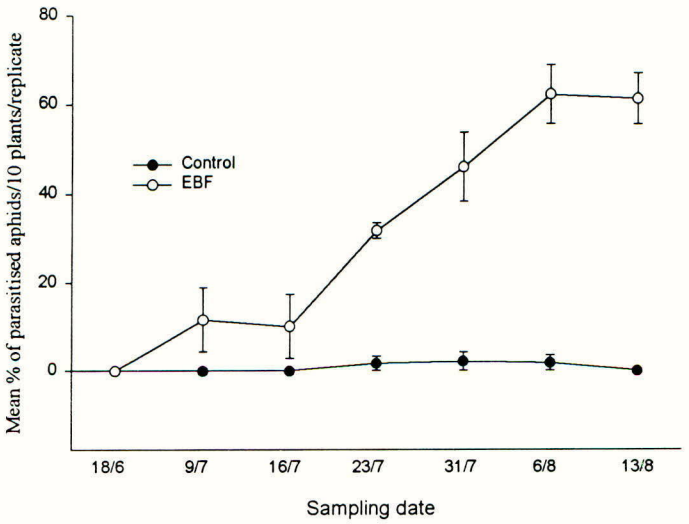

Figure 4. Mean % of parasitised aphids (± SE) in areas of a winter wheat crop with control dispensers (–●–, n = 2) and EBF dispensers (–O–, n = 4).

DISCUSSION

Synthesised aphid alarm pheromone, EBF, when released from dispensers in a winter wheat crop significantly alters the activity of an assemblage of carabid beetles, and increases the level of aphid parasitism by hymenopteran parasitoids. There is also a corresponding reduction in the level of aphid infestation. Semiochemical-mediated habitat location has been reported in several carabid beetle species by Evans (1988, 1994), and adults of the carabid beetle *Pterostichus melanarius*, which was the most common beetle found in this study, orient to olfactory cues arising directly from prey species such as crickets and blowfly larvae (Wheater, 1989) and from analogues of the pheromones of prey species such as (E)-β-farnesene (Kielty *et al.*, 1995; Kirkland & Evans, unpubl.).

Polyphagous carabids have an advantage in integrated pest management programs, in that they can survive in an area when there are no pest species present by feeding on alternative prey such as Collembola (Wheater, 1989). Therefore, inducing carabid beetle movement into cereal fields using semiochemicals early in the season before pest populations become established will not lead to starvation or death of the beetles. Consequently beetles will be present within the crop when pests such as aphids arrive in the spring/early summer, enabling the beetles to reduce the pest population when numbers are low preventing a build up to damaging levels later in the season.

Aphid counts in the vicinity of EBF dispensers were significantly lower than those around control dispensers. This may well be due to the natural effect of the EBF on the behaviour of aphids by disturbing settling behaviour and/or leading to aphids dispersing from wheat plants as reported by Phelan *et al.* (1976); Nault & Montgomery (1979) and Wohlers (1981). Additionally reduction in aphid numbers by EBF may well be due to predation by coccinellids and syrphids, but few of these were noted in the crop until aphid levels peaked, and none were found on the plants sampled. Numbers of aphids climbing back onto plants after dropping off in response to EBF may be reduced by injury caused by the relatively long drop to the soil surface, and by predation by ground-dwelling generalist predators such as carabid beetles which are attracted to EBF (Kielty *et al.*, 1996; Kirkland & Evans, unpubl.) and which are more active in areas where EBF is being released.

There was a significant level of parasitism of aphids on plants in the vicinity of the EBF dispensers. Up to 60% parasitism was recorded in the form of aphid 'mummies' being present. Parasitism in areas around the methanol dispensers did not exceed 2.5%. Whilst evidence exists for attraction of aphid parasitoids to the aphid sex pheromone (+)-(4aS, 7S, 7aR)-nepetalactone (Hardie *et al.*, 1994) there are no reports of attraction to EBF. By releasing EBF via field dispensers, movement of the parasitoids into the crop is enhanced, leading to greater levels of parasitism. Plants 70 m from the crop edge did not show any differences in their level of aphid infestation or parasitism from plants closer to the edge of the field.

By using semiochemicals such as EBF to enhance the suite of predatory and parasitic insects within a crop, pests may be prevented from reaching levels economically damaging to the crop without recourse to the use of pesticides.

ACKNOWLEDGMENTS

Thanks to the Ministry of Agriculture, Fisheries and Food for funding DLK, to Dr. H. McNab of the Chemistry Department, University of Edinburgh for use of the equipment needed to produce EBF, and to A. Hunter of BIOSS, Edinburgh for help and advice in the statistical analyses.

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Pheromone dispersion in the canopy trunk space

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ABSTRACT

Optimizing the placement of passive pheromone dispensers in the forest canopy trunk space requires understanding of the air movement in this space. This work concerns western pine beetle *(Dendroctonus brevicomis)* which typically fly in temperatures greater than 11 °C during daylight in the warm seasons. This study utilizes sulphur hexafluoride (SF_6) as a tracer to study the dispersion patterns in a ponderosa pine *(Pinus ponderosa)* trunk space in the southern Cascade Range in northern California. Four-hundred and fifty, 30-minute samples of SF_6 concentration were collected per test day inside of a circle of 30-m radius, resulting in over 3500 samples over 9 test days. Atmospheric conditions ranging from stable through unstable were sampled. Extensive canopy architecture as well as heat and momentum flux data were also collected. Results showed substantially different mixing regimes as a function of stability.

INTRODUCTION

The primary objective of this project is to provide pest managers with explicit guidance for the placement of passive pheromone releasers in the forest trunk space. Insect pheromones are naturally occurring chemicals and are increasingly being researched for use in pest management strategies. It is difficult to design a tracer experiment to investigate pheromone dispersion in the canopy without knowing the exact nature of the pheromone response mechanism of the insect. There is some evidence that response to a pheromone plume is a signal to noise ratio detection problem and that the insect follows a plume peak centerline concentration to its source. The flight of western pine beetle *(Dendroctonus brevicomis)* typically occurs during the day. The typical midday dispersion environment under a closed ponderosa pine canopy will be explicitly measured in this experiment. During the day, pheromone may be relatively uniformly mixed under the canopy implying that the beetles may encounter a threshold level of pheromone and use this as a proximity indicator. In this case, the insect could home in by flying upwind. The shaded stem space is stable and may not be well mixed, on the other hand, and the plume-following mechanism might suffice due to the narrower, more concentrated plume. The two approaches might lead to the same result as long as a detectable level of pheromone is encountered by the beetle. These mechanisms will, in all likelihood, be specific to the insect and the

specific pheromone being studied. However, the dispersion of any gaseous scalar quantity under this canopy can, at first pass, be treated generically assuming chemical passivity and it is largely determined by the dispersion environment which, in turn, is determined by meteorology, canopy density, and distribution. This discussion is guided by studies in the entomological and meteorological literature (Aylor, 1976; Aylor *et al.*, 1976; Murlis & Jones, 1981; Elkington *et al.*, 1984; Farbert *et al.*, 1997).

The degree of coupling of the in-canopy atmosphere and the 'free' atmosphere away from the canopy will depend upon canopy density and architecture as well as the state of the atmospheric surface layer. The stem space in a dense, closed canopy can be decoupled from the above-canopy planetary boundary layer (PBL) wind field with exchange between the canopy and the free atmosphere dominated by high-energy, low-frequency downward wind gusts or 'injection' events. The crown space converts mean-flow kinetic energy from above the canopy into turbulent kinetic energy (TKE) within the canopy, and much of this TKE is lost to drag due to canopy elements. Mean wind speeds below the crown space are less than those above the canopy. In closed canopies, the crown space receives much more direct-beam solar radiation during daylight hours than the stem space and warms more quickly. Thus an inversion layer is likely to form in the stem space in closed canopies during the daytime which further suppresses momentum transfer and turbulence. Local slope-related flows within the stem space and "chimney effects" due to breaks in the canopy may combine with mean motion above the canopy to drive both transport and dispersion within the stem space. In more open canopies, the mean wind speeds are less in-canopy due to a bulk drag effect of the canopy. However, in these canopies, a large amount of sunlight reaches the forest floor and the atmosphere is closely coupled with the free atmosphere as trees may act as individual obstacles to higher energy flows (higher mean wind speeds) causing large horizontal variability in the flow field. Forest canopies range in density from thick tropical jungle canopies which are completely closed to widely spaced trees in 'parklands' typical of much of the high arid western United States.

In some circumstances, during thermally neutral conditions, when the wind speeds above the canopy are greater than 5 to 10 m/s, plumes below the canopy follow the mean PBL wind direction. During stably stratified conditions, however, the plume direction in the canopy can be offset on the order of 45 degrees from the mean wind direction above the canopy. These numbers are specific to the canopy being studied, but they point out that there is a threshold wind event when the air above the canopy mixes with the in-canopy air and that the mean flow direction in-canopy can substantially deviate from the mean flow direction in the free atmosphere.

The degree of coupling will largely depend on the temperature structure of the surface layer or the 'stability' of the layer. In stable conditions, cold air is under warm air and vertical turbulence is damped. In these conditions, the stem space may be effectively decoupled from the above canopy atmosphere. A scalar quantity released under the canopy such as a pheromone would tend to stay under the canopy and the pheromone plume would remain concentrated due to the low mixing environment. In the case of an unstable environment, warm air is under cold air. Since the warm air is lighter, it rises through the cold air and mixes the unstable layer. In an unstable atmosphere pheromone would tend to rise and mix and might leave the target area where the insects are active. The canopy complicates this relationship because the stability will change with height in the canopy. Often, the upper leaf surfaces intercept the solar radiation and the shaded stem space will remain stable, but an unstable layer will develop at the canopy top. Generally, western pine beetle *(Dendroctonus brevicomis)* flight is limited to the stem zone and the mean attack height is around 6 m.

The combination of canopy structure and stability regime will determine optimum spacing of the pheromone assuming the entomological parameters and pheromone elution rate are known. The approach used in this study is to know the canopy structure and micrometeorology in detail and measure the gaseous dispersion field.

SITE DESCRIPTION

The site is in ponderosa pine *(Pinus ponderosa)* in the southern Cascade Range near the town of McCloud, California. This area offers various canopy densities by way of differing silvicultural treatments. This experiment was sited in a relatively close-crowned canopy on locally flat terrain at 1300 m elevation.

TRACER PROGRAM

There is evidence that plumes for near-instantaneous releases within a Douglas-fir canopy generally range in width from about 30 degrees to over 120 degrees. Instrument sampling arrays were deployed covering about 240 degrees to capture the plume. Even with 240 degree coverage, one plume edge was off the array during many of the tests. However, the plume centerline and maximum concentration were on the measurement array. The decision of which portion of the array to decorate for each test was based on then-current conditions and wind-direction forecasts for the period of the tracer test. Tests were conducted from June 20 to 28, 1998.

A set of three concentric circles centered on the emission point were marked for sampler deployment. The circles had 5, 10, and 30 m radii. Sampling locations were evenly spaced on each circle and between 47 and 53 samplers were used for each test. The 5-m-radius circle had 12 possible sampling locations, one every 30 degrees. To decorate 240 degrees, the 5-m circle had nine samplers. The 10- and 30-m-radius circles had 24 possible sampling locations each, 1 each 15 degrees. The 10- and 30-m circles had 17 samplers each to decorate 180 degrees. Each sampling location was identified with a circle radius and an azimuth angle. Four sampling towers were deployed, one on each arc as well as the 25-m meteorological tower. The three arc towers were 7 m high, and samplers were placed at 1.5 and 5 m. The meteorological tower had samplers at 1.5 m, 10 m, and 25m.

Krasnec et al., (1984) and Benner and Lamb (1985) provide detailed discussion of the syringe sampler and the analyzer respectively, as well as the general experimental approach. The syringe samplers were programmed to start simultaneously at all sampling locations. The sampling period was set for 30 minutes per sample. Since the samplers contain 9 sampling stations, this means that each test period was 4 hours and 30 minutes. Background concentrations of SF_6 could be above detectable levels, so background concentration levels were monitored.

The real-time analyzer used during the tests and for sample assay has a detection range of 10 ppt to 10 ppb (6.07×10^{-7} g/m^3). The lower end of the reliable quantification range is probably on the order of 50 ppt (3.04×10^{-5} g/m^3) . In order to stay below the 10 ppb limit at the plume centerline on the 5 m radius circle, the SF_6 emission rate was around 5.0×10^{-5} g/s and was adjusted in the field as data were analyzed. The analyzer was calibrated using no less than four audit standards both prior to and after analysis of each 30-minute test series.

METEOROLOGICAL MEASUREMENTS

Three 7-m towers collected mean meteorological data both under and outside the canopy. These towers had 2 levels of temperature, humidity, wind speed, and direction and one net radiometer. Three three-axis sonic anemometers were used to collect both mean and turbulence statistics. These instruments provide both momentum and heat flux as well as mean wind vectors and turbulent covariance statistics. These sensors were arrayed vertically up to 25 m and were collocated with pheromone samplers. A SoDAR was deployed to characterize the atmospheric boundary layer. It measured wind speed and direction between heights of 100 and 800 m.

CANOPY INVENTORY

Canopy density was measured on a regular grid pattern across the site. Two methods of measurement were used. The first is a LiCor 2000 LAI meter. The second was hemispherical photography. These measurements were taken on a regular grid and LAI was also measured vertically on the main meteorological tower up to 25 m. A detailed stem map of the plot was also drawn.

EXPERIMENTAL ERRORS IN THE TRACER PROGRAM

Two recognized errors entered into the field program. One was due to leakage from the test syringes because of incorrect capping and the second was due to a contaminant volatilizing from an O-ring lubricant in the syringe. The first error was studied in the field (it was corrected near the end of the program) and the affected syringe data can be corrected through a regression equation. The contamination error is not yet completely understood but it was directly evident in the field using the analyzer. It is apparently related to the syringe temperatures and occurs in around 25% of the data. These contaminated data are not discussed here, though it is hoped that after laboratory analysis of the syringes at varying temperatures, the effect of the contaminant can be removed.

RESULTS

Results are preliminary but begin to allow the development of guidance for the placement of passive releasers under this canopy. On still days, the temperature gradient under the canopy controls the vertical spread of the plume. The temperature gradient (or stability) in the trunk space is greatly influenced by the canopy architecture. The leaf area index (LAI, m^2m^{-2}) is very difficult to obtain in this type of canopy. Two methods of obtaining this quantity were pursued, as discussed previously. The hemispherical photographs have not been processed yet, so preliminary numbers are only available from the LiCor 2000. This instrument is known to be less accurate in canopies with substantial directional biases in the vegetation which is the case in the canopy here. This canopy extends up to an average height of between 25 and 30 m and is best characterized as individual raised traffic cones. The trunk space is open and easy to move through. A large percentage of the surface is illuminated by direct solar radiation during at least part of the day. The LiCor yields LAI s in this canopy between 2.5 and 3.

Due to the distribution of the canopy, surface heating commenced early and the stable layer was gone as indicated on the 30-m mast by around 10:30 a.m. The tracer release was at 1.5-m height,

and the vertical spread of the plume increased as the stability tended towards unstable. This information will be used to scale the plumes by the source strength to allow a direct indication of effective radius. The test period included 2 days with mean wind speeds of $>3ms^{-1}$ even at the lower anemometers. These days are neutral in terms of stability with the in-canopy flow highly turbulent. These conditions were unexpected. The nature of the canopy allows the mean velocity to remain high while moving through this field of discrete obstacles. Generally, the canopy should exert a bulk drag and the in-canopy velocity should be much lower than that above the canopy. The highest anemometer was not high enough to evaluate this effect and the SoDAR data were too high. The higher than expected frequency of these relatively high-velocity flows in this canopy require that attention be paid to this neutral stability condition which is highly turbulent due to the canopy obstruction, but quickly transports material long distances and prevents elevated concentrations.

The converse of the neutral situation was the very low velocity situation which existed on three test mornings, making it very difficult to position the array because the mean motion approached zero. Under these conditions, tracer that is released hangs together with little movement of the plume and low mixing, due to stable conditions. This cloud of concentrated material then is available to waft out of the canopy in the vertical motions (thermals) that begin to develop as the surface heats. More detailed analysis of the turbulence data should directly yield heat and momentum fluxes which can then be used to determine scalar (tracer) flux out of the canopy and subsequently be used to quantify the loss term from passive releasers under the canopy.

CONCLUSIONS

Conclusions are presented here in two sections. Since this paper largely focuses on methods, the experimental method is evaluated. This is followed by a discussion of preliminary results. Two sources of error exist in the data due to experimental procedures. The QA/QC performed during the experiment focused on the chemical analysis and the source/release system. The weak link from the experimental standpoint turned out to be the syringes themselves. Incorrect capping of the syringes turned out to be a subtle error and therefore was not picked up until well into the test program. Data were plotted and most suspicious outliers in this context were too high because of the frequency of zeroes or background in this type of work. The procedure was to reanalyze these outliers. When the reanalysis returned a lower concentration—which made more sense in the spatial context of the plume—errors were attributed to mislabeling (<1% of the data was deemed suspicious). The incorrect capping would have been spotted immediately if calibration gases had been put into syringes and carried out into the test plot and placed in the data stream. This was an oversight in the QA plan. The second error is more interesting because it may reveal a flaw in recommended equipment that was exactly specified. It was casually suggested that, as a precaution, the syringes be preheated in a ventilated oven to evaporate off any volatiles before the experiment. When this potential problem was discussed with experts, it was stated that the type of contamination seen in this study would not occur. As noted above, this error was immediately obvious at the analyzer when it occurred, and it requires further investigation before the discussion can proceed.

Preliminary results strongly support the idea that the dispersive environment is evolving as the day progresses, at least on the test days that there was not a strong synoptic gradient resulting in high winds at the site. A parallel study was being undertaken at the time this study was performed which indicated there was high beetle activity on the days that a cool, still (stable) morning

transitioned into a hot afternoon with a variable breeze (unstable). Therefore, the dispersion of the pheromone will be closely correlated to the time it is released. If the pheromone is released at night, a cloud or 'pool' of the material may accumulate near the infestation which acts as a volume source of material when the air begins moving significantly during the late morning. As the day progresses, a significant amount of a buoyant gas released in the trunk space will be lost upward and out of the canopy top.

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Entomopathogenic nematodes and fluorescent *Pseudomonas* rhizosphere bacteria inhibiting *Radopholus similis* invasion in banana roots

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ABSTRACT

The entomopathogenic nematode *Steinernema carpocapsae* and a strain of the rhizosphere bacterium *Pseudomonas fluorescens* were tested in two greenhouse experiments for inhibition of *Radopholus similis* invasion in banana roots. In contrast to previous experiments, in this study *S. carpocapsae* either applied as live or as sonicated nematodes caused no reduction in *R. similis* invasion. *P. fluorescens*, however, significantly reduced *R. similis* invasion by 50% in one experiment. When *S. carpocapsae* and *P. fluorescens* were applied together the effect on *R. similis* invasion was not different from when they were applied separately, indicating no interaction between the two organisms.

INTRODUCTION

Plant-parasitic nematodes can be among the most damaging pests of agricultural crops. On banana, one of the most important root pathogens is the burrowing nematode, *Radopholus similis*. The use of nematicides still remains the main method of control. However, with an increasing environmental awareness, the use of nematicides is being phased out in favour of an integrated pest management approach. Biological control in particular can be an important component of such an integrated approach.

Two organisms were investigated for their potential as biological control agents for *R. similis*: the entomopathogenic nematode *Steinernema carpocapsae* and a strain of the rhizosphere bacterium *Pseudomonas fluorescens*. Both entomopathogenic nematodes and rhizosphere bacteria have become increasingly important as biological control agents for various root pathogens. Entomopathogenic nematodes are commercially available for the control of mainly insect pests (Georgis, 1992). Fluorescent *Pseudomonas* rhizosphere bacteria have become known for their plant growth promoting ability as well as their inhibitory effect on many fungal root pathogens. Various rhizosphere bacteria are currently being developed commercially for the control of fungal root pathogens (Vidhyasekaran *et al.*, 1997). The effects of both entomopathogenic nematodes and fluorescent *Pseudomonas* rhizosphere bacteria either alone or in combination on *R. similis* invasion in banana roots were studied through greenhouse experiments.

MATERIALS AND METHODS

Entomopathogenic nematodes

Third stage 'dauer' juveniles of the entomopathogenic nematode *S. carpocapsae* (strain 252, UK) were used as inocula for experiments. They were produced *in vivo* using the Greater Wax Moth (*Galleria mellonella*) late instar larvae as described by Woodring & Kaya (1988). Both live and sonicated nematodes were used. Sonication of nematodes was for approximately 10 min using an ultra sonic bath. The suspension obtained was checked under a high powered microscope to confirm that nematodes were ruptured and gut contents were released.

Rhizosphere bacteria

P. fluorescens strain 95.3 isolated from banana roots in Dominica in the West Indies was used. The bacterium was routinely cultivated on King B agar (King *et al.*, 1954). Bacterial suspensions with an optical density at 640 nm ranging between 0.970 and 0.997 were used as inocula. The concentration of bacterial suspensions was checked by streaking 0.1 ml of tenfold serial dilutions onto triplicate King B agar plates. After 24 to 48 h at 28 °C, fluorescent colonies were counted under u.v. and results were expressed as colony-forming units per ml.

R. similis

R. similis was originally isolated from Plantain, *Musa* AAB, from Cameroon. The nematode was axenically cultured on carrot discs (O'Bannon & Taylor, 1968). As males are non-infective, only *R. similis* females and juveniles were counted for the inocula.

Plant material

Banana plants, *Musa* AAA, cv. Grand Naine, a variety known to be susceptible to *R. similis*, were grown *in vitro* on Murashige-Skoog medium (Murashige & Skoog, 1962). When 3 cm tall, plants were transferred onto vermiculite and kept under greenhouse conditions (25-35 °C ambient temp., 65% r.h., 16 h daylight). After acclimatisation, plants were repotted either in proprietory loam-based compost or fine sand.

Greenhouse experiments

For experiment 1, plants potted in a loam-based compost were divided over six treatments: *P. fluorescens*, live *S. carpocapsae*, sonicated *S. carpocapsae*, *P. fluorescens* + live *S. carpocapsae*, *P. fluorescens* + sonicated *S. carpocapsae*, and a control. *P. fluorescens* concentration was 1.7×10^9 bacteria/plant and live or sonicated *S. carpocapsae* concentration was 45,000 nematodes/plant. *S. carpocapsae* treatments were applied 4 days after *P. fluorescens* applications. One day after *S. carpocapsae* application, all plants were inoculated with 1,700 *R. similis*. All bacteria and nematode suspensions were applied in 3 ml water suspensions to 1 cm deep trenches cut around the bases of plants. Replication was fivefold and harvest of roots was 7 days after *R. similis* inoculation.

For experiment 2, four treatments were set up: *P. fluorescens*, sonicated *S. carpocapsae*, *P. fluorescens* + sonicated *S. carpocapsae*, and a control. Before plants were potted in sand they were submerged for 3½ h in an aerated suspension consisting of *P. fluorescens*, sonicated *S. carpocapsae*, both or sterile distilled water (SDW) (control treatment). For the *P. fluorescens*

root dip, 10 ml of a suspension with a concentration of 1.1×10^9 bacteria/ml was diluted to 50 ml with SDW giving a concentration of 2.2×10^8 bacteria/ml. For the sonicated *S. carpocapsae* root dip, 10 ml of 5,000 sonicated nematodes/ml were diluted to 50 ml with SDW giving a concentration of 1,000 sonicated nematodes/ml. For the combined bacteria/nematode root dip, 10 ml of both *P. fluorescens* and *S. carpocapsae* were diluted to 100 ml with SDW. After one day, all plants were inoculated with 210 *R. similis* in 1.5 ml applied to 1 cm trenches cut around plant bases. Treatments were replicated four times and harvest was 6 days after *R. similis* application.

At harvest, plant height and root fresh weight were recorded. Roots were washed out, stained in 0.1% acid fuchsin and macerated (modified from Bridge *et al.*, 1982). Aliquots from suspensions were taken and the number of *R. similis* were counted under an inverted light microscope (50x and 125x magnification). Total numbers of *R. similis* per root system were estimated.

Statistical analysis

Experiments were set up as completely randomized designs. Results were analysed in Minitab using one-way analysis of variance (ANOVA), Dunnett's multiple comparison test (treatments vs controls) and Tukey's pairwise comparison test (between treatments). The significance level was set at 5%.

RESULTS

Table 1. Experiment 1. Number of *R. similis* recovered per root system after treatment with *P. fluorescens*, live *S. carpocapsae* or sonicated *S. carpocapsae*.

Treatment	Plant height (cm)	Root fresh weight (g)	N° *R. similis* per root system
Control	3.7 ± 1.0 **a**	0.5 ± 0.2 **a**	182 ± 33 **a**
P. fluorescens	3.0 ± 0.8 **a**	0.5 ± 0.2 **a**	160 ± 32 **a**
Live *S. carpocapsae*	3.5 ± 0.7 **a**	0.6 ± 0.2 **a**	164 ± 38 **a**
Sonicated *S. carpocapsae*	3.7 ± 1.2 **a**	0.5 ± 0.3 **a**	190 ± 55 **a**
P. fluorescens + Live *S. carpocapsae*	4.1 ± 0.6 **a**	0.7 ± 0.2 **a**	168 ± 28 **a**
P. fluorescens + Sonicated *S. carpocapsae*	3.9 ± 0.7 **a**	0.7 ± 0.2 **a**	160 ± 34 **a**

Figures followed by the same letter are not significantly different from each other (one-way ANOVA; $p>0.05$).

In experiment 1, no difference was seen in numbers of *R. similis* between the control and *P. fluorescens* and/or *S. carpocapsae* treatments (Table 1). In experiment 2, however, plants

treated with the *P. fluorescens* root dip, either alone or in combination with *S. carpocapsae*, had significantly lower numbers of *R. similis* compared to control plants (Table 2). Percentage reduction in *R. similis* invasion was 50% and 55% with the *P. fluorescens* and the *P. fluorescens* + sonicated *S. carpocapsae* treatments respectively. The sonicated *S. carpocapsae* treatment alone did not cause significantly reduced *R. similis* numbers. In addition, the *P. fluorescens* and the *P. fluorescens* + sonicated *S. carpocapsae* treatments were not significantly different from each other.

Table 2. Experiment 2. Number of *R. similis* recovered per root system after treatment with fluorescent *P. fluorescens*, live *S. carpocapsae* or sonicated *S. carpocapsae*.

Treatment	Plant height (cm)	Root fresh weight (g)	N° *R. similis* per root system
Control	3.1 ± 0.9 **a**	0.5 ± 0.3 **a**	44 ± 11 **a**
P. fluorescens	3.8 ± 0.7 **a**	0.6 ± 0.1 **a**	22 ± 8 **b**
Sonicated *S. carpocapsae*	2.8 ± 0.3 **a**	0.4 ± 0.1 **a**	31 ± 10 **ab**
P. fluorescens + Sonicated *S. carpocapsae*	3.1 ± 0.6 **a**	0.6 ± 0.2 **a**	20 ± 11 **b**

Figures followed by different letters are significantly different from each other (one-way ANOVA, Dunnett's and Tukey's tests; $p<0.05$).

DISCUSSION

Antibiosis is defined as antagonism mediated by specific or non-specific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds, or other toxic substances (Fravel, 1988).

Although in the present study *S. carpocapsae* did not significantly reduce *R. similis* invasion, previous experiments have shown the inhibitory potential of live and sonicated *S. carpocapsae* to plant-parasitic nematode invasion (Aalten, 1996; Aalten, unpublished data). A suggested hypothesis was competition for space around the root zone as both entomopathogenic nematodes and plant-parasitic nematodes have an affinity for roots (Bird & Bird, 1986; Ishibashi & Choi, 1991; Matsunaga *et al.*, 1996). However, as sonicated *S. carpocapsae* can reduce plant-parasitic nematode invasion, substances associated with the nematode are more likely to be causal. *Steinernema* spp. are symbiotically associated with bacteria of the genus *Xenorhabdus*. Antibiotic activity associated with these symbiotic bacteria could account for the observed effect on plant-parasitic nematode invasion.

The rhizosphere bacteria *P. fluorescens* also proved inhibitory to *R. similis* invasion. The results suggest that such treatments may be more effective at lower *R. similis* inoculum levels. In this study, the higher inoculum level used was at least ten times greater than what might occur in the field if all nematodes were to invade. However, the actual infectivity of *R. similis* can be low and in this case it varied from 11% (experiment 1) to 21% (experiment 2). In

experiment 1, numbers of *R. similis* in roots were in excess of or at the higher end of field infestation levels. In experiment 2, numbers were lower but would still be considered as damaging (Fogain & Gowen, 1997). *P. fluorescens* application methods were also different between experiments. The results suggest that root dip (experiment 2) may be a more effective application method. Preliminary studies (Aalten, unpublished data) showed that *P. fluorescens* root colonization levels are significantly higher after root dip compared to root inoculation. It is suggested that degree of root colonization by rhizosphere bacteria is related to degree of disease suppression (Weller, 1988).

The present experiments support previous studies by Oostendorp & Sikora (1989 and 1990) who found root invasion by the cyst nematode *Heterodera schachtii* inhibited by rhizosphere bacteria. They suggested rhizosphere bacteria bind with root surface lectins thereby interfering with normal nematode-host recognition. However, the production of toxins could also play a role. Becker *et al.* (1988) observed the production of nematicidal compounds by rhizosphere bacteria which affected the root-knot nematode *Meloidogyne incognita in vitro* motility. Unpublished studies (Aalten *et al.*, submitted to Letters in Applied Microbiology) have confirmed *in vitro* repellency of *R. similis* and *Meloidogyne* spp. to *P. fluorescens* strains.

Both entomopathogenic nematodes and rhizosphere bacteria show potential as plant-parasitic nematode biocontrol agents. In an integrated pest management approach it is possible that several biocontrol agents may be used consecutively. The effects the two organisms have on each other were therefore also studied. In our experiments, *S. carpocapsae* treatments did not interfere with the reduced *R. similis* invasion due to the *P. fluorescens* treatment and no interaction between the two organisms was found.

Most bacteria are easily cultured and manipulated by genetic engineering techniques. The opportunity therefore exists for development of new bacterial strains with enhanced production capacities. Eventually it may be possible to isolate and produce the inhibitory compound by chemical processes and then apply the compound or its analogue as a nematicide.

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Biological control of *Botrytis cinerea* by suppression of sporulation

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ABSTRACT

The development of necrotrophic pathogens such as *Botrytis* spp. depends on the availability of necrotic tissues. Saprophytic fungi have been screened for their ability to successfully compete with *Botrytis* spp. during the colonisation of such tissues. *Ulocladium atrum* was most effective in suppressing *Botrytis* spp. colonisation. Experiments under commercial growing conditions were carried out to assess the potential of *U. atrum* to control *B. cinerea* in strawberry and in cyclamen as well as of *Botrytis* spp. in onion. Fruit rot of strawberry was significantly reduced by weekly applications of *U. atrum* in three out of four field experiments. The effect of the antagonist was as strong as of fungicide treatments. In a fourth experiment, the percentage of rotted fruit was low and treatments with *U. atrum* or fungicides had no significant effect. In cyclamen, treatments with *U. atrum* significantly reduced the number of diseased petioles as efficiently as fungicide treatments in ten out of 13 experiments. In three experiments, conducted in a greenhouse with conditions very favourable for *B. cinerea*, neither *U. atrum* nor fungicides controlled disease development. In onion, the number of leaf spots was significantly reduced by weekly *U. atrum* applications.

INTRODUCTION

Necrotrophic pathogens such as *Botrytis* spp. colonise necrotic tissues of lesions or plant debris. Conidia produced on such tissues are the source of new infections within the crop (Braun & Sutton, 1987; Köhl & Fokkema, 1998; Pfender *et al.*, 1993). At our institute, saprophytic antagonists have been selected which have the potential to colonise necrotic tissues rapidly and to compete with *Botrytis* spp. in such tissues. Exclusion of *Botrytis* spp. from such niches by antagonists will lead to a reduced production of conidia of the pathogen within the crop. The lower inoculum load will result in less new infections, and consequently the progression of the epidemic of *Botrytis*-incited diseases will be slower. The validity of this strategy has been demonstrated in onion where reduced sporulation of *Botrytis* spp. resulted in a lower aerial spore load in the treated plots compared to an untreated control (Köhl *et al.*, 1995a). The number of leaf spots caused by *Botrytis* spp. on onion leaves was significantly reduced.

Amongst saprophytic fungi, isolated from necrotic leaves, *Ulocladium atrum* proved to reduce sporulation of *Botrytis* spp. on necrotic tissues of a range of host plants including strawberry, onion, cyclamen and geranium. The antagonist was effective at the whole range of temperatures tested from 6 to 24°C and was not susceptible to interruptions of leaf wetness periods (Köhl *et al.*, 1995c). In a series of nine experiments, the antagonist consistently reduced sporulation of *B. cinerea* for more than 90% on dead lily leaves which had been exposed to field conditions under very different environmental conditions (Köhl *et al.*,

1995b). Other candidate antagonists including *Chaetomium globosum* and *Gliocladium catenulatum* failed to consistently control *B. cinerea* sporulation. Experiments in a lily crop showed that conidia of *U. atrum* have the ability to survive and to persist on surfaces of healthy leaves under field conditions for up to 21 days (Elmer & Köhl, 1998). After artificial induction of necrosis by using paraquat, the antagonist was able to colonise such leaves, outcompeting naturally occurring saprophytes such as *Cladosporium* spp. These experiments too showed that *U. atrum* has the necessary ecological characteristics to be a successful biological control agent of *Botrytis* spp.

There is no evidence that *U. atrum* produces antifungal metabolites or acts as a hyperparasite (Köhl *et al.*, 1997). It can be assumed from microscopical observations that the interaction is based on competition for nutrients or space.

The antagonist has been tested under field conditions in strawberry and under commercial greenhouse conditions in cyclamen (Köhl *et al.*, 1998). Preliminary results are presented in this paper.

In strawberry, fruits are very susceptible to grey mould caused by *B. cinerea.* Inoculum of the pathogen produced on dead leaves can contaminate flowers. Necrotic flower tissues can then be colonised by *B. cinerea*, from where young fruits can be infected later. The antagonist *U. atrum* is aimed at suppression of saprophytic colonisation of both dead leaves and flower parts by the pathogen.

In cyclamen (*Cyclamen persicum*), *B. cinerea* can cause severe damage of leaves and flowers reducing its ornamental value. The use of fungicides for disease control is limited by possible side effects of fungicides on plant development and fungicide resistance of the pathogen. Because of the high resistance of healthy leaves against *B. cinerea* infection, only a high inoculum pressure of the pathogen leads to successful infections. Naturally senesced leaves within the canopy are a prerequisite for disease initiation. *B. cinerea* can colonise such leaves saprophytically and subsequently infect adjacent healthy petioles or leaves. The antagonist applications are aimed at the exclusion of *B. cinerea* from such naturally senesced leaves.

In onion, *B. squamosa* and *B. cinerea* cause leaf spots. *Botrytis* spp. can sporulate abundantly on dead onion leaf tips, forming the inoculum for a next disease cycle. Several of such disease cycles can occur in a growing season. The antagonist application is aimed at the suppression of sporulation of *Botrytis* spp. in order to slow down the progression of the epidemic in the onion crop.

MATERIAL AND METHODS

Strawberry

Four experiments were carried out with strawberry, cv. Elsanta, at two locations. Weekly sprayings with *U. atrum* (2 x 10^6 conidia/ml) from planting up till harvest were compared with a control and the recommended standard fungicide spray (Köhl *et al.*, 1998). In the first experiment a fourth treatment consisted of weekly spraying with *Gliocladium roseum* (1 x 10^7 conidia/ml). In the other experiments this was replaced by weekly spraying with *U. atrum* (2 x

10^6 conidia/ml) during flowering only. The number of healthy and rotted fruits harvested per plot was counted for each harvesting date.

Cyclamen

In total, 13 experiments were carried out in different commercial greenhouses during spring and autumn of the years 1995 - 1997. Varieties and growing conditions differed considerably between growers, e.g. water was provided via wet mats from the bottom in some greenhouses or plants were irrigated overhead up to three times per week in others. Experiments started with 17 to 20 week old plants generally before naturally infections by *B. cinerea* occurred. In each experiment *U. atrum* applications (1 x 10^6 conidia/ml) at regular intervals from the beginning of the experiment until four weeks before marketing were compared with water applications at the same intervals as control. The interval between sprays of the antagonist was two weeks in the first experiment, but was increased to four weeks in later experiments. In some experiments, fungicides were applied as an additional treatment according to the grower's standards. In several experiments, the antagonist *Gliocladium roseum* was tested in an additional treatment (Köhl *et al.*, 1998). In all experiments, treatments were replicated four times with 24 plant per replication. For plants at marketable age, the disease incidence and the disease severity (DS), assessed as the number of petioles or leaves per plant showing *B. cinerea* sporulation, were recorded.

Onion

One field experiment was carried out in onion, cv Hyton. Conidial suspension of *U. atrum* (2 x 10^6 conidia/ml), vinclozolin or water were sprayed at weekly intervals. The number of leaf spots was counted at weekly intervals.

RESULTS AND DISCUSSION

Strawberry

In the first experiment spraying *U. atrum* gave significantly better control of fruit rot (5.7 % rot) than any other treatment (control 13.9 %, fungicide 12.0 % and *G. roseum* 15.6 %; Table 1). In the second experiment fungicide and weekly *U. atrum* treatment were equally effective (3.9 % and 4.7 %) as compared to the control and *U. atrum* at flowering only (8.1 % and 7.1 %). In the third experiment all treatments differed, with fungicides being most effective (1.3 %), followed by weekly *U. atrum* (2.6 %), *U. atrum* at flowering (3.4 %) and control (5.4 %). In experiment 4, coinciding with very hot weather, fruit attack by *B. cinerea* was much less than in the other experiments and there was no difference between treatments.

In conclusion, weekly treatments with *U. atrum* reduced grey mould of strawberry to the level of fungicide protection. In one experiment even spraying only during flowering, gave significant reduction of grey mould.

Table 1. Percentage of grey mould on strawberry fruits at harvest time; four trials (Boff, Jansen and Köhl, unpublished; trials in Breda were carried out in cooperation with Fruit Research Station (FPO), Wilhelminadorp, the Netherlands).

Treatment	Breda_1 Apr-Aug 1996	Breda_2 Apr-Jul 1997	Wag._3 Apr-Jul 1997	Wag._4 Jun-Aug 1997
Control	13.9 b	8.1 b	5.4 d	1.4
Fungicides	12.0 b	3.9 a	1.3 a	1.5
U. atrum at flowering	-	7.1 b	3.4 c	2.0
U. atrum weekly	5.7 a	4.7 a	2.6 b	1.1
G. roseum weekly	15.6 b	-	-	-

(Figures in columns followed by the same letter are not statistically different; $P<0.05$).

Cyclamen

In most experiments, applications of *U. atrum* in cyclamen reduced the disease incidence and disease severity significantly. The efficiency of the antagonist was as high as or higher than grower's fungicide programmes. Results of a representative experiment are shown as an examples in Table 2. In this experiment, biological control by *U. atrum* was as effective as chemical control consisting of five fungicide applications (dichlofluanid (2x), prochloraz-manganese (2x) and iprodione sprayed in alternation). *G. roseum* was less effective.
Only in three out of 13 experiments, treatments with *U. atrum* or two applications of iprodione did not reduce DS significantly. These three experiments were all conducted in the same greenhouse with conditions very favourable for *B. cinerea* development, resulting in DS's between 7.1 and 11.0 in the control treatment. Overhead irrigation seemed not to have a detrimental effect on the performance of the antagonist.

It can be concluded that *U. atrum* controls *B. cinerea* in cyclamen under practical growing conditions to a similar extent as standard fungicides.

Table 2. Effect of *Ulocladium atrum*, *Gliocladium roseum* and a fungicide programme on grey mould of cyclamen.

Treatment	Disease incidence (%)	Number of diseased petioles per plant (DS)
Control	84 a	3.5 a
Fungicides	46 b	0.7 b
Gliocladium roseum	69 a	1.8 a
Ulocladium atrum	40 b	0.9 b

(Figures in columns followed by the same letter are not statistically different; $P<0.05$).

Onion

The number of leaf spots was very low during the first weeks of the growing season. At the harvest time, 2.2 spots per 10 cm^2 of leaf area were counted on leaves of the water control. Leaves of both the antagonist treatment and the fungicide treatment had significantly less spots per 10 cm^2 of leaf area being 0.9 and 0.9, respectively.

CONCLUSION

The results of the experiments in cyclamen, strawberry and onion show that the antagonistic interaction between a saprophytic fungus and a necrotrophic pathogen during its saprophytic stage can be utilised for successful biocontrol of diseases incited by necrotrophic pathogens. Further research will include detailed studies on the competitive colonisation of necrotic tissues by *U. atrum* and *Botrytis* spp. Furthermore, the antagonist will be tested in other crops damaged by *Botrytis* spp.

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PCR-based detection of *Phytophthora fragariae* in raspberry and strawberry roots

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ABSTRACT

DNA was successfully extracted from *Phytophthora* isolates and from raspberry and strawberry root material using a CTAB extraction method. PCR on the extracts using primers P.FRAGINT and the universal primer ITS4 produced an amplicon approximately 800 bp in size for isolates of *P. fragariae* var. *rubi* and var. *fragariae* and for the closely related species *P. cambivora*. An identical band was produced from extracts taken from raspberry roots 1–40 days after inoculations with *P. fragariae* var. *rubi*. Greatest detection occurred between 1–5 days and declined thereafter. The reduction in detection by PCR coincided with a degradation of coenocytic mycelium and the development of thick-walled oospores in the roots; it was shown not to be associated with any increase in inhibitors of PCR produced from rotting root with time. In most samples greatest amplification was usually obtained when extracts were further diluted with TE buffer.

INTRODUCTION

Phytophthora fragariae has two varieties: var. *rubi* which causes the disease raspberry root rot; and var. *fragariae* which causes the EC quarantine listed disease strawberry red core. Both cause significant economic losses and can remain viable in soil for many years as thick-walled oospores. At present bait tests are used to test stocks for these pathogens (Duncan *et al.* 1993), but recently the use of the polymerase chain reaction (PCR) has been investigated (Bonants *et al.* 1997).

MATERIALS AND METHODS

Growth of isolates

Isolates of seven *Phytophthora* species (Table 1) were grown at 15°C in the dark on French bean agar (FBA), containing 1.5% Oxoid No. 3 agar, and 3% ground French beans (*Phaseolus vulgaris*). After 3 weeks incubation, c. 0.1 g of mycelium was scraped from the surface of each plate using a sterile glass slide and placed in a sterile mortar for immediate DNA extraction.

Studies on infected root material

Individual healthy raspberry plants, cv. Glen Moy, were inoculated in a 15°C growth room with *P. fragariae* var. *rubi* by eluting the soil of each pot with c. 100 ml spore suspension on each of three consecutive days (spore concentrations in zoospores/ml were: first inoculation, 1,900; second inoculation 4,200; third inoculation 15,700). Zoospores were obtained by floating 5 mm agar plugs, taken from the margins of cultures grown on FBA, in sedge peat water (1 litre water eluted through 1 kg sedge peat). Uninoculated plants acted as healthy controls. Five plants were destructively sampled on each of the following days after the last inoculation: 1, 2, 3, 5, 8, 12, 16, 20, 25, 30, 35, 40 and 50. The roots were washed free of soil and frozen prior to DNA extraction. At the same time, some roots were also examined microscopically for the presence of coenocytic mycelium and reproductive structures.

Extraction of DNA

DNA was extracted from c. 0.1 g of mycelium from each of the harvested cultures and from c. 0.1g of each root sample using a cetyltrimethyl ammonium bromide (CTAB) based method modified from Lodhi *et al.* (1994). The samples were ground up with liquid nitrogen in a sterile mortar containing 100 µl aliquots each of 1% Na_2SO_3, 1% insoluble polyvinylpolyrrolidone, 4% bovine serum albumin, and 1 ml extraction buffer. The extraction buffer contained 20 mM sodium EDTA, 100 mM Tris-HCl adjusted to pH 8.0 with HCl, 1.4 M NaCl and 2% CTAB. The ground samples were placed in microcentrifuge tubes and incubated at 65°C for 20 min. Tubes were immediately cooled on ice for 2 min then centrifuged at c. 11,000 *g* for 5 min. In a fresh microcentrifuge tube 500 µl of the aqueous phase was purified by combining it with 400 µl of a 24:1 mixture of chloroform:iso-amyl alcohol and mixed 20 times by inversion, then centrifuged as before. The aqueous phase was purified again as described above. In new microcentrifuge tubes, 400 µl of the doubly purified aqueous phase were combined with 200 µl of 5 M NaCl and 600 µl iso-propanol. The tubes were inverted 20 times, then cooled at -20°C for 15 min to precipitate DNA. The tubes were then centrifuged at c. 11,000 *g* for 5 min and the supernatant discarded. The pelleted DNA was placed in a desiccator overnight, then re-suspended in 100 µl TE buffer (10 mM Tris-HCl and 1 mM EDTA adjusted to pH 8.0). Ten and hundred fold dilutions were also prepared in TE buffer.

PCR amplification

Amplifications were performed in 200 µl tubes containing a reaction mix consisting of 0.5 µM of the primers P.FRAGINT (5' TCGATGTCAAACTTGA 3', based upon McReynolds, 1993) and universal primer ITS4 (5' TCCTCCGCTTATTGATATGC 3', White, *et al.* 1990), 200 µM dNTPs (Sigma), 5 µl 10 X PCR buffer (GeneAmp®, Perkin Elmer), 5 µl GeneReleaser™ (BioVentures, Inc.), 1.25 units AmpliTaq® (Perkin Elmer). Either 5 µl from one of the extracted DNA samples, or TE buffer acting as a negative control were added to each tube and the volume made up to 50 µl with sterile distilled water. A Perkin Elmer 9700 Thermal Cycler was used with the following programme: 2 min at 94°C, then 30 cycles of 40 s at 94°C, 1 min at 50°C and 2 min at 72°C. The programme was terminated with 4 min at 72°C and samples were then stored at 4°C.

To determine if greater inhibition of PCR occurred over time as roots decayed, aliquots of day 1 extract (diluted 10 fold) increasing in 0.5 µl units up to 2.5 µl, were mixed with 2.5 µl of the day 20 extract (10 fold dilution) and visa versa. These samples were then amplified as described above. Samples were also tested using the universal primer ITS3 (5' GCATCGATGAAGAACGCAGC 3', White, *et al.* 1990) and ITS4 to confirm that the extracts contained amplifiable DNA.

All amplified PCR products (12 µl) were mixed with 2 µl 0.1 % bromophenol blue loading dye and resolved on a 1.5 % agarose gel made up with TAE buffer pH 7.6 (40 mM Tris, 20 mM glacial acetic acid and 1 mM EDTA (pH 8.0)). The gels were then stained with 0.05% ethidium bromide and bands visualized on a transilluminator at 312 nm.

RESULTS

Cultures

Using primer P.FRAGINT with ITS4 a PCR amplicon c. 800 base pairs was produced for *P. fragariae* var. *rubi*, var. *fragariae* and *P. cambivora* but not for other *Phytophthora* spp. or TE buffer controls (Table 1). Primers ITS3 and ITS4 produced an amplicon c. 650 bp for DNA extracted from all the *Phytophthora* species.

Roots

PCR detection was achieved from the raspberry root material one day after the last inoculation (Table 2). This correlated with the observed presence of coenocytic mycelium in the roots. Detection continued up to day 40, but not at day 50. The greatest levels of detection occurred between 1–5 days, with the amount of PCR product generally declining after 8 days. This decline coincided with a degradation of coenocytic mycelium and the appearance of oospores in roots: coenocytic mycelium was seen only occasionally from 16–20 days, and rarely thereafter. In comparison, oogonia were present from 3–8 days and only occasionally at 12 days. Oospores began to form within 5 days and almost all developed thick-walls within 8–12 days.

PCR inhibition

Increasing the amount of DNA extract from day 1 to a constant amount of day 20 extract gave increased PCR amplification, while adding greater amounts of day 20 extract to a constant amount of day 1 extract had no effect on PCR amplification. Therefore the decrease in detection with time was not due to increased inhibition of the PCR reaction. For most of the samples, greatest amplification was usually obtained when the suspended DNA extract was diluted 10 fold with TE buffer. Bands of between 300 and 700 bp were produced using the universal primers ITS3 and ITS4 confirming DNA was extracted in all the samples following CTAB extraction.

Table 1. PCR detection of *Phytophthora* species using primers P.FRAGINT / ITS4 and ITS3 / ITS4 on DNA extracted from 3 week old cultures grown in the dark at 15°C.

Isolate	Code	P.FRAGINT / ITS4 PCR amplicon	ITS3 / ITS4 PCR amplicon
P. fragariae var. *fragariae*	cc 1216	+	+
P. fragariae var. *fragariae*	cc 931	+	+
P. fragariae var. *fragariae*	cc 1293	+	+
P. fragariae var. *rubi*	cc 1217	+	+
P. fragariae var. *rubi*	cc 1218	+	+
P. fragariae var. *rubi*	cc 1219	+	+
P. fragariae var. *rubi*	cc 947	+	+
P. cambivora	cc 1221	+	+
P. cambivora	cc 1223	+	+
P. cambivora	cc 1233	+	+
P. cinnamomi	cc 1213	-	+
P. cinnamomi	cc 1215	-	+
P. cinnamomi	cc 1220	-	+
P. cinnamomi	cc 1222	-	+
P. cinnamomi	cc 1224	-	+
P. cinnamomi	cc 1225	-	+
P. cinnamomi	cc 1226	-	+
P. cinnamomi	cc 861	-	+
P. cryptogea	cc 1211	-	+
P. cryptogea	cc 1212	-	+
P. citrophthora	cc 1252	-	+
P. citrophthora	cc 1253	-	+
P. cactorum	cc 1255	-	+
P. cactorum	cc 1254	-	+
TE buffer	-	-	-

+ Amplicon present.
- Amplicon absent.

Table 2. PCR detection of *Phytophthora fragariae* var. *rubi* in inoculated roots in relation to the development of fungal structures over time.

	Time roots harvested after last inoculation (days)													
	H_0[a]	1	2	3	5	8	12	16	20	25	30	40	50	H_{50}[a]
Structures observed														
Coenocytic mycelium	-	+	+	+	+	+	+	-/+	-/+	-	-	-	-	-
Oogonia & antheridia	-	-	-	+	+	+	-/+	-	-	-	-	-	-	-
Immature oospores	-	-	-	-	+	+	-/+	-	-	-	-	-	-	-
Mature oospores	-	-	-	-	-	+	+	+	+	+	+	+	+	+
No. of positive PCR tests (5 replicates)	0	4	5	5	5	3	2	1	2	2	2	2	0	0

[a] Inoculated with distilled water as healthy controls. Roots harvested at day 0 (H_0) and day 50 (H_{50}).
- Structure absent.
+ Structure present.

DISCUSSION

When primer P.FRAGINT was used with the universal primer ITS4, *P. fragariae* var. *rubi* and var. *fragariae* isolates could be distinguished from all the other *Phytophthora* spp. tested except the closely related species *P. cambivora*. This cross-reaction may cause problems for the diagnosis of *P. fragariae* var. *rubi* in raspberry roots when host material is infected with *P. cambivora*. However, it should not be a problem for the PCR diagnosis of strawberry red core as *P. cambivora* is not known to affect strawberries (Erwin & Ribeiro, 1996). Other methods and primers are being evaluated to distinguish *P. fragariae* from *P. cambivora*.

The decrease in PCR detection in raspberry roots with time reflected the degeneration of coenocytic mycelium occurring after the switch from active pathogenesis to sexual reproduction, rather than any increase in PCR inhibition with time. Clearly, once the coenocytic mycelium degenerates, most of the fungal DNA in the root is locked up inside thick-walled oospores. Since oospores are not easily disrupted by conventional methods, the target DNA remains unavailable for PCR. Novel methods for disrupting oospores are currently under evaluation.

Preliminary experiments show primer P.FRAGINT will detect *P. fragariae* in roots of alpine strawberry (*Fragaria vesca*). Currently a time course experiment, similar to that described above is being carried out using alpine strawberry material.

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Chemical and physical alternatives to methyl bromide and their combination in the control of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* in the open field

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ABSTRACT

In three experimental trials carried out in North Italy the effectiveness of methyl bromide (MB), metham sodium (MS) and dazomet (DZ) applied alone or in combination with a 2 week period of soil solarization against *Rhizoctonia solani* and *Sclerotinia sclerotiorum* in the open field were evaluated. MB, at 30 g/m^2 under virtually impermeable film confirmed its efficacy; covering the soil after the application of MS and DZ with low density polyethylene films improved the efficacy of the two fumigants, while reducing some of their negative features. The combination of soil solarization with any of the three tested fumigants resulted in an effective control of the two pathogens, permitting a reduction in the duration of solarization and application of the fumigants at half dosage.

INTRODUCTION

Soil-borne pathogens, as well as nematodes and weeds, can be controlled by using methyl bromide (MB), metham sodium, dazomet or soil solarization (Katan, 1984). Soil fumigation with MB, applied at 60 g/m^2 under low density polyethylene (LPDE) plastic film, is a common practice for the control of a wide spectrum of soil-borne pathogens in many crops. However, during recent years, concern regarding MB ozone depletion potential led to its inclusion in the list of ozone-depleting substances controlled by the Montreal Protocol (Bell *et al.*, 1996) and stimulated the search for methods of soil disinfestation applicable under different cultural and environmental conditions. Among available chemical alternatives, metham sodium (MS) and dazomet (DZ) are generally chosen when soil-borne fungal pathogens are the main target (Garibaldi & Gullino, 1995). Solarization, carried out by covering the soil with transparent film during the hot season, has been widely exploited in a number of warm and marginally suitable countries (Katan & DeVay, 1991). In North Italy, particularly in the open field, where the temperatures reached are only partially effective against soil-borne pathogens, it is interesting to improve its effectiveness by combining it with other control measures (Garibaldi & Gullino, 1991). The present work was done in order to evaluate the effectiveness of methyl bromide (MB), metham sodium (MS) and dazomet (DZ) applied alone under plastic film or in combination with solarization against *Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

MATERIALS AND METHODS

Layout of the experimental trials

Three experimental trials were carried out at Albenga (North Italy) at the Centro Regionale di Sperimentazione e Assistenza Agricola (CeRSAA) of the Chamber of Commerce of Savona in

1996, as described under Table 1. The trials were carried out in the open field in a sand:silt:loam (75:20:5) soil, pH 8, by following a randomized block design with 3 or 4 replicates (60 m^2/plot).

Soil infestation with pathogens

In order to achieve a more uniform soil infestation and, consequently, a higher and even disease incidence, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* were incorporated, as infected wheat kernels, at 30 g/m^2 into the all plot surface prior to disinfestation as indicated in Table 1. Moreover, in order to evaluate the direct effect of the treatments on the survival of *R solani,* the inoculum of the pathogen was buried into the soil, in selected location of the plots. Seven g of infected kernels were put into small bags (5 cm diameter), prepared with gas permeable tissue and buried into the soil 12–18 h before the treatment. Two bags were introduced into each plot, one at 10 and one at 20 cm depth. The bags were taken out 7 days after fumigation or at the end of solarization.

Soil preparation, fumigation and solarization treatments

Soil was levelled, drippers (19 mm diameter) were placed on its surface at a maximum distance of 35 cm from each other. Soil was mulched with standard LPDE (Eiffel, Fontanellato, Italy, 40 μm thick) or the virtually impermeable film (VIF) Bromotech (LMG Smith Brothers, Bristol, UK, 30 μm), coded as LMG. Soil was manually uncovered seven days after the fumigation treatments or at the end of the chosen solarization period. MB (Metabrom, Bromine Compounds, Israel, 98% a.i) was applied by licensed fumigators using the hot gas method, at the dosages reported under Tables 2–8. Metham sodium (MS) (Vapam, SIPCAM, 32,7 % a. i.) was applied as water suspension, by using 30-40 l/m^2 at the rates reported in Tables 2–6. Dazomet (DZ) (Basamid, Basf, 99 a.i.) was incorporated into the soil by rototilling at the rates reported in Tables 2-6; the soil was then covered and irrigated with 30–40 litres/m^2. Solarization lasted 14 or 28 days. In the case of its combination with MB, in trial 2, fumigation was carried out before or after solarization.

Pathogen inoculum survival

When soil was unmulched, the bags containing *R.solani* inoculum were taken out from the soil and immediately processed. One hundred infected kernels/bag were directly plated on Petri plates (10 kernels/plate) on a Rhizoctonia semi-selective medium (Migheli *et al.,* 1990). Inoculated plates were incubated for 5–7 days at 24°C. The number of kernels from which living mycelium was developing was counted and the data so collected are expressed as percent of infected kernels, with control made equal to l00.

Crops grown

Bean and lettuce, crops sensitive to the pathogens artificially introduced into the soil, were grown at a density of 50 seeds/m^2 for bean, (cvs Bobis, Canellino and Anellino), 10 plants/m^2 for lettuce cv. Gheisa and 300 seeds/m^2 for lettuce cv. Foglie di quercia. The cultural practices adopted by commercial growers in the region were applied.

Disease evaluation

Disease severity was evaluated at regular intervals by counting the number of infected plants or by using a Disease Index scale from 0 (healthy plant) to 5 (dead plant). Data collected were statistically analyzed, according to Duncan's Multiple Range Test.

RESULTS

All tested treatments effectively reduced the survival of the inoculum of R. *solani* buried into the soil, at both depths (Table 2). The best results were observed with MB at full dosage or at 40 g/m^2 under LMG. In trial 2, 30 g/m^2 of MB under LMG, alone or combined with 2 weeks of solarization, did not provide complete inhibition of the pathogen (Table 2).

Table 1. Layout of the experimental trials.

Trial number and code	Soil infestation carried out on	Cultural practices	Crop grown and date of planting	Number of replicates
1. ST 1	*R. solani*, 30 g/m^2 infested kernels (24 May 96)	MB fumigation: 14 Jun DZ fumigation: 17 Jun MS fumigation:18 Jun Unmulching: 27 Jun Rototilling: 9 Jul	Bean (cvs Bobis, Anellino) Sown : 1st 15 July 2nd 12 August 3rd 15 Septembert	3
2. ST 2	*R. solani*, 30 g/m^2 infested kernels *S. sclerotiorum*, 30 g/m^2 infested kernels (24 May 96)	Irrigation: 24 Jun; 2 Jul MB fumigation 27 Jun; 3 Jul MS fumigation:28 Jun Unmulching: 22 Jul Rototilling: 25 Jul	Bean (cvs Bobis, Canellino) 28 July Lettuce (cv. Gheisa) 29 July	3
3. SC 2	*R. solani*, 30 g/m^2 infested kernels *S. sclerotiorum*, 30 g/m^2 infested kernels (1 Aug 96)	Irrigation: 12 Aug MB fumigation: 6 Aug Unmulching: 13 Aug; 6 Sept Rototilling: 18 Sept	Bean (cvs Bobis, Canellino) 10 September Lettuce (cvs Gheisa, Foglie di quercia) : 10 Sept	4

Table 2. Effect of different treatments on the survival of soil buried inoculum of *R. solani*.

TREATMENT	% kernels infected with *R. solani* at a depth of					
	Trial 1		Trial 2		Trial 3	
	10 cm	20 cm	10 cm	20 cm	10 cm	20 cm
--	100 b *	100 b	100 d	100 c	100 b	100 c
Soil solarization 28 dd	n.t.	n.t.	0 a	0 a	n.t.	n.t.
MB 60 LPDE	0 a	0 a	1 a	0 a	0 a	0 a
MB 40 LMG	0 a	0 a	n.t.	n.t.	0 a	0 a
MB 40 LPDE	4 a	4 a	n.t.	n.t.	0 a	0 a
MB 30 LMG	n.t.	n.t.	37 c	0 a	n.t.	n.t.
MB 30 LPDE + SS 14 dd	n.t.	n.t.	n.t.	n.t.	10 a	33 b
MB 30 LMG + SS 14 dd	n.t.	n.t.	12 a	100 c	10 a	33 b
SS 14 dd + MB 30 LMG	n.t.	n.t.	25 b	0 a	n.t.	n.t.
Dazomet LPDE 100	4 a	5 a	0 a	17 a	n.t.	n.t.
Metham sodium LPDE 192	0 a	1 a	0 a	0 a	n.t.	n.t.
Metham sodium 81 LPDE+SS 14 dd	n.t.	n.t.	12 a	0 a	n.t.	n.t.
Dazomet 50 LPDE + SS 14 dd	n.t.	n.t.	0 a	67 b	n.t.	n.t.

*Means in the same column, followed by the same letter, do not differ significantly, following Duncan's test ($P = 0.05$).

Table 3. Effectiveness of different treatments against *R. solani* on bean (cvs Bobis and Anellino), expressed as per cent of emerged plants (E), per cent of infected plants (IP) and disease incidence (DI). Trial 1, first crop.

Treatment	cv. Bobis			cv. Anellino		
	% E	% IP	DI	% E	% IP	DI
--	83.7 b *	29.3 b	0.7 b	94.0 a	36.3 b	1.1 b
MB 60 LPDE	95.5 a	5.0 a	0.1 a	95.7 a	9.6 a	0.2 a
MB 40 LMG	95.8 a	4.3 a	0.0 a	95.0 a	5.6 a	0.1 a
MB 40 LPDE	93.7 a	0.7 a	0.0 a	95.7 a	1.9 a	0.0 a
Dazomet LPDE 100	93.7 a	3.6 a	0.1 a	95.8 a	4.5 a	0.1 a
Metham sodium LPDE 192	92.2 a	3.8 a	0.1 a	91.0 a	6.6 a	0.0 a

* see Table 2

DZ and MS, applied at full dosage under LPDE film, effectively controlled *R. solani* on bean in three subsequent crops (results of second crop are not shown), providing results similar to those offered by MB applied at full dosage under LPDE. Also the dosage of 40 g/m^2 of MB under LPDE or LMG was effective (Tables 3–4).

On lettuce, in trial 2, the best control of both *R solani* and S *sclerotiorum* was achieved by soil solarization, MB at 30 g/m^2 under VIF and by combining soil solarization for 14 days with 30 g/m^2 of MB under LMG (Table 5). MS alone did not adequately control R *solani*. Its efficacy was slightly improved when applied at half rate in combination with two weeks of solarization (Table 5). DZ applied alone was slightly more effective than MS, its efficacy was not improved when it was combined, at half rate, with two weeks of solarization (Table 5). The positive effect of combining 2 weeks of soil solarization with MB fumigation was confirmed on

cv Foglie di quercia of lettuce in trial 3 (Table 7). Such a combination proved highly effective also against *R. solani* on bean both in trials 2 and 3 (Tables 6 and 8). When solarization was combined with MB, the control of *R solani* on bean was similar when the fumigant was applied before or after soil mulching, except in the case of S. *sclerotiorum* on lettuce. From a practical point of view, when fumigation is carried out before the start of solarization, plastics are less damaged.

Table 4. Effectiveness of different treatments against *R. solani* on bean (cv Bobis), expressed as percent of emerged plants ((%E) and of infected plants (%IP) and disease incidence (DI). Trial 1, third crop.

Treatment	cv Bobis		
	% E	% IP	DI
--	54.3 b *	60.2 b	1.3 b
MB 60 LPDE	88.8 a	24.7 a	0.4 a
MB 40 LMG	88.2 a	28.7 a	0.5 a
MB 40 LPDE	92.0 a	33.1 a	0.5 a
Dazomet LPDE 100	84.0 a	38.8 a	0.7 a
Metham sodium LPDE 192	90.8 a	25.5 a	0.4 a

* see Table 2

Table 5. Effectiveness of different treatments against *R. solani* and *S. sclerotiorum* on lettuce. Trial 2.

Treatment	% of plants infected with		
	R. solani	*S. sclerotiorum*	TOTAL
--	4.3 ab *	1.3 ab	5.6 b
Soil solarization LPDE 28 dd	1.3 a	0.5 ab	1.8 a
MB 60 LPDE	3.4 ab	0.5 ab	3.9 ab
MB 30 LMG	1.6 a	0.3 ab	1.9 a
MB 30 LMG + SS 14 dd	1.1 a	1.8 b	2.9 ab
SS 14 dd + MB 30 LMG	1.6 a	0.0 a	1.6 a
Metham sodium 192 LPDE	6.4 b	0.5 ab	6.9 b
Metham sodium 81 LPDE + SS 14	2.3 ab	0.5 ab	2.8 ab
Dazomet 100 LPDE	3.2 ab	0.3 ab	3.5 ab
Dazomet 50 LPDE + SS 14 dd	3.9 ab	0.3 ab	4.2 ab

* see Table 2

Table 6. Effect of the different treatments against *R. solani* on bean (cvs Bobis and Canellino) expressed as percent of emerged plants ((%E) and of infected plants (%IP) and disease incidence (DI). Trial 2.

Treatment	cv. Bobis			cv. Canellino		
	% E	% IP	DI	% E	% IP	DI
--	87.3 b *	30.9 c	0.6 a	64.0 b	40.4 c	1.0 a
Soil solarization LPDE 28 dd	91.5 ab	18.8 b	0.4 a	73.8 b	25.8 bc	0.5 a
MB 60 LPDE	94.2 a	6.8 a	o.1 a	86.2 a	16.2 ab	0.3 a
MB 30 LMG	93.3 a	2.8 a	0.0 a	81.5 a	9.8 ab	0.2 a
MB 30 LMG + SS 14 dd	91.8 ab	3.8 a	0.1 a	87.7 a	12.6 ab	0.3 a
SS 14 dd + MB 30 LMG	91.8 ab	7.4 a	0.1 a	84.8 a	14.9 ab	0.3 a
Metham sodium 192 LPDE	93.3 a	4.8 a	0.1 a	89.7 a	4.6 a	0.1 a
Metham sodium 81 LPDE + SS 14	92.8 a	4.9 a	0.1 a	82.8 a	5.6 ab	0.1 a
Dazomet 100 LPDE	93.5 a	4.5 a	0.1 a	80.0 a	8.1 ab	0.1 a
Dazomet 50 LPDE + SS 14 dd	93.0 a	5.4 a	0.1 a	83.3 a	8.0 ab	0.1 a

* see Table 2

Table 7. Effect of different treatments against *S. sclerotiorum* on lettuce (cv. Foglie di quercia). Trial 3.

Treatment	% infected plants
--	14.8 b *
MB 60 LPDE	3.4 a
MB 40 LPDE	2.6 a
MB 40 LMG	9.5 ab
MB 30 LPDE + SS 14 dd	2.2 a
MB 30 LMG + SS 14 dd	7.8 ab

* see Table 2

Table 8. Effectiveness of different treatments against *R. solani* on bean (cvs Bobis and Canellino), expressed as per cent of emerged plants (E) and disease incidence (DI). Trial 3.

TREATMENT	cv. Bobis		cv. Canellino	
	% E	DI	% E	D I
--	89.8 a *	1.2 b	84.5 a	1.4 c
MB 60 LPDE	91.5 a	0.9 b	85.9 a	1.1 bc
MB 40 LPDE	87.7 a	0.8 b	84.9 a	0.9 bc
MB 40 LMG	88.6 a	1.0 b	78.8 a	0.8 b
MB 30 LPDE + SS 14 dd	93.4 a	0.1 a	87.2 a	0.2 a
MB 30 LMG + SS 14 dd	89.5 a	0.3 a	87.0 a	0.3 a

* see Table 2

DISCUSSION

The only partial activity of soil solarization when adopted in the open field was confirmed in trial 2, probably because soil temperatures did not reach sufficiently high levels (Garibaldi & Gullino, 1991).

MB confirmed its efficacy in the control of soil-borne pathogens at both full dosage (60 g/m^2) under LPDE or at reduced dosages (30 or 40 g/m^2) under gas impermeable film, thus allowing a reduction in the emissions of the fumigant into the atmosphere (Gamliel *et al.*, 1997; Gullino *et al.*, 1996). MS and DZ, at full dosages did provide a satisfactory but not always complete disease control. Covering the soil with plastic reduced the escape of unpleasant smells, particularly in the vicinity of houses. Moreover, in the case of DZ, such practice avoids further rolling of the soil, thus reducing labour costs and damage to soil structure. In the case of MS, plastic mulching avoids the need for further watering, normally carried out in order to reduce the escape of the fumigant from the soil cracks.

The combination of 2 weeks of soil solarization with fumigation with half dosage of MB or MS or DZ proved effective in most cases and allowed a reduction in the non-cultivation period. However, it cannot be expected that reducing the length of solarization from 4 to 2 weeks will provide the same positive results with all pathogens, since a shorter period of such treatment may reduce its effect.

Such a strategy could indeed increase the number of growers using solarization as a disinfestation method, helping to reduce the present dependance on chemicals. It must be stressed, however, that the usage of half dosage of fumigant in combination with a shorter period of solarization remains essential, at present, in order to achieve an acceptable level of disesase control.

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Immunodiagnosis as an aid to the timing of fungicide sprays for the control of *Mycosphaerella graminicola* on winter wheat in the UK

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ABSTRACT

Over 85% of the "grain-filling" capacity of the wheat plant is attributed to the top two leaves. Therefore, if full yield potential of the crop is to be realised these leaves must be protected from disease. *Stagonospora nodorum* and *Mycosphaerella. graminicola* are often present as a disease complex early in the growing season, but *M. graminicola* is more prevalent during the "grain-filling" stage. Immunodiagnostics allowing pre-symptomatic detection of Septoria diseases have been used as an aid for improving the timing of fungicide sprays. Field trials, over five years, have established that pre-symptomatic detection of *M. graminicola* on leaf three can be an accurate indicator of the optimum timing of a second fungicide spray for the protection of the top two leaves for the duration of "grain-filling" and this timing is related to cultivar susceptibility.

INTRODUCTION

Mycosphaerella graminicola (Septoria leaf blotch) and *Stagonospora nodorum* (leaf and glume blotch) comprise the Septoria disease complex on wheat and are of major economic importance in temperate regions world-wide. Disease surveys in the UK from 1976-1988 (Polley & Thomas, 1991) recognised that *S. nodorum* was the more widespread of the two pathogens. Because of changes in the cultivars grown, husbandry practices and fungicide usage, *M. graminicola* is now more prevalent causing severe epidemics in some seasons and substantial yield losses (King, 1977). Growers prefer quality wheats that are often susceptible to Septoria infection and, therefore, fungicides are the main disease control strategy. *S. nodorum* is often present in the late winter and early spring on the winter wheat crop in the UK but *M. graminicola* becomes the more prevalent pathogen later in the season. As symptom expression is preceded by latent periods of 7-14 days for *S. nodorum* and 21-35 days for *M. graminicola*, and symptoms are often non-specific and easily confused with other foliar diseases, visual diagnosis of initial infection is not possible (Royle *et al.* 1986). Generally, similar triazole fungicides are used for control of both Septorias and applied at timings linked to stages in crop development, regardless of disease pressure or cultivar susceptibility. Although, triazole fungicides vary in their disease control spectrum and curative ability, and the latent periods differ between the two Septoria diseases, chemical control is limited to early stages in the life cycle. Over 85% of the grain-filling capacity of the wheat plant is attributed to the top two leaves. Therefore, if yield potential is to be realised, accurate fungicide timing is required in order to protect these leaves from infection.

Immunoassay technology is frequently used for the diagnosis and quantification of many human infectious diseases. Recently, this technology has been applied to plant diseases (Miller *et al.*, 1988; MacDonald *et al.*, 1990) and specifically to Septoria diseases using the *M. graminicola*- and *S. nodorum*-specific polyclonal antibody-based immunoassays developed by DuPont (Joerger *et al.*, 1992) and Novartis (Smith *et al.*1994). Our paper describes recent work examining the use of immunodiagnostics for the pre-symptomatic detection of Septoria diseases on field-grown winter wheat as an aid to improving the timing of fungicide sprays and evaluating fungicide performance.

MATERIALS AND METHODS

A series of field experiments were carried out over 3 years (1993-1995), using the Septoria susceptible cultivar Riband, to develop a threshold for a single flusilazole (Sanction) spray using the DuPont Advisor kit for Septoria diseases of wheat (Joerger *et al.*, 1992). The trial site was divided into three areas and 30 whole plants were collected randomly from each area at weekly intervals. Plants were separated into leaf layers and disease incidence measured visually and by immunoassay, for both *M. graminicola* and *S. nodorum* on the top three leaves. Septoria antigens for both diseases were extracted from the leaves (30 x 3) from each leaf layer by macerating them in the buffer provided (5ml/leaf). The extracts were applied to the immunoassay plates along with the standard antigen concentrations included with the kit. The amount of antigen present in each sample was estimated from the calibration curves provided by the standards and disease measured as antigen units/ml (au/ml). The development of both Septoria diseases on the top three leaves from untreated plots was monitored throughout the 1993 and 1994 seasons (Figure 1). In 1993, one full-rate flusilazole spray was applied to replicate plots when the immunoassay readings moved above the base-line (<5 au/ml) to either 10 *S. nodorum* or *M. graminicola* antigen units/ml in leaf 2 (GS59). As results from this trial indicated that *M. graminicola* was the more prevalent disease during the "grain-filling" stage and the spray timing was not optimum for this disease (Figure 2), the threshold was altered for the following season (1994) to 10 *M. graminicola* au/ml in leaf 3 (Figure 3). In 1995, the threshold was altered again to *5 M. graminicola* au/ml in leaf 3. After spraying, plants were sampled (10 plants/plot) every two weeks on treated and untreated plots until harvest, and disease progress assessed.

In 1996, the field experiment was changed in order to further evaluate the immunodiagnostic threshold established for *M. graminicola* in 1995, of 5 au/ml in leaf 3. Treatments included an early spray 19 days before the diagnostic threshold, determined from experience of antigen build-up from previous seasons and the local weather data, 9- and 14-day post-diagnostic spray timing. In 1996, the diagnostic threshold was reached at GS 41. Sampling before and after treatment and assessment of disease progress on the top three leaves were carried out as in previous seasons (Figure 4).

Another field experiment in 1997 investigated whether the thresholds developed for cv. Riband could be applied to other wheat cultivars. The experiment included cvs. Riband and Hereward, a cultivar tolerant to *M. graminicola*. Spray timings were 10 days pre-diagnostic, the diagnostic timing and 14 days post-diagnostic. The diagnostic threshold for cv. Riband was reached at GS 39 but, due to bad weather, both the pre-diagnostic and diagnostic timings were sprayed at GS 45 (Figure 5a). The "pre-diagnostic" spray on cv. Hereward was applied at GS 45 and the

"diagnostic" at GS 59 (Figure 5b). Sampling methods and disease assessments were carried out as in previous years.

RESULTS AND DISCUSSION

Measurement of both Septoria diseases by immunoassay on the top three leaves in 1993 (Figure 1) indicated that although *S. nodorum* was evident early in the season, the epidemic did not develop and disease incidence on the top two leaves remained low (5-10 au/ml). However, the *M. graminicola* epidemic increased in severity from GS 59 throughout the period of "grain-filling" and by GS 65 it was the more prevalent disease on the upper leaves.

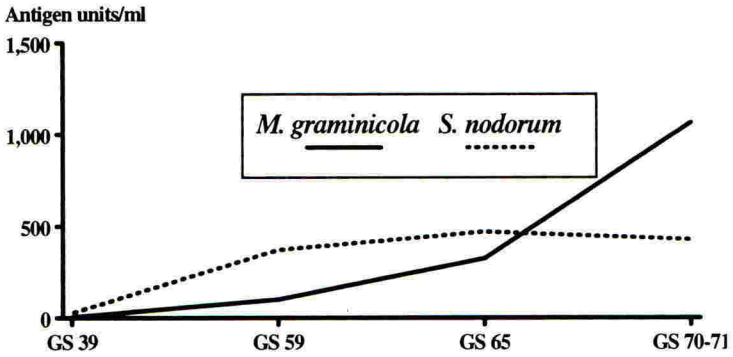
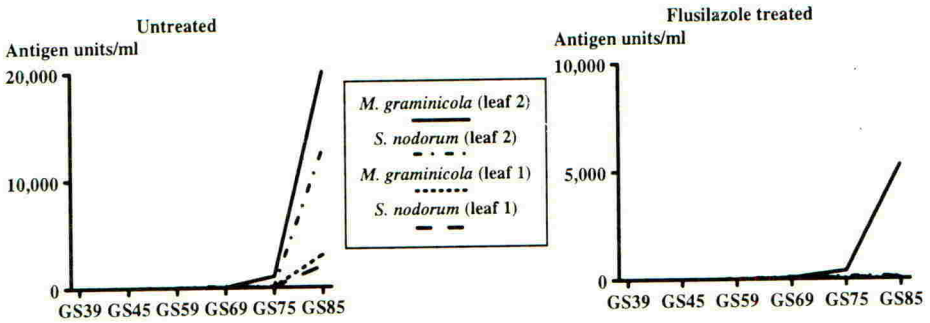


Figure 1. Incidence of *M.graminicola* and *S. nodorum* on the top three leaves of wheat (cv Riband).

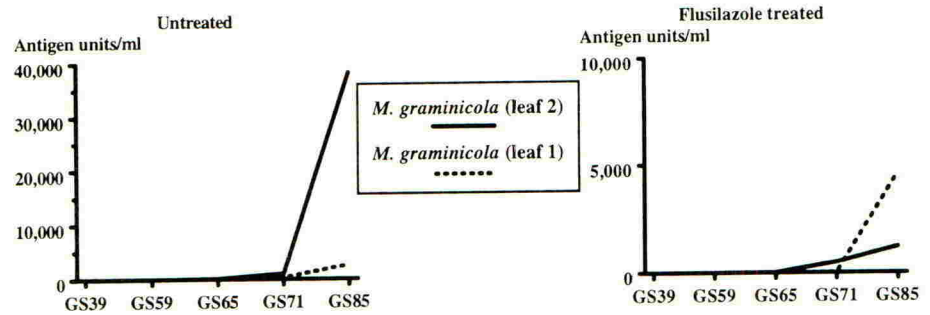
In 1993, the pre-determined immunodiagnostic threshold (10 au/ml) was met by the detection of *M. graminicola* antigen in leaf 2 at GS 59. Assessment of disease at the end of "grain-filling" (GS85) showed that application of flusilazole at this threshold controlled both *M. graminicola* and *S. nodorum* on the flag leaf (98% and 95%, respectively). However, only *S. nodorum* was well controlled on leaf 2, whereas significant amounts of *M. graminicola*, infection occurred in this leaf (Figure 2).

Previous glasshouse studies examining the performance of the immunodiagnostic for the presymptomatic detection of both *M. graminicola* and *S. nodorum* showed that the growth response as measured by immunoassay was not linear. Measurement of antigen remained at the base-line until the initiation of pycnidiospores. At this stage in disease development, there was an abrupt rise in antigen levels reaching the maximum at symptom expression (unpublished data). The immunoassay detects disease in later stages of infection when fungicide intervention is often less efficient and, infection of the upper leaves often occurs step-wise from lower in the crop canopy. Therefore, in order to protect the two top leaves from infection, the leaf used for the disease threshold should be a leaf layer lower (leaf 3). Consequently, for the more aggressive *M. graminicola* pathogen it was concluded that the diagnostic threshold was too late and should be moved to leaf 3.



1993 threshold - 10 *M. graminicola* antigen units/ml in leaf 2 = GS59

Figure 2. Efficacy of flusilazole applied at a threshold of 10 *M. graminicola* antigen units ml^{-1} in leaf 2 (cv Riband).



1994 threshold - 10 *M. graminicola* antigen units/ml in leaf 3 = GS39

Figure 3. Efficacy of flusilazole applied at a threshold of 10 *M. graminicola* antigen units ml^{-1} in leaf 3 (cv Riband).

Using the results from the 1993 field experiment, the immunodiagnostic threshold was modified for the field experiment in 1994 to 10 *M graminicola* au/ml in leaf 3. The disease pressure was higher during this season and this threshold was met at GS 39. Assessment of disease by immunoassay during the "grain-filling" stage showed that *S. nodorum* was controlled on both the flag leaf and leaf 2 but *M.graminicola* was only controlled on leaf 2. There was no control of the disease on the flag leaf although these leaves remained green until late in the "grain-filling" period (Figure 3). Using leaf 3 as the "indicator" leaf for the immunoassay gave better overall control than that obtained using the leaf 2 threshold; *M. graminicola* in the flag leaf resulted in leaf necrosis before the end of "grain-filling" (GS 85).

Because of the lack of control of *M. graminicola* in the flag leaf in 1994, the threshold in 1995 was again modified to 5 *M. graminicola* au/ml in leaf 3. This criterion was met at GS 41 but, because of adverse weather conditions, sprays were delayed by five days. During the "grain-filling" stage there was good control of both Septorias on flag leaves but, because sprays were late and because of limitations in the curative activity of flusilazole, there was no control of *M. graminicola* on leaf 2. However, the results obtained indicated that, despite the delayed spray timing, the threshold, 5 *M. graminicola* au/ml in leaf 3, gave better control of both Septorias than previous thresholds.

Further evaluation of this immunodiagnostic threshold in 1996 showed that at GS 69 there was no control of disease on leaf 3 with any spray timing because fungicides were applied when *M. graminicola* pycnidia were forming and, therefore, were less effective. Sprays applied at immunodiagnostic threshold (GS 41) gave better disease control on both the flag leaf and leaf 2 during "grain-filling" than any of the other spray timings. If sprays were delayed later than the immunodiagnostic threshold, disease control was lost on the upper leaves (Figure 4).

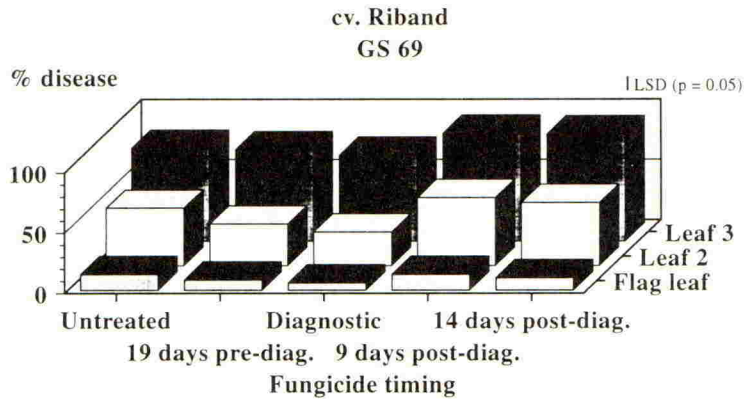


Figure 4. Efficacy of flusilazole at different immunodiagnostic timings for the control of *M. graminicola*.

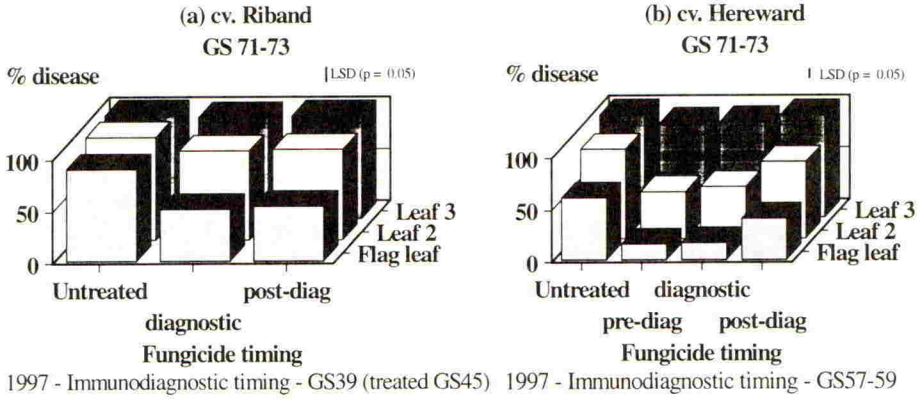


Figure 5 Efficacy of flusilazole at different immunodiagnostic timings for the control of *M. graminicola* in winter wheat cvs Riband (a) and Hereward (b).

Adverse weather conditions delayed fungicide application on cv. Riband in 1997 (from GS 39 to GS 45) and, consequently, there was no disease control on both leaf 2 and leaf 3 when assessed at GS 71-73. Control on the flag leaf was similar to that of the post-diagnostic timing and, although there was significantly less *M. graminicola* than on the untreated, control was not at acceptable levels (Figure 5a). For cv. Hereward, as expected, there was no control of disease on leaf 3 at GS 71-73 from sprays applied at any spray timing. Treatment at the diagnostic timing (GS 57-59) and 10 days earlier gave significant control of *M. graminicola* on both the flag leaf and leaf 2. However, the 14-day post-diagnostic treatment achieved no disease control on leaf 2, whereas disease control was better than untreated following the 14-day post-

diagnostic spray but significantly worse than that from an earlier timing on the flag leaf (figure 5b).

Optimum fungicide timing is related to cultivar susceptibility. In the seasons 1995-1997, the threshold criterion for optimum fungicide timing for the susceptible cultivar Riband, was between GS 39 and GS 41. The same criterion was met by the tolerant variety, Hereward, at GS 57-59 in 1997. Timing is also critical, regardless of cultivar susceptibility, if spray timings are delayed, control of disease on the upper leaves is lost. In 1995 and 1997, spray timings for Riband were delayed because of adverse weather conditions. The pre-diagnostic gave significantly better disease control than post-diagnostic applications. Therefore, in order to counteract problems associated with bad weather, the threshold may be reduced further allowing a wider spray 'window'. Timings will differ depending on cultivar susceptibility, inoculum levels and local weather conditions. In some seasons, where less susceptible cultivars are being grown, the immunodiagnostic information should aid decisions on whether reduced fungicide rates are an option or, indeed, if fungicide application is necessary at all.

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