7.
Posters

POISONING OF WOODPIGEONS ON WOODWALTON FEN

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ABSTRACT

Since 1982, incidents involving woodpigeons (Columba palumbus) have been recorded relatively frequently in Woodwalton Fen National Nature Reserve following the local drilling of winter wheat under wet soil conditions. Incidents are characterised by birds being uncoordinated and unable to fly properly. Chemical analysis implicates the seed-dressings chlorfenvinphos and, to a lesser extent, fonofos. Previously, incidents involving woodpigeons and these seed-dressings had been reported only very rarely under the Wildlife Incident Investigation Scheme.

INTRODUCTION

Bunyan et al. (1971) found the feral pigeon (Columba livia) to be particularly sensitive to chlorfenvinphos poisoning and implied that under certain circumstances grain dressed with chlorfenvinphos might poison this species. This proved correct and a small number of incidents was recorded during each of the following 10 years in the Wildlife Incident Investigation Scheme operated by MAFF (eg see Stanley & Bunyan, 1979; Fletcher & Hardy, 1983). During this time, however, incidents involving woodpigeons (Columba palumbus) were very rare, seemingly only one being described in the literature (Stanley & Bunyan, 1979).

During the period 1976-1982, regular visits to Woodwalton Fen National Nature Reserve in Cambrideshire had revealed a number of incidents involving erratic behaviour by woodpigeons. Typically these occurred at times of winter drilling on the surrounding farmland. Pigeons with no sign of external injury were caught floundering on the ground; if killed, they were found to have grain in the crop. In view of the known toxicity of chlorfenvinphos to feral pigeons and its widespread use on winter-sown cereals, it was considered that this seed-dressing chemical might be to blame. Following discussions with MAFF staff a project was started in 1982 to determine the frequency and cause of such incidents.

METHODS

About 30 visits were made to the Reserve each winter from October to March; each visit was made at dusk and lasted at least one hour. The Reserve was searched reasonably uniformly irrespective of the location of the main woodpigeon roosts. Instances of unusual behaviour were noted, and attempts made to catch affected birds. An "incident" was defined as a visit during which unusual behaviour was displayed by one or more birds. The location of any pigeon remains was also recorded. On rather more than half of the visits, birds were counted in the main roosts in the south of the Reserve. These transect counts underestimate the total numbers present (as it is never possible to count all roosting areas during a single visit) but provide a guide to seasonal and annual changes.

RESULTS AND OBSERVATIONS

Results for each winter (Table 1) reveal that 82% of the incidents and 93% of birds showing unusual behaviour were recorded in three winters: 1982/3, 1983/4 and late 1987. This trend cannot be explained by more birds being present (Table 1). However each of these winters was characterised by wet conditions which will have made drilling of winter grain more difficult and increased the risk of poisoning from cereal seed-dressings: eg see comments by Fletcher & Hardy (1984) relating to late 1983. During the winters of 1982/3 and 1987/8 conditions were sufficiently wet for the Reserve, which can act as a water storage area, to receive flood-water from the local river system. Incidents occurred from November to January (Table 2) coinciding with the drilling of winter grain. In the three worst winters, incidents occurred during 18 (44%) of the 41 visits made from November to January, with 50 birds being affected.

Affected birds were uncoordinated, being unable or reluctant to fly or, in less severe cases, being able to fly just sufficiently to evade capture. The most affected birds were unable to right themselves if on their backs. No bird appeared injured in any way. Of the 54 birds affected, 21 (39%) were caught, while 26 (48%) stayed in trees or flew away. The remaining 7 (13%) could not be located in dense ground vegetation or were otherwise difficult to reach, landing in or beyond dykes and rivers. The only other birds seen behaving unusually were individual stock doves (Columba oenas) in January 1983 and January 1985.

Two birds from each of two incidents in the first winter (1982/3) were analysed by MAFF under the Wildlife Incident Investigation Scheme: all four birds were confirmed as chlorfenvinphos casualties with 50-170 mg/kg in their crop or gizzard contents. The brain esterase level of three of these birds was measured and ranged from 12 to 17% of the control value. During the same winter, two racing pigeons belonging to the Reserve warden became ill, vomiting grain. One bird, which like the severely affected woodpigeons had been unable to perch, died and was analysed by MAFF: the chlorfenvinphos concentration in the gizzard contents was 112 mg/kg and the brain esterase level was 35% of the control value.

Because one of the warden's racing pigeons recovered, an attempt was made during the second winter (1983/4) to determine whether sick woodpigeons might recover if maintained under good conditions. Eight birds from five separate incidents during November and December 1983 were kept in cages in an unheated building and provided with food and water. Each was fit for release in 1-3 days (six were released after one day).

During the three winters 1984/5-1986/7, only four incidents were recorded, each involving single birds. The only bird sent to MAFF during this period was a freshly-dead woodpigeon collected in November 1986. This was not counted as an incident in this study as no unusual behaviour was seen that day. However the bird was submitted as it had grain in the crop and analysis showed it to be a fonofos casualty.

The autumn of 1987 was very wet and four incidents, involving 10 birds, were recorded in December. Five birds from three incidents were collected for MAFF investigations: two birds contained chlorfenvinphos alone, one contained fonofos and one contained both residues.

TABLE 1

Information on incidents and results of transect counts presented for each winter

Winter	No of visits	No of incidents (% of visits)	No of birds behaving unusually	Transect counts No	Mean ± SE
1982/3	27	6(22)	22	26	930 ± 110
1983/4	30	8(27)	18	16	1140 ± 180
1984/5	30	1(3)	1	16	860 ± 180
1985/6	33	3(9)	3	16	1480 ± 280
1986/7	34	0(0)	0	15	870 ± 170
Oct-Dec 1987	18	4(22)	10	11	1170 ± 270
	172	22(13)	54		

TABLE 2

Information on incidents and results of transect counts presented by month, October 1982 - December 1987

Winter	No of visits	No of incidents (% of visits)	No of birds behaving unusually	Transect counts No	Mean ± SE
October	26	0(0)	0	15	290 ± 70
November	30	2(7)	8	17	1340 ± 230
December	39	12(31)	29	23	1380 ± 130
January	25	8(32)	17	15	1190 ± 220
February	29	0(0)	0	18	1080 ± 160
March	23	0(0)	0	12	780 ± 170
Total	172	22(13)	54		

DISCUSSION

Incident investigations have implicated seed-dressing chemicals: usually chlorfenvinphos but sometimes fonofos. As fonofos did not receive provisional clearance until 1985 for use when drilling winter cereals, it is unlikely to have been involved in incidents during the early part of the study.

This study has revealed that at this site, such seed-dressing incidents occur much more frequently than had previously been indicated by the Wildlife Incident Investigation Scheme. Only one chlorfenvinphos/woodpigeon incident was recorded prior to 1982 (Stanley & Bunyan, 1979). The only previously documented fonofos/woodpigeon incident was during a wildlife trial with the chemical (Fletcher & Hardy, 1983) and also occurred on the Fens.

Similar incidents have been recorded in the neighbouring National Nature Reserve at Holme Fen (R Harold, pers comm; M Massey, pers comm). It is possible that such incidents occur elsewhere but are overlooked or are dismissed as shooting-injury. While shooting-injury may have been responsible for some of the unusual behaviour recorded at Woodwalton, it cannot have been a significant factor as none of the birds handled appeared injured and affected birds soon recovered. Moreover, shooting is not consistent with the seasonal and annual changes that were noted (for instance, February is the main month for shooting).

It is possible that in areas of the Fens where no oilseed rape is grown, woodpigeons have to concentrate more on newly-sown cereal fields in mid-winter and so place themselves at greater risk from poisoning. However, the Reserve is on the Fenland edge with the nearest rape fields only 1-2 km away.

Without detailed focused studies of this type, the Wildlife Incident Investigation Scheme may underestimate the relative importance of certain types of incident that may be ignored or overlooked in the field. In this respect, concern might be afforded to passerines taking dressed grain, as they may be more sensitive even than pigeons to chlorfenvinphos poisoning (Stanley & Bunyan, 1979). Death may occur in sensitive species in only 30 minutes (Bunyan et al 1971), and to be confident about understanding the extent of effects on small easily-overlooked species one needs detailed regular surveillance of drilled fields and/or roosting areas combined with post-mortem investigations.

Although it has been shown that affected birds can recover, pigeon remains in the Reserve (95 were counted during the study) indicate that some are predated or scavenged by foxes. It is hard to believe that such losses have any appreciable effect on population levels. Although the transect count fell from more than 2000 to 200-400 immediately after the incidents in 1987, this change was likely to have been due to a temporary change in roost location as numbers recovered within one month.

ACKNOWLEDGEMENTS

I am grateful to Lynne Farrell and Ron Harold for help in the field and to MAFF staff for undertaking the post-mortem investigations.

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APPROACHES TO HAZARD ASSESSMENT FOR SMALL MAMMALS IN CEREAL FIELDS

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ABSTRACT

Small mammals such as wood mice and voles are too small and secretive to observe directly, or to rely on finding carcases as means of detecting pesticide impacts. Instead, they may be trapped, marked and released in order to provide information on population changes following a pesticide application. This paper discusses the advantages of combining ecological interpretations (eg. recapture histories of marked animals) with a limited programme of sampling from the population for histological, biochemical and residue analyses. Examples are taken from a series of field trials at a single location, involving several different pesticides. This provides insight into the problems of obtaining appropriate control data in small mammal studies.

INTRODUCTION

Assessing pesticide effects on wild mammal populations involves a variety of practical challenges. Species of small rodents cause particular difficulties, being too small and secretive to permit observational studies of behaviour and distribution. Dead animals are unlikely to be found during systematic carcase searches. Consequently, most investigations involve programmes of trapping, to mark, release and recapture animals on a trial site (eg. Edwards 1977).

Data obtained from these exercises allow assessment of various population parameters, and hence can identify any changes that coincide with an experimental pesticide application. The purpose of this paper is to explore the potential and limitations of these ecological interpretations, using data on wood mice *Apodemus sylvaticus* from several recent field trials. In addition, we indicate the value of planned sampling of animals from the population, for laboratory studies of physiological and biochemical effects (Westlake *et al.* 1980, 1982) and residue analysis. Setting the compromise between minimal disturbance or populations, and the need for laboratory material, is a vital part of the design of field trials with small rodents.

FIELD METHODS

The most frequently-used mode of trapping involves groups of baited Longworth or other live traps (Twigg 1975a) dispersed through the trial site, checking at least daily for the presence of captured rodents. Design of the trapping programme involves many issues, which depend on the nature of the habitat and the local mammal populations. Decisions on the arrangement of trapping points (eg. in lines along hedgerows, or in regular grids over fields); their density and total numbers; and the number of traps placed at each point, depend on the importance given to achieving a maximum capture-rate. Provision of a suitable bait also has an influence

on trapping success.

In this paper we refer to two studies conducted in autumn on fields of winter cereals treated with molluscicide pellets (Study I) or with an insecticidal seed dressing (Study II). Both investigations were based on regular trapping in grids of trap-points covering much of the field surface (Table 1). Animals caught in traps were identified to species, age-class and sex, weighed and uniquely marked by fur- or toe-clipping (see Twigg 1975b).

Table 1

Summary of trapping effort, and small mammals captured in two field trials

	Study 1	Study II
Crop	Winter Wheat	Winter Wheat
Pesticide treatment	pelleted molluscicide	insecticidal seed treatment
Dates	Oct-Dec	Sept-Dec
Trapping grids	2 field grids 20 m x 20 m 2 traps per point 290-314 traps per night	1 field grid, 3 grids in adjacent woods, 20m x 20m 1 trap per point 155-224 traps per night
Total trap-nights	6864	5502
Small mammal captures (no of animals/no of captures)		
Wood mouse *Apodemus sylvaticus*	129/404	112/198
Yellow-necked mouse *Apodemus flavicollis*	6/15	?/100
Bank vole *Clethrionomys glareolus*	0/0	?/245
Short-tailed vole *Microtus agrestis*	0/0	0/0
Harvest mouse *Micromys minutus*	?/2	0/0
House mouse *Mus musculus*	?/4	0/0
Common shrew *Sorex araneus*	0/0	?/35
Pygmy shrew *Sorex minutus*	0/0	?/12
Number collected for laboratory studies :	50	74

ECOLOGICAL ANALYSIS

In any capture-recapture study, it is tempting to apply models which provide an estimate of population size, based on the relative numbers of marked and unmarked animals in each day's catch (see Southwood 1978).

Thoughseveral such models have been used in small mammal studies (eg. Green 1979, Montgomery 1980), the underlying assumptions often cannot be met, placing doubt on the resulting population estimates. Most seriously, small rodent populations are rarely closed, so that immigration and emigration inflate the size of the population calculated to be 'resident' (Flowerdew 1978), and may mask changes in numbers due to local pesticide effects. Also, animals may be segregated by limited overlap in their home ranges, and the trapping technique itself carries bias because animals of some species are liable to become trap-shy or even 'trap-happy' after first capture (Gurnell 1982). Numbers of animals caught within a trapping area can provide estimates of population density (eg. Gurnell 1978).

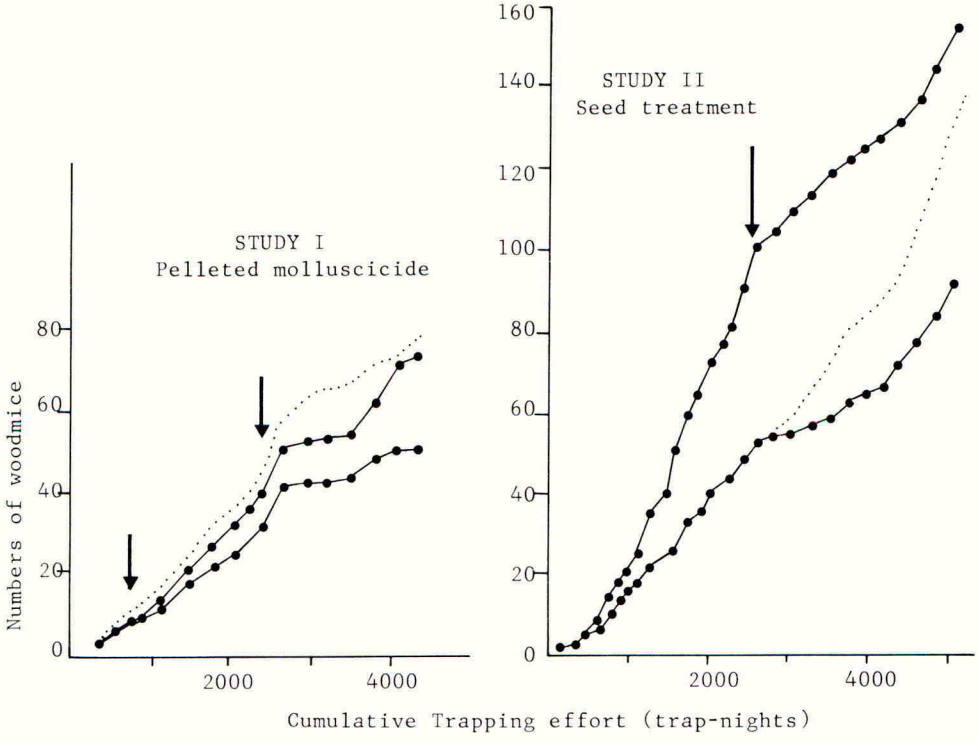

FIG.1. Cumulative captures of wood mice in two field trials. Upper solid lines indicate all captures (N), lower solid lines indicate numbers of individuals (I), and broken lines are an adjustment to I, to account for removal of animals for laboratory study. Arrows show dates of pesticide applications.

There are useful alternatives to population estimates. Figure 1 illustrates the cumulative numbers of wood mice captured in our studies, separating the previously-unmarked animals from the total catch. This presentation shows three relevant points. First, the shape of the curves provides information on the local population - in a closed population of resident mice, the curve of individuals (ΣI) would eventually reach a plateau corresponding to the local population size. That is clearly not the case in either study. Second, the difference between the two curves ΣI and ΣN depends on trapping efficiency, population size and the amount of immigration/emigration at the site, which are hard to distinguish. Third, the effect of events such as pesticide applications may be seen in changes of the slope of the curves. In Study I, there is a reduction in capture-rate following the second of two molluscicide applications. However, the fact that this is evident in both ΣI and ΣN argues that it may have been a change in trapping success, rather than removal of some resident animals by poisoning.

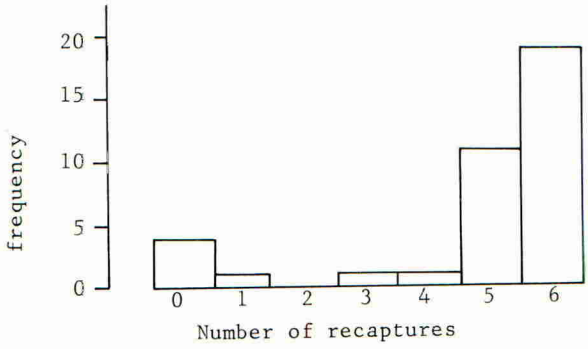

FIG.2. Frequency of recapture over six days of wood mice marked on the first or second days' trapping in Study I in December.

The capture histories of individual mice can help to separate residents from itinerants; in Study I, most of the mice marked early in the trapping period were recaptured frequently during the following week (Fig.2), indicating that they used the field on a daily basis.

Distribution of captures within a trapping grid is useful in two respects. First, the overall numbers of captures at each point may indicate patterns of use, and hence of potential exposure to pesticides, at different distances from the field edge, for example (Fig.3). Plots of the locations of successive captures of individual mice illustrate restrictions of their ranging behaviour, and overlap in ranges is reflected in the number of different animals trapped at each point.

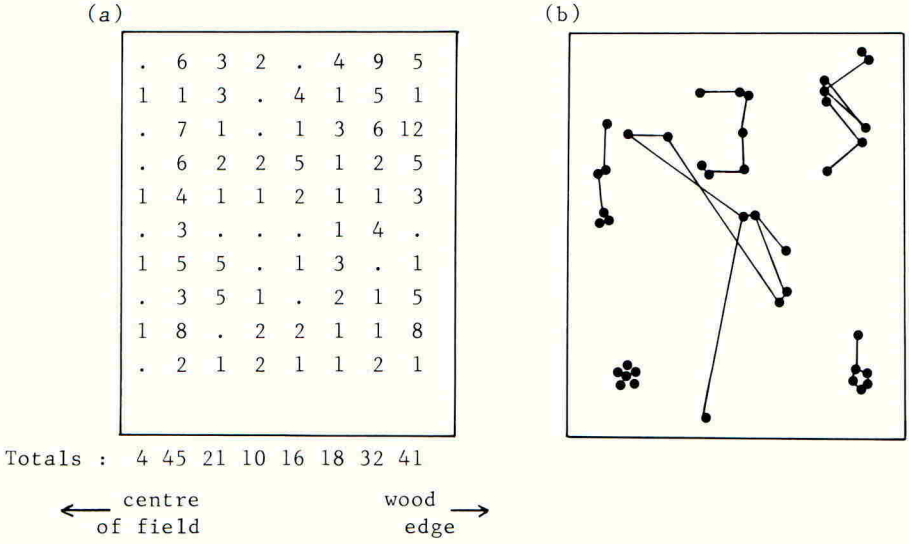

FIG.3. Locations of captures of wood mice on one trap-grid in Study I; (a) numbers of captures over 8 days trapping in December, (b) examples of the movements of individual marked mice.

Body weights are easily recorded while trapping, and may reveal changes in body condition, or in the composition of the population (eg. adult/juvenile ratios). The variability of body weight is generally so great that only drastic changes due to pesticide effects are likely to be detectable, however.

SAMPLING FOR LABORATORY REQUIREMENTS

In addition to searching for changes in the number and distribution of rodents after an application, there is great value in taking animals from the field, for laboratory studies. This has formed the principal reason for trapping in some studies (Bunyan *et al.* 1981). Animals may be selected randomly, or chosen according to a predetermined programme based on age, sex or other criteria. Examination of these animals alongside any that are found dead provides a means of confirming exposure to the pesticide that cannot be matched by field observations and trapping data alone. Studies typically include post-mortem examinations to detect gross pathological symptoms and assess histological changes (Tarrant 1988), and analysis for residues of the pesticide or its metabolites (Bunyan *et al.* 1981). In addition, some compounds have effects that can be seen in biochemical tests, such as inhibition of acetylcholinesterase (AChE) by organophosphorus and carbamate pesticides (Westlake *et al.* 1980, 1982). Table 2 summarises the results of laboratory studies undertaken in the

two field trials referred to above. In Study I, a substantial number of wood mice betrayed evidence of having fed on molluscicide pellets, but few showed major toxic effects. In contrast, mice captured on the treated field in Study II had heavily reduced brain and plasma enzyme activity, corresponding to the presence of pesticide residues in their guts (Westlake *et al.* 1982). However, mice trapped within fringing woodland were little affected, suggesting a marked local restriction of risk that could have been revealed only by a very large-scale trapping programme.

Table 2

Results of laboratory investigations of wood mice in Study I (unpublished) and Study II (Westlake *et al* 1982)

	Study I	Study II
Number of woodmice collected from the field* :	51	81
% showing pathological signs of poisoning on post-mortem	14.3% (n = 49)	not assessed
% showing depression of brain acetylcholinesterase activity	2.1% (n = 48)	40.7% (n = 81)
% with pesticide residues in stomach & intestines	10.2% (n = 49)	61.2% (n = 80)

***Note** : includes all mice collected after pesticide application, and those found dead in traps or during carcase searches

Removing animals from the field carries obvious implications for the interpretation of trapping results. It may not be clear whether those removed are likely to be replaced by immigrants, or whether their absence affects the behaviour of those remaining. Similar questions apply to the consequences of mortality due to poisoning, and it is important to find a way of adjusting for the removal of animals taken for laboratory studies.

One approach is to set limits to the bias by assuming, at one extreme, that all mice removed were immediately replaced by immigrants, which experienced a similar pattern of residence. In that case, the observed curves should correspond to those seen if there had been no removals. At the other extreme, if none of the mice were replaced, numbers equivalent to the total removed must be added to ΣI and ΣN; this is indicated by the broken lines in Fig.1. These bounds can be narrowed by further adjustments (eg. to take account of delays in replacement, measured immigration rates, and changes in the home ranges of survivors), but it is clear that a heavy sampling programme leads to uncertainty in interpreting population changes. Small changes in the slope of the cumulative capture curves become hard to assess.

SETTING A COMPROMISE

Deciding on the scale of sampling for laboratory work depends on several priorities. A minimum number for appropriate statistical treatment of residue and esterase measurements must be achieved, but biological interpretation can be strengthened by obtaining as wide a range of data as possible from each animal. However, larger samples may need to be taken if interest is centred on the time-course of a recognised hazard, for example,

or if the priority is to determine accurately what proportion of the population has been exposed to a pesticide. Subject to these constraints, the aim should be to minimise disruption to the field population; in practice, this can be aided by regular removal of a small number of animals rather than sudden removal of many, and by spreading the losses across sex, age and body-weight categories.

COMPARISONS WITH CONTROL DATA

It is implicit in hazard assessment studies that information from a population exposed to a pesticide is compared to 'normal' levels - whether these are AChE activity, population density, individual ranging behaviour, etc. The major question is whether it is better to use matched 'control' sites or to regard each trial as its own control, by making pre- and post-application contrasts. There is no general answer, since differences between sites relative to within-site variability may be large or small, depending on species and habitat. Ideally, both approaches should be followed, so that the merits of the two forms of control data can be evaluated. We have done this in a third recent study, in which differences between two treated fields and an untreated control field were no greater than the before/after comparisons on each field, even though there were no appreciable pesticide effects (Table 3).

Table 3

Comparison of pre- and post-application control data, and data from an independent untreated control field, from a study involving two molluscicide pellet treatments (fields A and B). Results are for all species combined.

	Field A	Untreated Control	Field B	Untreated Control
Before application				
Daily capture rate :	2.75	5.60	1.50	3.00
No collected/no caught :	3/11	9/28	1/3	3/9
After application				
Daily capture rate :	4.50	7.25	3.57	8.00
No collected/no caught	14/31	5/29	11/25	11/48

The two trials in Table 1 present an unusual opportunity to compare effects of two pesticide applications on a single population, since they were conducted at the same site, albeit eight years apart. There were obvious differences in species composition and population density between the sites (Fig.1, Table 1), which were evident before as well as after pesticide appliations, suggesting that they relate to long-term population fluctuations rather than to pesticide effects. The scale of changes in capture rate, however, were similar in the two studies, despite much greater effects (Table 2) and heavier sampling of mice in Study II. This indicates the resilience of wood mouse populations to even major mortality, attributable to movements from neighbouring habitats.

Study II also permits a between-species control comparison, since substantial numbers of wood mice, bank voles and field voles were trapped, but only wood mice were removed for laboratory studies. Table 4 shows that the capture rates for all three species were lower after application, but the reduction was statistically significant only for wood mice, suggesting an effect of removing animals to the laboratory, although there

may also be species-differences in behavioural responses to pesticides.

Table 4

Rates of capture of three species of small rodents in Study II, on 10 trapping days before, and 10 days after pesticide application.

	Wood mouse *Apodemus sylvaticus*	Yellow-necked mouse *Apodemus flavicollis*	Bank vole *Clethrionomys glareolus*
Proportion of animals collected for laboratory studies after application	79/89	none	none
Average daily capture rate			
- before application	8.00	3.10	8.70
- after application	4.80	2.30	7.50
Significance of difference (Mann-Whitney U test)	$P<0.01$	NS	NS

Usually it is most convenient to rely on within-site comparisons, which are unlikely to lead to serious misinterpretation if effects are being assessed qualitatively (ie. hazard or no-hazard). However, detailed quantitative analysis of reduced esterase activity, for example, may be very sensitive to minor differences in the control values used.

CONCLUSION

We have attempted to show that useful information can be obtained by live-trapping small rodents before and after pesticide applications, even if it is not possible to derive reliable estimates of population size. The most appropriate approach is to form a compromise between the ease of interpretation of capture-recapture data, and the removal of adequate samples of animals for laboratory studies.

ACKNOWLEDGEMENTS

We are grateful to colleagues who contributed to the studies mentioned, and to Dr. J.R. Flowerdew for comments on the manuscript.

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HISTOLOGICAL IDENTIFICATION OF THE EFFECTS OF PESTICIDES ON NON-TARGET SPECIES

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ABSTRACT

Histological examination of tissue taken from live-trapped animals in the field provides a valuable means of identifying sub-acute toxicological effects of pesticides, which would otherwise not be detected as field mortality or from analysis of tissue for chemical residues. Quantifiable measurements of cell morphology as indicators of toxicity are discussed. The full interpretation of histological results from field trial data may be augmented by comprehensive laboratory studies including histological and biochemical measurements. This provides further data on dose-related systemic effects and chemical residue levels in specific organs, which may be correlated with observed effects in the field.

INTRODUCTION

Pesticides play an important role in agriculture by limiting damage to crops caused by pests, weeds and diseases. Although some pesticides have only a specific use on a given crop (eg. nematicides) many are generally used on a large scale (cereal seed treatments) and even low applications of, non-persistent pesticides may have a transient effect upon non-target species. Pesticides with short environmental half lives may lead to short term exposure of sedentary animal populations such as wood mice (Apodemus sylvaticus) and nesting birds. Sub-lethal effects would not be detected by searching for mortality. Instead, exposure may be demonstrated by quantifiable histological changes, which may be reversible as exposure to the active agent decreases. This paper reviews current methods of detecting the histological effects of the field use of pesticides.

POPULATIONS AT RISK FROM THE FIELD USE OF PESTICIDES

Wild free-ranging animal populations may be exposed to pesticides through a number of routes, and histological data are generally most easily interpreted as part of a multi-disciplinary approach.

Laboratory studies may have to be carried out to obtain initial background data upon the compound under review, which will greatly help to interpret the field results. Initially those animal populations at risk must be identified, according to the proposed use of the pesticide. Seed dressing may present a potential risk to seed eating birds and small mammals (Westlake et al. 1980, Greig-Smith 1988). Insecticide sprays present a potential hazard to insectivorous birds such as Tree Sparrows (Passer montanus and Grey Partridge chicks (Perdix perdix). Coloured granules coated with a nematicide may be attractive as a gritting agent to several bird species and may also prove hazardous to soil invertebrates, causing a risk of secondary poisoning if prey species are affected. Poison on a cereal base as used in molluscicide pellets and rodent baits, may provide

an attractive food source to non-target species (Tarrant & Westlake 1988). Poisoned rodents may also provide a route for secondary poisoning in birds of prey and mammalian carnivores.

If non-target mortality occurs, an adequate post-mortem examination should be carried out to discover any non-chemical cause of death such as disease and physical trauma (eg. shooting or collisions with farm machinery or buildings). Useful information can also be obtained by examining stomach contents. This will show whether coloured baits or seed dressings have been ingested and identify crops upon which the animal has been feeding.

SELECTION OF HISTOLOGICAL FEATURES TO QUANTIFY

Pesticides with a specific mode of action will allow a logical choice of which target organ to select for histological evaluation. For example, the rodenticide calciferol acts by mobilising body calcium to produce calcium deposits in kidney and heart tissue, which can be quantifiably stained with the calcium-specific alzarin dye (Tarrant & Westlake 1984).

The liver exhibits a variety of histologically quantifiable effects when exposed to toxic compounds. Exposure may also produce induction of liver enzymes which can be measured using various biochemical methods (Westlake et al. 1979). Wood mouse liver cells may become enlarged, shown in field and laboratory samples as an increase in cell area, after ingestion of foliage treated with a herbicide such as diclofop methyl. Lipid accumulation in liver cells as triglyceride may produce a pronounced lipid vacuolation which in avian species is characteristic of poisoning resulting from an organochlorine metabolite of seed dressing (Tarrant et al. 1983).

Liver cells may also respond to a toxic insult by increasing cell replication, observed as a quantifiable increase in liver cell bi-nucleation. This has been observed in wild rabbits (Oryctolagus cuniculus) dosed with the carbamate aphicide demeton-S-methyl (unpublished data) and in wood mice exposed to the herbicide diclofop-methyl (Westlake et al. 1988).

If exposure to a toxic compound is prolonged and cells are exposed to the parent compound or its metabolites, then internal or external cell membranes may become disrupted with subsequent leakage of cell contents. This will have two measurable effects. Initially, material lost from the cell will activate tissue defence mechanisms and macrophagic cells will accumulate around the damaged cells. The number of such inflammatory foci per unit area of the liver can be quantified and compared to control tissue. Concurrent with this effect there is a release of tissue-derived enzymes such as glutamate oxaloacetate transaminase (GOT), glutamate dehydrogenase (GDH) and sorbitol dehydrogenase (SDH) which can be measured in the plasma from animals exhibiting histological damage (Westlake et al 1983). This has been observed in starlings (Sturnus vulgaris) exposed to methiocarb in cherry orchards and in small mammal species (Westlake et al 1988). Such effects are dependent upon duration and rate of exposure. Toxicological effects may not present a linear response (eg. Fig.1). Laboratory studies involving toxic effects upon the liver have shown that lower dose levels may present a greater visible effect upon cell morphology than higher dose levels that cause rapid cell death with resultant accumulations of mononuclear leucocytes. If a metabolite has a greater toxicological activity than the parent compound then there will be an initial delay before any histological effects can be observed or measured.

Fig.1. Changes in mean plasma enzyme levels in quail fed on a diet containing DDMU, a metabolite of an organochlorine seed dressing.

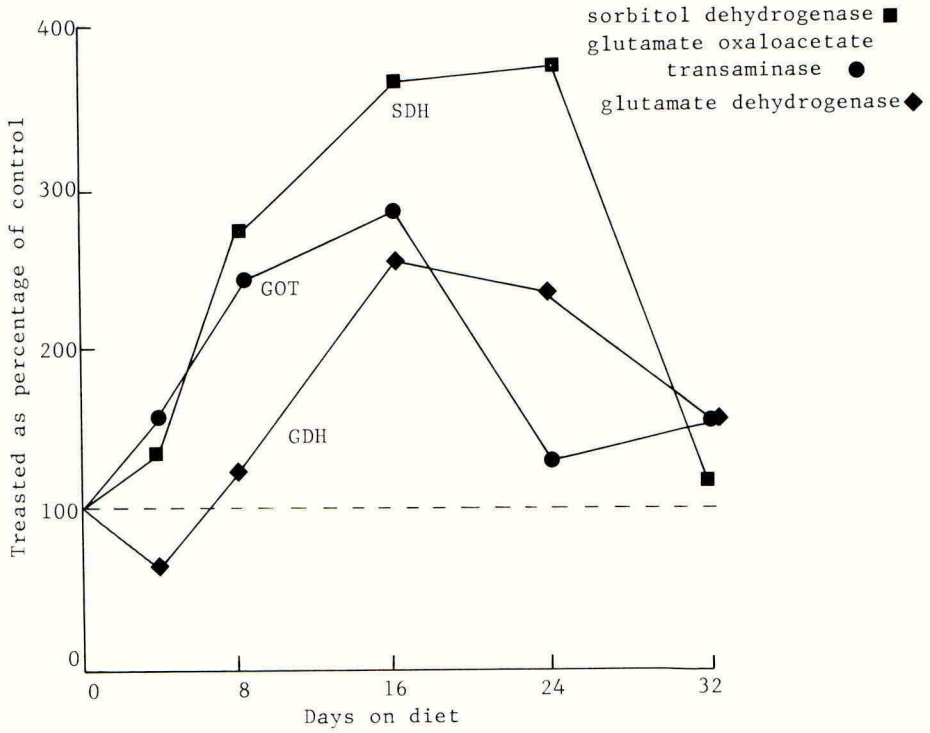

Table 1 summarises the range of liver tissue evaluation techniques used in one recent study. These changes can usefully be evaluated with the aid of image analysis systems, allowing large numbers of observations to be quantified, for statistical comparison against untreated control animals.

CONCLUSION

Sampling of animals from a wild population for histological examination can provide the valuable information which contributes to the assessment of field hazards from pesticides in agricultural use. The sensitivity of histological techniques can identify cellular changes associated with exposure. This is particularly important as the generation of pesticides now in use are generally less persistent in the environment and are not associated with heavy mortality in indicator species. Laboratory studies are also useful to develop new techniques and to obtain background data to help in the field assessment studies. If field evaluation can also incorporate enzyme studies on blood taken by non-lethal sampling from indicator species, additional valuable information can be obtained to aid the assessment of environmental hazard.

Table 1

Method for the evaluation of liver sections

	Quantification of histological change : applied to control and test liver sections					
1	Cell enlargement, with loss of cytoplasmic staining	0 Non observed	1 few cells	2 Many cells	3 Majority cells	4 All cells
2	Nuclear size changes	a Majority small	b mainly small some large	c majority large	d Definite focal areas of small or large	
3	Anuclear cells in areas of hypertrophy : specify lobular position	1 few cells	2 many cells		3 Majority of cells	
4	Kupffer cell activity	+ Small numbers of enlarged active cells		++ Many enlarged active cells observed over most of section		
5	Fibrosis increased fibrotic content of connective tissue at the triads or inter lobular trabeculae areas	0 none	1 minor	2 pronounced	3 Extensive	
6	Presence of poly-nuclear or mono-nuclear leuco-cytes amongst cord cells	1 Diffuse single	2 Diffuse chains or collections in sinusoids		f or c Definite foci or cuffing	
7	If definite collections of leucocytes		total count/tissue section			
8	Nuclear inclusion bodies		total number/tissue section			

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EFFECTS OF THE FOLIAR FUNGICIDE PYRAZOPHOS ON CEREAL COLLEMBOLA

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ABSTRACT

The organophosphorus systemic foliar fungicide pyrazophos significantly increased the mortality of *Sminthurinus aureus* (Collembola: Sminthuridae) in the laboratory. Pyrazophos reduced the numbers of four out of eleven species of Collembola in a field of winter barley and for these species the magnitude of the insecticidal activity of pyrazophos was comparable with that of the broad-spectrum insecticide dimethoate. In both cases, however, significant effects could not be detected in individual species after eleven weeks.

INTRODUCTION

The use of foliar fungicides has increased steadily since their introduction in the early 1970s. Over 10 million 'spray hectares' (i.e. including more than one spray/field/season) are now treated with foliar fungicides (Hardy, 1986). Some information exists about the side-effects of fungicides on cereal arthropods (Catling, 1969; Reyes & Stevenson, 1975; Vickerman, 1977; Vickerman & Sotherton, 1983; Sotherton & Moreby, 1984). The insecticidal activity of pyrazophos has been demonstrated by various workers but only Sotherton, Moreby & Langley (1987) demonstrated its toxicity to Collembola (as a group), in this case in winter barley.

Since many Collembola are mycetophagous (often facultatively) (Macnamara, 1924; Hale, 1967; Butcher, Snider & Snider, 1971), the collembolan diet is a route for the uptake of systemic fungicides into the cereal microfauna.

Although the importance of Collembola as decomposers is uncertain (Hale, 1967; Butcher, Snider & Snider, 1971) they are numerically important and fall prey to other beneficial invertebrates, particularly the Acarina (Wallace, 1953; Harris, 1975), Araneae (Sunderland, 1986) and Coleoptera (Ernsting & Jansen, 1978; Griffiths, 1983). Where these predators reduce the numbers of insect pests (cereal aphids, for example) there is the risk that the knock-on effects of fungicides on Collembola could influence the balance of pest species in cereals. The aim of this work was to determine whether any of the foliar fungicides commonly used on cereals are toxic to Collembola under agronomically realistic conditions.

MATERIALS & METHODS

Laboratory toxicology

Details of the laboratory toxicology are given by Frampton (1988).

The test species, *Sminthurinus aureus*, was taken from a culture which had been maintained under controlled conditions for *c.* 9 months prior to the toxicological experiments. Substrates, each in an experimental enclosure, were treated with the broad-spectrum fungicide pyrazophos ('Missile', Hoechst U.K. Ltd., 30% a.i.) at a dilution and a rate / unit area equivalent to that recommended for use in the field (2.0 l/ha in 220 l/ha water) using the Potter Precision Spray Tower (Busvine 1971). Control substrates were sprayed with distilled water at a rate/unit area equivalent to 220 l/ha.

Ten adult *S. aureus* were trapped on the treated or control substrate in each experimental enclosure and five substrate replicates were used per treatment. All experimental enclosures were then maintained under identical controlled conditions and the subsequent mortality of the animals was recorded.

Survival curves of control and pyrazophos-treated animals were compared using grouped data proportional hazards models similar to that described by Bartlett (1978). Because of the marked effects of pyrazophos in the laboratory (see Results), this compound was further evaluated in the field.

Field evaluation

Trial design and fungicide application

The study was conducted in 1985 and the study site is described by Frampton (1988). A 29.5ha field of winter barley (cv Halcyon) was divided into nine plots, each of *c.* 2.7ha in area. The plots were arranged in a 3 x 3 Latin square design to give three replicates of each of pyrazophos, dimethoate ('Rogor E', Schering Agrochemicals) and control (unsprayed) treatments, dimethoate being used as a potential insecticide toxic standard. Pyrazophos and dimethoate were applied to the appropriate plots on 2 May, 1985 (G.S. 32; Zadoks, Chang & Konzak, 1974) at their recommended field dilutions (pyrazophos 600 g.a.i./ha, dimethoate 400 g.a.i./ha) and rates/unit area (200 l/ha) (Frampton, 1988).

Sampling, extraction and identification of Collembola

A dietrick Vacuum Insect Net (D-vac) was used for sampling epigeal, hemiedaphic and epedaphic Collembola. Ten samples were taken along transects through the centre of each plot and each sample was transferred to 70% alcohol in the laboratory within 24h, after storage at 4°C. Eleven species of Collembola were caught (four symphypleone and seven arthropleone) on five sampling dates: 30 April (pre-treatment), 10 May, 29 May, 13 June and 23 July, 1985. Details of the species caught and methods of their extraction are given by Frampton (1988).

Statistical analysis

A $\log_{10}$ $(\underline{x}+1)$ transformation was found to be suitable for the data counts $(\underline{x})$. Pre-treatment variability in the numbers of Collembola between experimental plots was taken into account during the subsequent analysis of variance by subtracting the $\log_{10}$-transformed mean pre-treatment counts

from their corresponding $\log_{10}$-transformed mean post-treatment counts for each species on each date.

RESULTS

Laboratory toxicology

Survival curves for *S. aureus* after pyrazophos and control treatments are shown in Fig. 1. The curves are significantly different ($P<0.001$). The effects of pyrazophos were potent with 100% mortality in pyrazophos-treated *S. aureus* after 13h whereas control animals survived for more than 300h (Fig.1).

Field evaluation

Of the 11 Collembola species caught, the Latin square analysis of variance revealed significant treatment effects on the four symphypleone species *Sminthurus viridis*, *Sminthurinus elegans*, *S. aureus* and *Jeannenotia stachi*. The seven arthropleone species were not significantly affected by the pyrazophos treatment (Frampton, 1988) but many of these species were caught in low densities in the control plots.

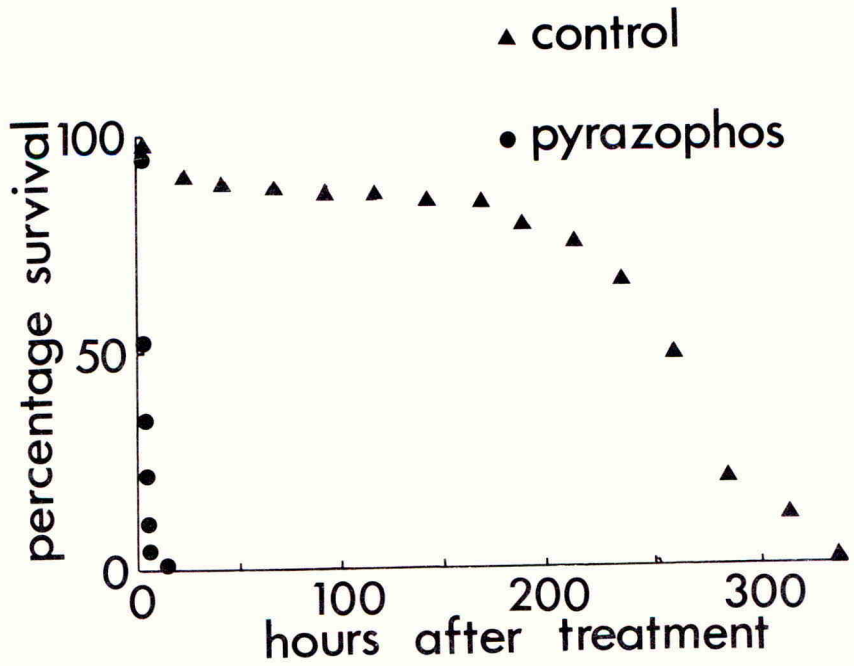

Fig.1. The time-survival relationship for *S. aureus* following laboratory treatment of the substrate with pyrazophos.

The change in the numbers of the symphypleone species (grouped as the total Symphypleona) relative to the pre-treatment numbers are shown in Fig.2. In the control plots these species reached peak numbers on 13 June. The effects of dimethoate were more immediate than those of pyrazophos; one week after treatment the lowest numbers of these species were found in dimethoate-treated plots whereas six weeks after treatment they were lowest in the pyrazophos-treated plots. The insecticidal activity of pyrazophos on the symphypleone Collembola was similar to that of dimethoate (Fig.2). *S. viridis*, *S. elegans* and *J. stachi* were eliminated from dimethoate and pyrazophos-treated plots on some of the sampling dates (Frampton, 1988) but only in the Symphypleona as a group were significant effects still detected 11 weeks after treatment.

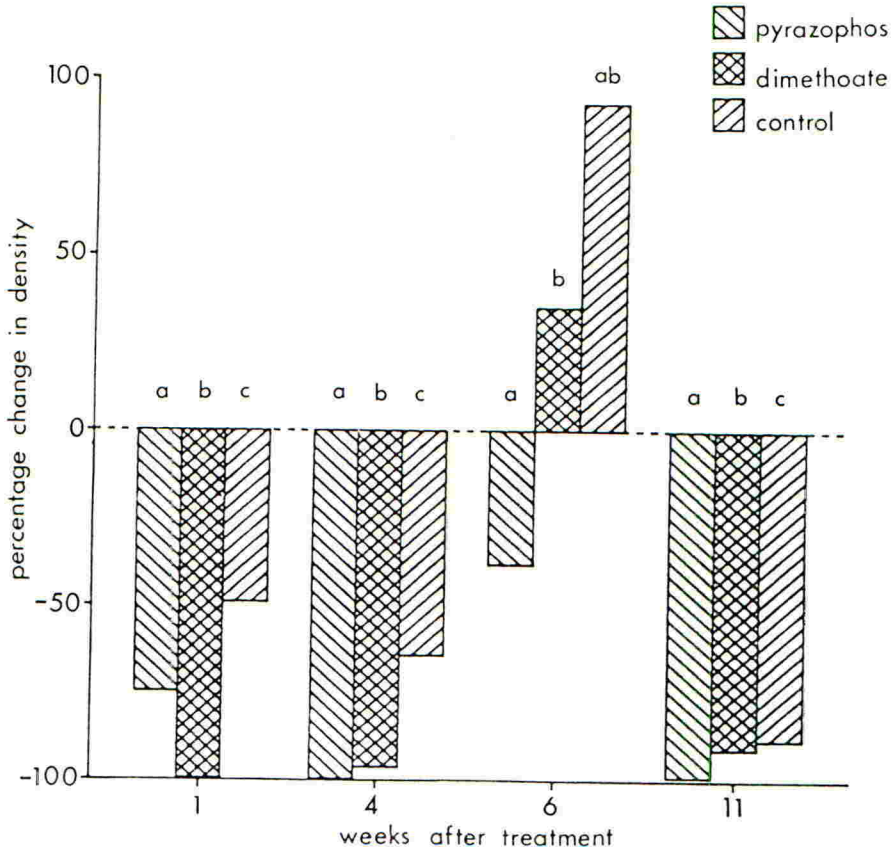

Fig.2. Percentage changes in mean Collembola numbers (per m^2) on four post-treatment dates. Positive changes shown an increase in numbers with respect to pre-treatment numbers; negative changes indicate a decrease. All error mean squares calculated from the analysis of variance on Log_{10} counts were less than 20% of the log_{10} mean. Histograms sharing the same letter are not significantly different ($P<0.05$). The densities of control animals on the four dates were 864, 605, 3264 and 198 respectively.

DISCUSSION

Of the four commonly-used foliar fungicides selected for laboratory screening against *S. aureus*, all were found to increase significantly the mortality of this collembolan. Since the Collembola could have browsed on the treated filter paper substrate or on microorganisms on the treated substrate, it was not possible to determine whether the effects of the fungicides were direct or indirect (via the diet). In the field, the more immediate effects of dimethoate are to be expected since this is a broad-spectrum insecticide (Vickerman & Sunderland, 1977). The lag phase with pyrazophos might be attributed to indirect effects through a reduction in food, for example a reduction in saprophytic fungi.

Relationship between laboratory toxicology and field evaluation

In the laboratory, substrates were treated with pyrazophos at rates / unit area and dilutions equivalent to those normally used in the field. Under field conditions it is likely that epigeal Collembola will receive a reduced dose of pyrazophos through foliar interception so fungicides not affecting Collembola significantly in the laboratory were considered unlikely to exhibit effects in the field; those exhibiting significant effects on Collembola in the laboratory were considered worthy of further evaluation in the field.

The field trial design

This study was intended to investigate the action of pyrazophos on Collembola under agronomically realistic conditions. Ideally, the minimum plot size should be an entire field so temporal and spatial redistribution of Collembola within a field would be unlikely to obscure treatment effects. Such large scale experiments are usually prohibitive (Wratten *et al.*,1988) and in this case the available land was restricted to a 29.5ha field. However, since Collembola are thought to be relatively immobile in comparison with some predatory insects, a large number of small plots would have been acceptable to provide better information on and control of spatial variability in the numbers of Collembola. For small plots, redistribution of Collembola between plots would require monitoring or checking with inclusion-exclusion barriers.

In order to accomodate an independent study of the effects of pyrazophos on mobile predatory insects (Wratten *et al.*,1988) at the 29.5ha site, a plot size of *c.* 2.7ha with 9 plots arranged in a 3 X 3 Latin square matrix was chosen for use by both studies. With the 'large' plot size, the use of inclusion-exclusion barriers was considered unnecessary for the Collembola study and sampling from the centre of each plot was to minimise edge-effects.

Importance of trends in the results

Eleven weeks after the treatment, no significant effects of pyrazophos could be detected in individual species nor in the total (symphypleone plus arthropleone) Collembola. However, pyrazophos apparently still influenced the numbers of some Collembola species since significantly fewer of the Symphypleona (as a group) were caught in pyrazophos-treated than in control

plots on this date (Fig. 2). This exemplifies the problem that statistical validation of the field effects is only plausible when sufficient densities of animals are caught and where there is reasonable homogeneity between samples. It is therefore important also to consider trends in the numbers of different species even where effects are non-significant. In the four symphypleone species (*S. viridis*, *S. elegans*, *S. aureus* and *J. stachi*), for example, the numbers caught were always lower in pyrazophos-treated than in control plots eleven weeks after treatment and yet only when the species data were pooled was this effect significant (Fig. 2).

Comparison with other studies

The results of this field study are supported by those of Sotherton, Moreby & Langley (1987) in which large (>20ha) plots were used. In both studies the effects of pyrazophos on Collembola were detected within one week of treatment and persisted at least for six weeks.

In view of the toxicity of pyrazophos to the Symphypleona demonstrated in this study and its toxicity to other insects (Sotherton, Moreby & Langley, 1987; Wratten *et al*, 1988) it is surprising that no significant effects of pyrazophos were detected on the Arthropleona.

Of the seven arthropleone species caught, four of these species were much rarer in samples than any of the symphypleone species. *Isotoma notabilis*, one of the 'rare' arthropleone species, was nevertheless consistently caught in lower numbers in pyrazophos-treated than in control plots. Likewise, the numbers of *I.virdis*, a species caught in higher densities but with higher spatial variability (perhaps a consequence of its hydrophilic habit) were lower in pyrazophos-treated than control plots four, six and eleven weeks after treatment. The most abundant of the arthropleone species. *Lepidocytrus cyaneus*, was always caught in lower numbers in pyrazophos-treated plots than in control or dimethoate-treated plots and only in pyrazophos-treated plots did its numbers fall below the pre-treatment levels.

These similarities in the effects of pyrazophos on the symphypleone and on some arthropleone Collembola could be further investigated by comparison of the effects of pyrazophos on insects from each sub-order in the laboratory. Such a comparison between *S. aureus* and *I. viridis* was attempted in the laboratory but was unsuccessful as a result of high *I. viridis* control mortality (G. K. Frampton, unpublished data).

Statistical analysis alone indicates a short-term persistence of pyrazophos except in the Symphypleona (as a group). As stated by Sotherton, Morley & Langley (1987), such apparent short-term persistence does not imply safety of the compound, but merely reflects the scale of the experiment. Although the plot size used in this study was considered acceptable for the study of Collembola, any effects of the redistribution of insects between plots will depend on the duration of the experiment as well as the plot size. In this context it would have been useful to have compared the effects of pyrazophos eleven weeks after treatment in this study with the effects eleven weeks after treatment in the larger plots of Sotherton, Moreby & Langley (1987).

Whilst it is difficult to assess how low densities, variability in numbers and movement of Collembola influenced the effects of pyrazophos on these insects in this field trial, the observed effects will have been mitigated rather than accentuated by these factors. The results presented here therefore do not necessarily represent the maximum effect of pyrazophos on Collembola in a field of winter barley. In view of the potential importance of Collembola in the cereal ecosytem and the continued increase in the use of foliar fungicides in the U.K., a better understanding of the ecology of Collembola in arable land and that of the ecotoxicology of the fungicides is needed.

ACKNOWLEDGEMENTS

This work forms part of a M.A.F.F.-funded studentship supervised by Drs. G.P. Vickerman and S.D. Wratten to whom thanks are due for their help. Thanks are also due to Mr. H. Gough of I.C.I. Plant Protection Division and Dr. M. Wetton of the British Museum for taxonomic advice and Mr. J. Perry of Rothamsted Experimental Station for statistical advice. This work could not have been carried out without the help of the Leckford Estate, especially Mr. B. Gibbons and of Hoechst (UK.) Ltd., especially that of Dr. R.T. Hewson.

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EFFECTS OF THE FUNGICIDE PYRAZOPHOS ON PREDATORY INSECTS IN WINTER BARLEY

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ABSTRACT

The effects of an application of the fungicide pyrazophos on 'non-target' invertebrates in winter barley are described. Plot-size was c. 2.7 ha and dimethoate was used as a toxic standard. Numbers of some species of Carabidae, Staphylinidae and Araneae were reduced by both pesticides, and effects of pyrazophos could be detected for up to 82 days post-spraying for some taxa. No effects could be detected in the following year and the biological, statistical and methodological factors contributing to differences between the results of this trial and others are discussed.

INTRODUCTION

Although pyrazophos has been shown to have insecticidal properties against some glasshouse insect pests (Parrella 1983; Ledieu & Helyer, 1983) and against some invertebrate predators in the laboratory or glasshouse (Hassan, 1974; Van Zon & Wysoki, 1978), the short- and long-term consequences, if any, on beneficial insects of the field use of this compound have received little attention. Sotherton et al. (1987) demonstrated within-season reductions in the densities of some predatory insects following treatment of large plots up to 24 ha in barley. The trial reported here also took place in 1985, with further observations in 1986. It differed in some respects from that of Sotherton et al., 1987. Interpretation of the results of such trials depends heavily on trial design, sampling methods and frequency, local meteorological conditions and local invertebrate phenologies; these aspects will be briefly compared in the Conclusions section below and are further discussed by Sotherton et al. (1988). The aims of the work were to evaluate the effects of pyrazophos against 'non-target' arthropods in winter barley and to interpret these in relation to the long-term consequences of the use of such a compound in arable land.

MATERIALS AND METHODS

Trial site

The trial was carried out in a field of winter barley (cv. Halcyon) on a commercial farm in northwest Hampshire. The barley was drilled on 24 September 1984 and harvested on 29 August 1985. Details of the pesticide and other applications made to the whole crop are given in Table 1. The field was divided into nine plots in a 3 x 3 matrix, with each treatment (pyrazophos, dimethoate (as a potential toxic standard) and control) being assigned three replicate plots in a Latin Square arrangement. Plot size ranged from 2.6 to 2.8 ha.

Treatments

Method of application

Details of the compounds used in the trial are as follows: pyrazophos (as 'Missile', 30% EC) applied at 600g a.i./ha in 220 l water. Dimethoate (as Hortichem Croptex Dimethoate, 40% EC) applied at 440g a.i./ha in 220 l water. Applications were made using a 'Chafer' tractor-drawn sprayer with a 15.4m boom fitted with hollow cone nozzles. The tank pressure was 2.0 bar and the tractor ground speed was 10km/h. Compounds were applied on 2 May 1985, when the crop was at growth stage 32 (Zadoks, et al., 1974).

TABLE 1
Crop treatments besides trial pesticides

Date	Product	Function	Rate
1985			
24/9/84	Methiocarb	Carbamate molluscicide	1.67 kg/ha
26/9/84	Chlorpyriphos	OP insecticide	1.5 l/ha
31/10/84	Chlorsulphuron and metasulphuron	Herbicide	100 g/ha) Tank
	Chlormequat chloride	Growth regulator	0.75 l/ha) mix
1/11/84	Sumicidin	Pyrethroid insecticide	0.2 l/ha
14/4/85	Fluroxypyr	Herbicide	1 l/ha (headlands only)
16/4/85	Benomyl	Systemic fungicide	5 kg/ha)
	Chlormequat chloride	Growth regulator	1.5 l/ha) Tank mix
	Tridemorph	Systemic fungicide	1.5 l/ha)
24/4/85	Cu solution	Trace element	2.5 l/ha
	Mg solution	Trace element	2.5 l/ha
3/5/85	Sulphur	Inorganic fungicide	10 kg/ha
17/5/85	2-chloroethylphosphonic acid and mepiquat	Growth regulator	2 l/ha
31/5/85	Propiconazole	Systemic fungicide	0.5 l/ha
1986			
7/11/85	Sumicidin	Pyrethroid insecticide	0.2 l /ha
16/12/85	Chlorsulphuron and metasulphuron	Herbicide	100 g/ha
25/4/86	Benomyl	Systemic fungicide	500 g/ha
	Propiconazole	Systemic fungicide	0.5 l/ha
22/5/86	2-chloroethylphosphonic acid and mepiquat	Straw strengthener	2 l/ha
7/6/86	Propiconazole	Systemic fungicide	0.5 l/ha

Sampling methods

Sampling of invertebrates took place towards the centre of each plot, making use of tramline number and cane plot-markers to identify the position in the field. Three sampling methods were employed: i) suction sampling (D-Vac) to collect fauna from the ground and from the aerial components of the crop, (ii) pitfall trapping to collect soil surface fauna, (iii) quadrat sampling to collect fauna from the soil, the soil surface and the base of plants. Methods i) and iii) theoretically provide absolute estimates of population density.

Suction sampling

Ten D-Vac samples were taken from the central area of each plot on each sampling occasion. Each sample consisted of five cumulative sub-

samples each lasting for 10s and covering 0.092 m^2, giving a sample area of 0.46m^2 and a total area of crop sampled in each plot of 4.6m^2.

Invertebrate samples were emptied into labelled polythene bags, which were kept cool and returned to the laboratory for the contents to be freeze-killed before transfer to tubes containing absolute alcohol for subsequent identification and counting.

Pitfall trapping

Ten pitfall traps, constructed from polystyrene beakers (12 cm deep and 9 cm in diameter at the top) were dug into the ground at 10-m intervals at the plot centre and were filled to a depth of 4-5cm with a 4% formalin solution.

The traps were first opened between 3 and 6 days before the pre-treatment sample but after that the sampling dates varied, with a one-week trapping period on each occasion (see Table 3). The trap contents were emptied into labelled plastic bags which were sealed and transported back to the laboratory.

In the laboratory, the samples were filtered using a fine mesh sieve and the retained material placed in tubes of absolute alcohol for subsequent sorting and identification.

Quadrat sampling

On each sampling occasion arthropods were collected from ten randomly chosen 0.1m^2 quadrats at the plot centre. The quadrats, 4-5m apart, were squares of 33cm side, and hence enclosed 0.1m^2. Using a mouth-operated aspirator (a pooter), arthropods were collected from the base of plants, the soil surface and the soil itself to a depth of 3-4cm. The fauna collected was immediately transferred to labelled tubes of absolute alcohol and later the same day returned to the laboratory for subsequent identification and counting.

All three methods were used to take pre-treatment samples a few days prior to the application of pyrazophos and dimethoate (Table 3) and on four post-application dates in 1985 prior to harvest. The same plots were relocated in 1986 (winter barley c.v. Halcyon, as in 1985, see Table 2) and two D-vac and two pitfall samples were each taken on two dates, one in May and one in June.

Statistical analysis

The mean number of individuals/taxon/replicate/sample date/sample method was transformed using $\log_{10}(n+1)$. A date-by-date Latin square analysis of variance was then carried out on these mean transformed values. To remove variation arising from differences in arthropod density between plots prior to treatment, the mean log-difference method of Sotherton *et al*. (1987) was used. For each post-treatment date, the $\log_{10}$ (n+1) mean number found per replicate was subtracted from the $\log_{10}$ (n+1) mean number present during the respective pre-treatment sample. Where either analysis showed a significant F-ratio (at or above the 5% level), Tukey's Test (Snedecor, 1962) was carried out to determine whether pyrazophos, dimethoate or both compounds resulted in significantly different arthropod numbers relative to the control.

RESULTS

The results of the analysis of variance for 1985 data are summarised in Tables 2, 3 and 4. Pitfall-trap catches were dominated by Staphylinidae, Araneae and Carabidae. The heavier carabid beetles were less well represented in D-vac samples, in which Staphylinidae dominated, while quadrat sampling produced mainly Staphylinidae, though data were variable, with many zeros and generally low numbers. In the whole 1985 trial, more than 30000 individual invertebrates were sorted and identified, usually to species; in the 1986 follow-up sampling, a further 10000+ were similarly dealt with.

TABLE 2

Results of Latin Square analyses of variance for D-Vac data.

Latin Square ANOVA * log (n+1) transformation
* log (n+1) difference transformation
* or * F value significant at 5% level
** or ** F value significant at 1% level
( ) F value significant, but pair-wise comparison of treatment means from Tukey's test was insignificant
P = pyrazophos D = dimethoate C = control

DAYS POST SPRAYING	8		27		42		82	
	P<C	D<C	P<C	D<C	P<C	D<C	P<C	D<C
Staphylinidae								
Tachyporus spp. adults	**	**						
Cypha spp.			*					
Total Staphylinidae adults			(*)					
Tachyporus larvae					**	**		
		D>C						
Araneae								
Linyphiidae adults		*						
Linyphiidae immatures							*	*

Taxa analysed were those which reached 1.0 individuals/0.46 m2 on at least one sampling occasion after treatment. Taxa analysed on this basis but for which there were no significant effects were Tachyporus hypnorum, Tachyporus nitidulus, callow Tachyporus spp., Oxytelus spp., Meioneta rurestris, Coccinellidae, Dolichopodidae, Aphidius spp.

In 1985 there were no significant pre-treatment differences in arthropod numbers. After treatment, pitfall and D-vac data revealed that dimethoate and pyrazophos reduced the numbers of some groups. In all, 67 taxa were identified from D-vac samples, 90 from pitfalls and 39 from the quadrats, most to the species level. Of those, pyrazophos significantly reduced the numbers of four of the D-vac taxa, seven of the pitfall taxa and none from the surface searching, although only fourteen D-vac taxa were analysed statistically, because of low and variable numbers in the other groups. Thirty-two pitfall-trap taxa and six quadrat taxa were analysed. The criterion for selecting a taxon for statistical analysis was that there should be one individual/sample on at least one post-treatment sampling

date, where a 'sample' is a single quadrat, one pitfall trap or a 0.46 m^2 D-vac sample.

Numbers of staphylinid adults were significantly reduced soon (9 days) after treatment by both pyrazophos and dimethoate; the species most affected were Tachyporus hypnorum and T. chrysomelinus; significantly lower numbers of larval and second-generation adult Tachyporus were found later in the season.

A similar pattern existed for linyphiid spiders, especially the most abundant species, Erigone atra. Numbers of total linyphiid immatures were significantly reduced in the pyrazophos plots 82 days after treatment.

TABLE 3

Results of Latin Square analyses of variance for pitfall data.

DAYS POST SPRAYING	9		23		46		83	
	P<C	D<C	P<C	D<C	P<C	D<C	P<C	D<C
Staphylinidae								
T. hypnorum	* *	* *		*	*			
T. chrysomelinus	* *	* *						
T. nitidulus						*		
Callow Tachyporus spp.							* *	* *
Tachyporus spp. adults	*	*		(*)	*			
Total Staphylinidae adults	* *	* *						
Tachyporus spp. larvae						**		
Carabidae								
Bembidion lampros				*				
Bembidion obtusum					*			(*)
Araneae								
Erigone atra	**	**						
Total Linyphiidae adults				*				*
			P>C					
Lycosidae - adults			*	*				

Taxa analysed were those which reached 1.0 individuals/trap on at least one sampling occasion post-treatment. Other details as in Table 2. No significant effects: Staphylinidae: Cypha spp., Oxytelus spp., Philonthus spp. - adults, Philonthus spp. - larvae., unidentified sp. - larvae; Carabidae: Amara spp., Harpalus rufipes, Loricera pilicornis, Calathus fuscipes, Calathus melanocephalus, Pterostichus melanarius, total Carabidae adults: Araneae: Meioneta rurestris, Bathyphantes gracilis, Oedothorax spp., Lycosidae: immatures, Coccinellidae: larvae, Elateridae; Chilopoda. Symbols as in Table 2.

Compared with pyrazophos in 1985, the effects of dimethoate were more extensive, both taxonomically and temporally; however, for both compounds, an effect on the majority of the faunal groups analysed statistically could not be demonstrated.

In 1986, arthropod taxa and densities were generally similar to those in 1985 and there were no significant differences between the numbers of all taxa identified in the pyrazophos and control samples. In the dimethoate plots, the numbers of the carabid Bembidion obtusum in pitfall traps were higher than in controls in June, while numbers of the predatory dipteran Platypalpus spp. were reduced significantly, also in the June samples.

When the invertebrate 'densities' from the 1985 D-vac samples were used to analyse changes in the fauna which is part of the diet of partridge (Perdix perdix) chicks, no effect on predicted partridge survival could be detected, using the model of Potts (1980).

TABLE 4

Results of Latin Square analyses of variance for surface search data.

DAYS POST SPRAYING	7	22	41	77
	- -	- -	- -	P>C P>C
Carabidae				
Total Carabidae adults				* * * *

Taxa analysed were those which reached 1.0 individuals/0.1 m2 on at least one sampling occasion post-treatment. Log (n+1) difference transformation not carried out as most pre-treatment densities were close to zero for this sampling method. Latin Square ANOVA (log (n+1) transformation). No significant effects: Oxytelus spp., Tachyporus spp. adults, total Staphylinidae adults, Meioneta rurestris, total Linyphiidae adults. Symbols as in Table 2.

DISCUSSION

Although some taxa were affected by pyrazophos on some dates, the effects were temporally and taxonomically sporadic and less extreme than those of dimethoate. In comparison with the trial of Sotherton et al., (1987), also conducted in the south of England in 1985, the effects of pyrazophos appeared to be less severe. Sotherton et al. (1987) revealed effects of pyrazophos lasting for more than 45 days, with groups most affected being aphid-specific predators, polyphagous predators (but excluding Araneae) and parasitoids. The numbers of game-bird food-items were also reduced significantly with effects again lasting for more than 45 days.

Both trials have demonstrated that pyrazophos has insecticidal effects in arable crops but the apparent extent of this effect appears to differ between them. Biological, statistical and methodological factors could contribute to these differences, and Table 5 summarises these possibilities.

It seems likely that, even when large (>20 ha) plots are used in trials evaluating the effects of pesticides on beneficial insects, the high levels of mobility of some faunal groups can contribute to an apparent 'recovery' of population levels (Sotherton et al., 1987). When whole fields are sprayed (see Vickerman, 1988) orthodox statistical replication may be sacrificed in favour of spatial realism (Sotherton et al., 1988).

TABLE 5

Biological and statistical differences between the present trial and the 1985 trial of Sotherton et al. (1987).

	This trial	Sotherton et al (1987)
1. Trial design	Latin square;3 treatments; 3 plots/treatment; plot size c.2.7ha;one field	Two treatments;4 plots treatment; 10-20 ha plots; two fields
2. Pyrazophos application date	May 2 1985	May 14 1985
3. Sampling methods	D-Vac, surface-searching and pitfall trapping	D-vac and surface searching
4. Statistical analysis	Latin square ANOVA of transformed densities for each post-treatment sample date subtracted from densities pre-treatment. Also, date-by-date Latin Square ANOVA. $F_{2,2}$=19.00 at P=0.05. Tukey's range test.	Two-way ANOVA of data transformed and corrected as opposite. $F_{1,3}$ = 10.13 at $F^{=}$ 0.05 No range test (two treatments).
5. Faunal densities (m^{-2};D-vac)		
a) pre-spraying (all plots)		
Staphylinidae adults	12.33	26.9
Tachyporus spp.	5.81	50.1
Carabidae	0.1	4.0
Araneae	1.0	11.0
b) 27 days (this trial) 15 days(Sotherton et al.) post-spraying (i.e. May 29): control nos.		
Staphylinidae adults	7.7	63.8
Tachyporus spp.	0.8	57.7
Carabidae	0.1	4.3

Significant but relatively short-lived effects of pesticides in arable land have also been demonstrated using deltamethrin and dimethoate (Vickerman et al., 1987). The Latin square design was used because of probable heterogeneity in the colonisation of predators from field boundaries prior to treatment. However, this sacrifices statistical sensitivity in terms of the F-value needed for significance compared with a two-way ANOVA. Low densities in this trial also affected the likelihood of obtaining significant effects (see Table 5).

Even if significant row and column effects are identified and therefore isolated from treatment effects, the role of these spatial factors cannot be known at the trial-design stage. From a fundamental scientific viewpoint, the alternative ways of addressing the problems identified in Table 5 (i.e. spatial scale/recolonisation and the spatial heterogeneity and variable phenologies of the fauna) could include, i)

long-term monitoring over a wide geographical area (Potts & Vickerman, 1974), ii) farm-scale replicated experiments within a season (not yet attempted), or iii) within-farm un-replicated or 'pseudo-replicated' experiments which continue for a number of years and in which temporal trends in faunal numbers are recorded (e.g. the U.K. Boxworth Project, Vickerman 1988). From a commercial point of view, however, these options are expensive and time-consuming and will never be applied to more than a few products. Jepson (1988) proposed the development of alternative manipulative experimental designs in an attempt to develop a more rational basis for evaluating potential side effects.

This trial was sponsored by Hoechst U.K. Ltd and we thank Dr. R.T. Hewson of that company for technical support.

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THE USE OF POLYTHENE BARRIERS TO STUDY THE LONG TERM EFFECTS OF PESTICIDES ON GROUND BEETLES (CARABIDAE, COLEOPTERA) IN SMALL-SCALE FIELD EXPERIMENTS

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ABSTRACT

A series of experiments are described to investigate the side-effects of the molluscicide, methiocarb, on populations of ground beetles in winter wheat. Polythene barriers were used to reduce movement of animals between experimental plots, but still allow seasonal dispersal between field margin and crop. Effects of methiocarb were determined by frequent pitfall trapping in treated and untreated plots. In 1987-88, results from barriered plots were compared with those obtained in unbarriered areas. Smaller numbers of ground beetles were caught over a period of several months after methiocarb treatment in autumn. The presence of barriers had relatively little impact either on the magnitude or duration of these pesticide effects, at least over winter from November to January, although the reduction in catch size in unbarriered plots was less pronounced immediately after treatment and again in February. Further studies are being done to confirm this trend.

INTRODUCTION

Ground beetles (Carabidae) are important predators of slugs, snails, wireworms, aphids, stem-boring Diptera, lepidopterous larvae, and other pests of arable crops. They, therefore, constitute a significant part of the polyphagous predator complex and should be considered in most integrated pest management systems.

Many governments have registration requirements that involve the submission of data assessing the toxicity of pesticides to these beetles both in the field and in the laboratory. Field studies of the impact of pesticides on ground beetles are often hindered by re-invasion of treated plots from untreated areas, due to the beetles' considerable mobility. Re-invasion may be reduced by using either very large plots (probably at least 0.5 ha) or containment barriers (Edwards, 1977). Barriered plots can be as small as 10 m square. The use of such enclosed plots has received much attention in recent years (e.g. Edwards *et al.*, 1984; Matcham & Hawkes, 1985; Powell *et al.*, 1985; Shires, 1985).

This paper aims to examine the possible advantages of using polythene barriers in long term environmental studies (minimum one year). Any long term study should take account of migration to and from semi-natural habitats, but avoid possible movement between experimental plots.

METHODS

In 1985, a field of winter wheat, near Long Ashton, was divided into five plots (each about 0.7 ha). Plots were separated on both sides by a polythene barrier extending from the field margin to approximately 100 m into the crop, but left open at both ends (see Fig. 1). The polythene was buried to a depth of 20-25 cm using a modified sub-soiler on loan from Boxworth EHF, and supported to a height of 40 cm by wooden posts and fencing wire. Plots were treated with methiocarb slug pellets (220 g a.i./ha) broadcast on 18 October, or cypermethrin (25 g a.i./ha) sprayed on 4 November, or were left untreated.

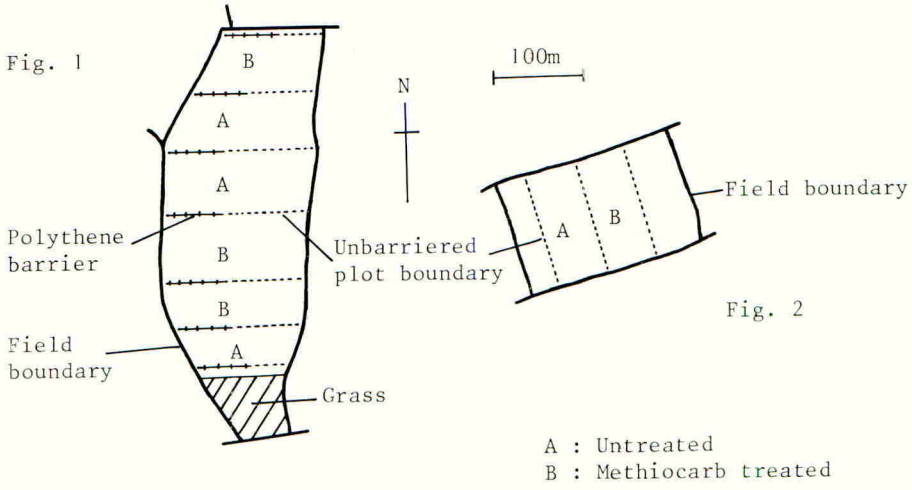

Figure 1. Layout of the 1986-87 and 1987-88 barriered field experiments.
Figure 2. Layout of the 1987-88 unbarriered field experiment.

Since autumn 1986, the field has been divided into six plots (Fig. 1), separated in a similar fashion to the previous year. The polythene was partially buried to 20-25 cm, supported to a height of 80 cm, and extended 60 m into the crop. Three plots were treated with methiocarb, broadcast on 2 November 1986 and 29 October 1987, and three plots were left untreated. In autumn 1987, two plots of similar size in a different winter wheat field were treated in the same way, one plot treated with methiocarb and one plot left untreated, but were not separated by polythene barriers (Fig. 2).

Numbers of ground beetles were monitored by using a grid arrangement of pitfall traps (24 per plot in 1985-86; 9 per plot in 1986-87 and 1987-88). The traps, consisting of white, plastic beakers (10 cm diameter x 5.5 cm depth), were placed 25 m (15 m in 1985-86) apart. Effects of methiocarb on the ground beetles were compared between barriered and unbarriered plots.

RESULTS

The ground beetles caught in pitfall traps, in all three years and in both fields, were dominated by four species, Nebria brevicollis (F.), Pterostichus melanarius (Ill.), P. cupreus (L.), and Trechus quadristriatus (Schrank). These four species formed 81.0% of 2552 ground beetles caught in 1985-86 and 70.5% of 3659 beetles caught in 1986-87.

In 1985-86 and 1986-87, pitfall catches of adult and larval ground beetles from barriered plots showed a significant reduction for 4-5 months after treatment with methiocarb, based on the least significant difference at 95% level (Figs 3a, b). Differences between treated and untreated plots sometimes persisted up to seven months after treatment.

Preliminary analysis of 1987-88 data for adult ground beetles revealed a similar trend in both barriered and unbarriered field experiments in the three months sampled so far (Figs 3c, 4). Numbers of ground beetles caught were reduced following treatment with methiocarb, although the per cent reduction was less marked in unbarriered plots immediately following treatment and three months later, in early February, than in barriered plots (Fig. 5).

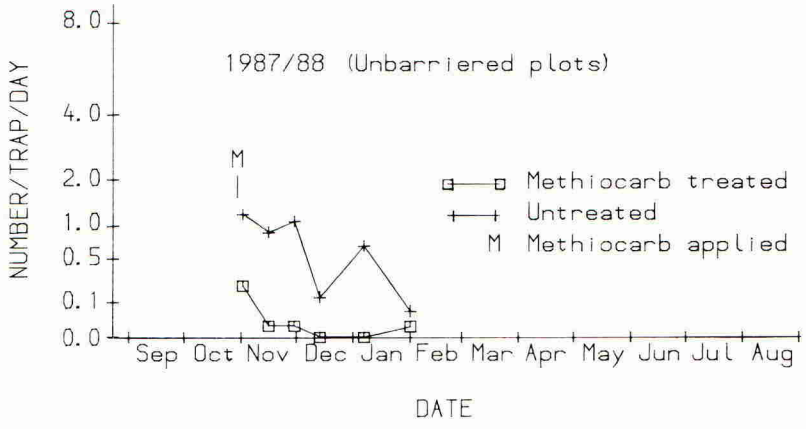

Figure 4. Seasonal pitfall trap catches of adult ground beetles in unbarriered, methiocarb treated and untreated plots, based on square-root transformed data for 1987-88.

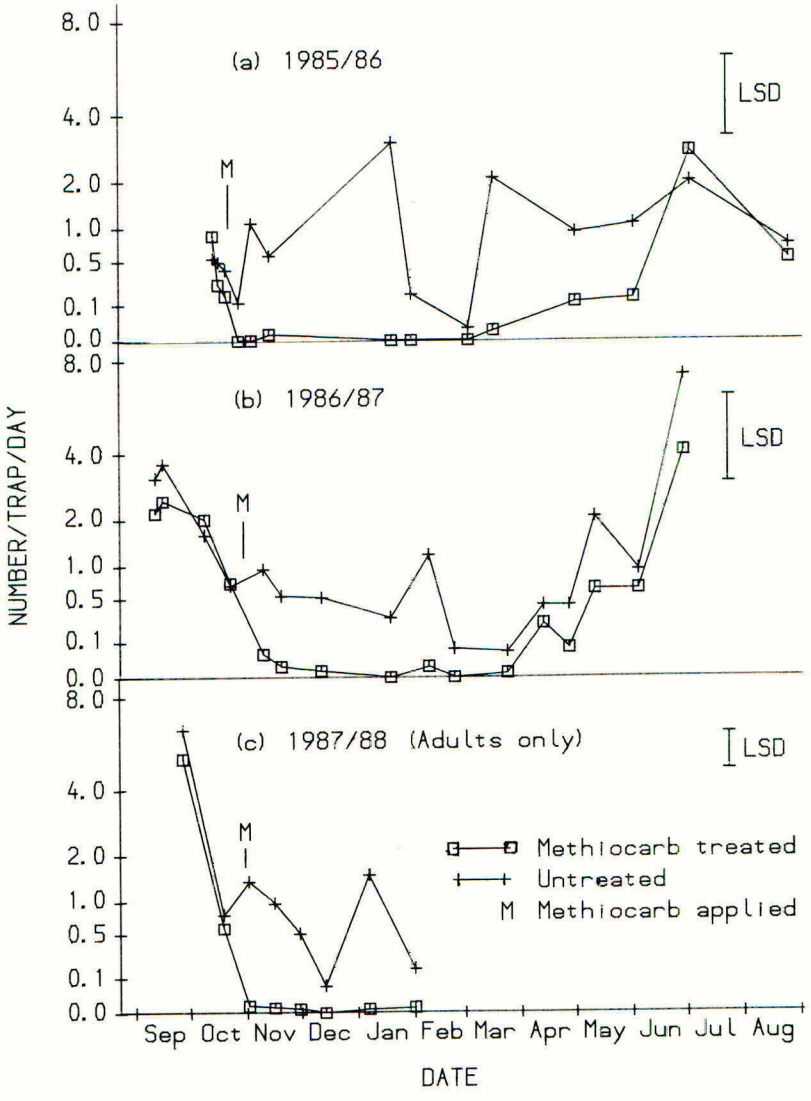

Figure 3. Seasonal pitfall trap catches of adult and larval ground beetles in barriered, methiocarb treated and untreated plots, based on square-root transformed data (a) 1985-86, (b) 1986-87, (c) 1987-88 (adults only). LSD - least significant difference at 95% level.

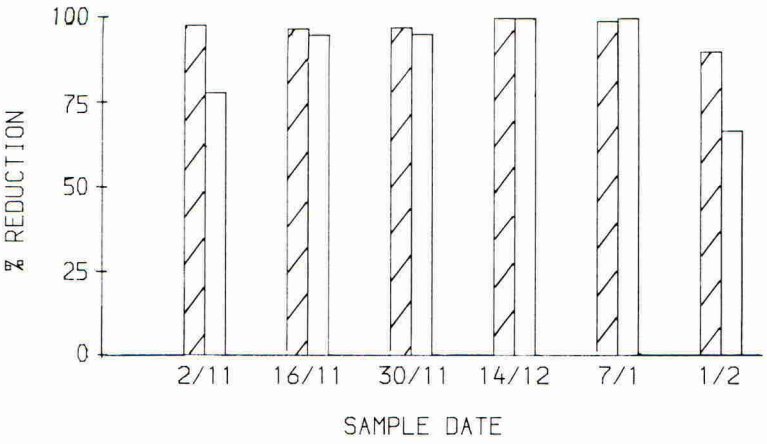

Figure 5. Per cent reduction in pitfall trap catches of adult ground beetles, in methiocarb treated plots compared to untreated plots. Unbarriered plots ☐; barriered plots ▨.

DISCUSSION

It is expensive and laborious, both to erect and maintain polythene barriers. For example, in 1986-87, 840 m of layflat polythene tubing, 420 m of fencing wire and 50 wooden posts cost about £300. Erecting the barriers took 4.5-man days and maintaining them throughout the year took about 20-man days. The presence of barriers doubled the time necessary for cultivations and overall treatments. Due to the expense and labour, careful consideration should be given to the possible advantages and disadvantages involved in using barriers.

Large plots, separated from adjoining plots by physical barriers but retaining their associated field boundary, are probably more representative of whole fields than unbarriered plots. Migration into such plots would be limited to dispersal from and through field boundaries, as would be the case for whole fields, with avoidance of immigration from adjoining areas of crop. Large, replicated plots within the same field are likely to give results which vary less than those of whole fields, and can be statistically analysed.

With plots exceeding 0.5 ha, the preliminary data analysed so far do not show any great advantage, in terms of magnitude and duration of effect, in using barriered rather than unbarriered plots. The small differences that were observed between barriered and unbarriered plots, in early November and early February, may have been due to higher levels of mobility than during November-January, leading to higher rates of re-invasion. Further work is currently being done to determine the value of barriers during spring and summer, when mobility and abundance of

ground beetles is increased. Barriered plots may be more useful than unbarriered plots when studying agrochemicals with less marked side-effects, which are quickly obscured by rapid re-invasion.

ACKNOWLEDGEMENTS

The author would like to thank staff at Long Ashton Research Station for their technical assistance, in particular Dr B.D. Smith and Dr D.A. Kendall for their supervision and comments on the manuscript. This study was supported by an Agricultural and Food Research Council studentship.

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