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Effects on Invertebrates (2)

Chairman: Dr R. A. Brown

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METHODS FOR STUDYING ABUNDANCE AND SPECIES DIVERSITY OF INVERTEBRATES IN ARABLE SOILS

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ABSTRACT

A wide range of invertebrates inhabit arable soils. These include microarthropods (Acari and Collembola), Millipedes (Diplopoda), earthworms (Lumbricidae) and dipterous larvae. Sampling design over heterogenous field sections, size of auger used, and rational extraction techniques, are discussed in relation to the required accuracy, taxa of concern and the labour required. Aspects of area and vertical distribution are illustrated by some examples including mesostigmatic mites. Results of long-term comparisons between different field treatments are presented.

The abundance of millipedes in crop-covered soils is described using different methods. Assessment of earthworm abundance is briefly reviewed referring to the most suitable method for use on fields.

Dipterous larvae are important components of soil eco-systems. Preliminary results of quantitative assessments of larval populations are reported with reference to improvements in the floatation technique.

INTRODUCTION

Current agricultural practices including use of pesticides have caused serious impacts on soil inhabiting organisms (Edwards 1984, Paul 1986, Foissner 1987). Quantitative evidence for this finding is still very limited. Methodological and statistical obstacles make assessments and evaluation of such effects, rather difficult. The following contribution describes some aspects of methodology for studying invertebrates in arable soils, reported and evaluated according to various results obtained within the long-term on-farm investigations at Lautenbach (Steiner *et al*. 1986).

METHODS

General considerations

Soil animals are mostly aggregated (Hughes 1962, Joose 1971, Usher 1976, Koehler 1984). Any series of samples taken from any natural population will have a high statistical error. However, the range of error decreases when sample number increases. High accuracy consequently needs a high number of replications, which is, however, time- and cost-consuming (Southwood 1978). More information might be gained by studying the areas with less accuracy.

Many fauna groups are affected by the previous vegetation on the field, organic content, soil tillage and pesticide treatments. Uniformity of the experimental field should be ensured,before initiating comparisons. In addition, plots should be laid out on the same soil type. Thus, soil maps are of great value for such studies. Accordingly replicates of single treatments cannot be "completely" randomized.

Timing of soil sampling should correspond with the activity peaks of the fauna group of interest. This includes considering soil humidity, temperature, diapause and aestivation aspects. Timing has a great influence on the vertical distribution of some soil organisms (Brown 1982, Schmelcher 1983).

Sampling and extraction

Acari and Collembola

Extraction techniques, corer design and sample number have been discussed in many publications (Macfadyen 1961, Dhillon & Gibson 1962, Wallwork 1976, Gottfriedsen 1987). It is not the purpose of this paper to re-evaluate the documented methods. It should be noted that the results reported are obtained using a dynamic extraction technique. A modified Berlese-Tullgren-apparatus (El Titi 1984, 1986) was used. Field sampling was carried out using a "split auger" (Bieri et al. 1978), except in the case of "comparison of sampling devices".

Millipedes

For estimating the population density of Diplopoda three methods were compared, and only one was used later on; that is the "Bait Trap" (Klimm 1984).

Earthworms

Handsorting is known to be the most effective method for extraction of earthworms (Edwards 1972, Peters 1984). However, the method is laborious. When Lumbricus terrestris is dominant, chemical extraction is easier and more efficient. A 0.5% Formalin-solution is very effective in bringing this species to the soil surface.

Diptera

Adoption and modification of extraction techniques used in forestry studies to suit arable soils are still under investigation (Wubbeler, in preparation). It seems that an improved floatation method could be used for arable soils to show differences in larval population of two sites.

RESULTS

Microarthropods

To evaluate optimal size of coring device two augers "Split and Pin" have been compared on the same plot. Soil samples of a total of 2.5 l were taken by both devices from each of three replicates. The results obtained are summarized in the following Table.

TABLE 1

The abundance of microarthropods in arable soil sampled with two different corers at Lautenbach (FRG), 1983

Mean of microarthropods per liter soil		
	'Pin' auger	Split corer (Bieri)
Acari		
Mesostigmata	19	101
Cryptostigmata	105	139
Astigmata	6	16
Other mites	4	14
Collembola		
Onychiuridae	18	80
Isotomidae	1	34
Other springtail	17	21

The split auger produces better extraction results than the pin corer. Abundance and diversity of all extracted microarthropods was greater, when the "split" corer was used.

The corer was used also to study the distribution pattern (Temporal and vertical) of mites and collembola on a 15 x 60 plot over the year. Ten samples were taken monthly in three soil layers (0 - 10 cm, 10 - 20 cm and 20 - 30 cm) and microarthropods were extracted. The results obtained are illustrated for mesostigmatic mites and collembola in Fig. 1.

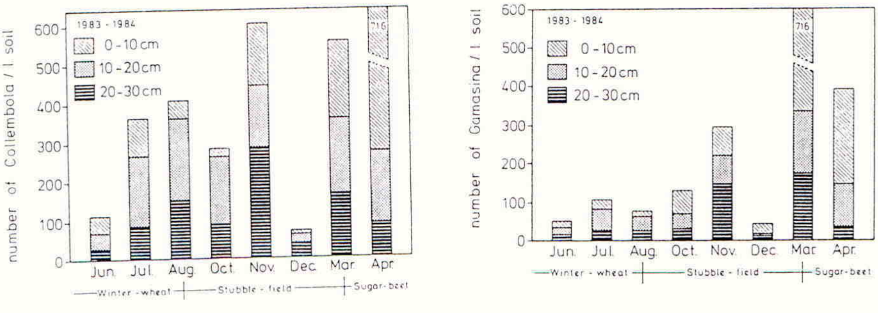

Fig. 1. Seasonal variation in the vertical distribution pattern of Collembola and Gamasina in arable fieldsat Lautenbach over 8 month period.

The density of Gamasids and Collembola showed fluctuation over time in the three soil layers. A decrease in the gamasid density in the topsoil in July/August can be due to shortage of moisture: in winter, hence it can be related to a behavioural response to weathering conditions. Collembola (mainly Onychiouridae and Isotomidae) seem to move into deeper soil layers in autumn/winter.

The "clumped" distribution pattern of mites and collembola in arable soils is "repeatedly" shown also by the data collected at Lautenbach, for example of 1986 (Fig. 2). Similar distribution patterns are recognized on three sampling occasions for two different farming systems.

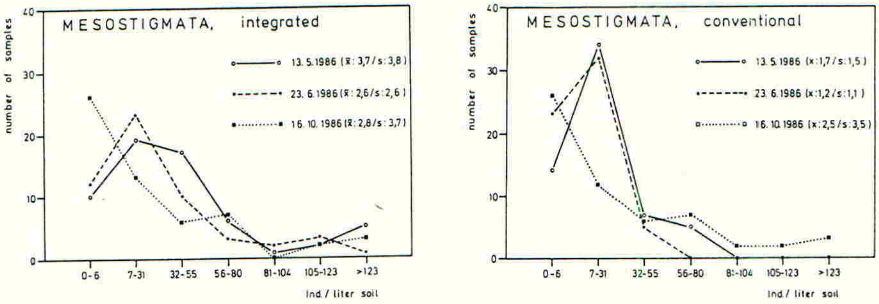

Fig. 2. Range of aggregation of mesostigmatic mites, sampled on 3 different occasions, on six plot-pairs "integrated/conventional" 1986 (n = 60 samples per date and treatment)

Results of comparing Gamasid density, in soil of two farming systems over 8 years time using "plot-pairs" are shown in Fig. 3.

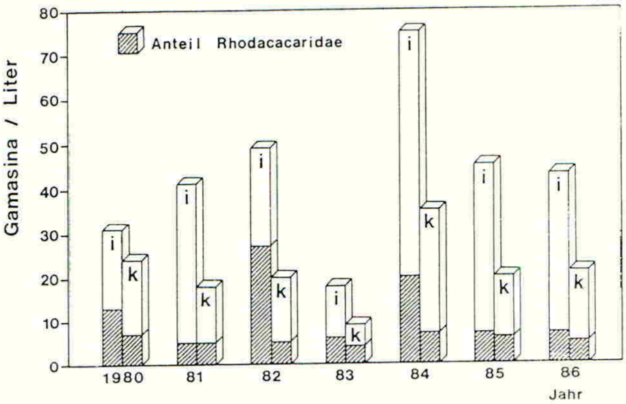

Fig. 3. Average number of Gamasids in "integrated (i)" and "conventional (k)" farming systems at Lautenbach between 1980-1986. (Mean of 60 samples/year, taken on 3 occasions).

Millipedes

Extraction by "handsorting" and sieving produced extremely poor results in all five replicates. Immense labour was needed. This method cannot be recommended. Trials were then devised to compare two non-quantitative methods. These are "bait" and "pit-fall" traps.

Bait traps (potato-slices under wood squares) attracted a higher number of millipedes belonging to more families than the pit-fall techniques.

TABLE 2

Comparison of two methods for estimating the abundance of millipedes on the field

Comparison of two methods for estimating the abundance of millipeds on the field

Exposure time: 7 weeks	Bait trap (5 traps/field)		Pitfall technique (10 traps/field)	
	WW	SB	WW	SB
total Diplopoda	16	435	11	286
number of ssp.	4	6	1	5
missed ssp.	-	-	C.londinensis U.foetidus B.superus	B.guttulatus
dominant ssp.	P.denticulatus U.foetidus	P.denticulatus P.germanicus	P.denticulatus	P.denticulatus U.foetidus

Earthworms

In contrast to millipedes, handsorting, possibly combined by floatation is considered to be the most effective method to extract earthworms. However, the technique is rather laborious and ineffective in extracting L. terrestris.

At Lautenbach, L. terrestris is the dominating species. In this particular case the formalin method was more effective to study earthworm populations. The results are illustrated in Table 3.

TABLE 3

Average weight of Lumbricidae (Formalin-Extraction on 4 fields) in "integrated" and "conventional" farming at Lautenbach

Year		1980	81	82	83	84	85	86	87	$\bar{x}$
Biomass (g/m^2)	i:	15,6	29,2	43,7	31,8	15,9	18,3	20,5	24,3	24,9***
	k:	7,9	6,4	9,1	6,5	7,0	6,4	3,2	4,2	6,3
Density ($no./m^2$)	i:	(-)	24,5	42,3	20,8	15,8	34,5	37,8	27,5	29,0***
	k:	(-)	16,0	11,3	5,8	2,5	10,0	8,8	6,3	8,7

i: integrated k: conventional LSD: Biomass = 0,41/ Density = 0,65 (*** P= 0,01)

Both numbers and biomass indicated highly significant effects of farming systems on earthworms.

Diptera

A soil corer (5 - 8 cm diameter, 30 cm long) was used in these studies (Wubbeler, personal communication). Soil columns were subdivided in three layers of 10 cm each and examined separately. The preliminary results showed differences in the larval abundance of two sites and changes in the vertical distribution. Most of the larvae belonged to the families Ceccidomyiidae, Sciaridae, Chironimidae, Rhagionidae, Empedidae, Dolichopodidae, Petauristidae, Lonchopteridae and Muscomorpha. In spring and summer most of the larvae were extracted from the top soil (0 - 10 cm); late in autumn, mainly after ploughing, however, in the 20 - 30 cm deep layer. Gallmidge-larvae respond most clearly to farming system.

DISCUSSION AND CONCLUSION

Some methods of studying soil invertebrates are more efficient than others. No single method emerges as a complete answer to the question of population assessment of soil organisms. Hence, any method will be more or less, a compromise between labour demand, accuracy required and organism taxa of interest.

On-farm studies require some special precautions. Design and measurement of plots should fit into the husbandry operations on the fields. These start in adjusting plot size to drill and sprayers width, tramlines, etc.

When effects of pesticides on entire groups of species, are to be studied, it is essential to determine the number of sampling units required. According to Hairston & Byers (1954) this will be that number, above which no significant increase in the species diversity is achieved. However, the clumped distribution of soil microarthropods, causing big variances might be "flattened up" to some extent by uniting a number of subsamples to a sample unit on one hand and excluding extreme deviations in soil properties on the other hand. The close "pairing" of treatments and controls can be a helpful compromise.

Similar soil physical properties and organic matter contents distinguish a particular habitat, which in turn has comparable effects on soil inhabitants and their coenoses (Wallwork 1975). Topography of the field area can have the same effects. Variations in the altitude within a field can determine the erodability of soil and nutrients, from one section to the other. This results often in considerable differences in diversity of vegetation and organisms.

Studies on pesticide effects should consider the same corer type. As reported here and elsewhere (Brown 1982) more arthropods with greater diversity were obtained by "split auger", compared with the "pin corer".

Mites and Collembola respond to changes in the moisture content of the top soil often by moving down to deeper layers. In the case of Onychiuridae they move in autumn downwards, where they overwinter. Such behavioural responses should be taken into account, when experimentall comparisons are designed. This issue gives timing a rather important value. The most suitable time, will be that, when the majority of soil arthropods are accumulated in the top soil. In this case it is justified to restrict sampling to the top 10 cm layer. Different abundances in different soils can be detected in long-term and short studies, as illustrated in these studies.

For studying the effects of pesticides on earthworms it is essential to know the dominant species in the particular case so the most rational method can be chosen. The results obtained in our studies using the formalin-extraction reflects clearly different population densities due to the two farming systems.

On the other hand no quantitative method can be recommended to estimate the millipede population. Bait traps can provide reasonable data if they are used on barriered field area in which subsoil movements of millipedes are precluded.

Methodologicall improvements are discussed in relation to extraction of dipterous larvae. The improved floatation technique seems to produce good "quantitative data", as illustrated. Changes in the population size as well as in the relative abundance of single groups (= families) can be detected by this sampling and extraction technique.

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REFERENCES

Bieri, M.; Delucchi, U.; Lienhard, C. (1978) Beschreibung von zwei Sonden zur standartisierten Entnahme von Bodenproben fur Untersuchungen an Mikroarthropoden. Mitteilungen der Schweizerischen Entomblogischen Gesellschaft 51, 327-330.

Brown, R.A. (1982) The ecology of soil-inhabiting pests of sugar-beet, with special references to Onychiurus armatus. Ph.D. Thesis, University of Newcastle-upon-Tyne.

Dhillon, B.S.; Gibson, N.H.E. (1962) A study of the Acarina and Collembola of agricultural soils. 1. Numbers and distribution in undisturbed grassland. Pedobiologia 1, 189-209.

Edwards, L.A.; Lofty, J.R. (1977) Biology of earthworms. Chapman and Hall, London, 333 pp.

Edwards, C.A. (1984) Changes in agricultural practices and their impact on soil organisms. Proceedings of ITE Symposium Agriculture and the environment, Monks Wood Experimental Station, 28-29 Feb.

El Titi, A. (1984) Auswirkungen der Bodenbearbeitungsart auf die edaphischen Raubmilben (Mesostigmata: Acarina). Pedobiologia 27, 79-88.

El Titi, A. (1986) Einfluss von Bodeninsektiziden und organischer Dungung auf Vertreter der Raubmilbenfamilie Rhodacaridae (Mesostigmata: Acarina). Zeitschrift fur Pflankenkrankheiten und Pflanzenschut 93, 503-508.

Foissner, W. (1987) The micro-edaphon in ecofarmed and conventionally farmed dryland cornfields near Vienna (Austria). Biology and Fertility of Soils 3, 45-49.

Gottfriedsen, R. (1987) Die edaphische Milben-Fauna in unterschiedlich bewirtschafteter Feldflur im Raum Heilbronn (Baden-Wurttemberg). Diplomarbeit der Universitat Tubingen, Fakultat fur Biologie.

Hairston, N.G.; Byers, G.W. (1954) The soil arthropods of a field in southern Michigan: a study in community ecology. *Contributions from the Laboratory of Vertebrate Biology of the University of Michigan* (cited in Brown 1982) *64*, 1-37.

Hughes, R.D. (1962) The study of aggregated population. In: *Progress in Soil Zoology*, Murphy, P.W. (Ed.), Butterworth, London, 51-55.

Joose, E.N.G. (1971) Ecological aspects of aggregation in Collembola. *Revue d'ecologie et de biologie du sol* *8*, 91-97.

Klimm, B. (1985) Analyse des Diplodenbestandes in der Feldflur von Lautenbach. Untersuchungen in Rahmen eines Projektes zum integrierten Pflanzenschutz. *Diplomarbeit der Universitat Tubingen, Fakultat fur Biologie*.

Koehler, H. (1984) Methodische, Okologische und Experimentelle Untersuchungen zur Sukzession der Mesofauna der Abdeckschicht einer Bauschuttdeponie unter besonderer Berucksichtigung der Gamsina (Acari, Parasitiformes). *Dissertation, Universitat Bremen*.

Macfadyen, A. (1962) Soil arthropods sampling. *Advances in Ecological Research* *1*, 1-34.

Matt, M. (1986) Die edaphische Collembolen-Fauna in unterschiedlich bewirtschafteter Feldflur in Raum Heilbronn (Baden-Wurttemberg). *Diplomarbeit der Universitat Tubingen, Fakultat fur Biologie*.

Paul, W.D. (1986) Vergleich der epigaischen Bodenfauna bei wendender bzw. nichtwendender Grundbodenbearbeitung. *Mitteilungen der BBA*, H.232, 290.

Peters, D. (1984) Faunatische und okologische Untersuchung der Lumbriciden, Diplopoden und Chilopoden auf verschieden bewirtschafteten Flachen der niederrheinischen Tiefebene. *Dissertation Universitat Bonn*.

Schmelcher, M. (1983) Untersuchungen zur Milbenfauna verschiedener Weinbergboden im Oberen Rheingau unter besonderer Berucksichtigung der Raubmilbe l(Acarina, Gamasina). *Diplomarbeit der Universitat Bonn*.

Southwood, T.R.E. (1978) Ecological methods with particular reference to the study of insect populations. Chapman and Hall, London, 524 pp.

Steiner, H.; El Titi, A.; Bosch, J. (1986) Integrierter Pflanzenschutz im Ackerbau. Das Lautenbach-Projekt I. Versuchsprogramm. *Zeitschrift fur Pflankenkrankheiten und Pflanzenschut* *93* (1), 1-18.

Usher, M.B. (1976) Aggregation responses of soil arthropods in relation to the soil environment. *Proceedings 17th Symposium British Ecological Society*, Anderson and MacFadyn (Eds), 61-94.

Wallwork, J.A. (1976) The distribution and diversity of soil fauna. Academic Press, London, New York, San Francisco, 355 pp.

Wubbeler, H. (in preparation) Terrestrial Diptera under integrated and conventional farming systems in Lautenbach.

A METHOD TO STUDY THE EFFECTS OF CHLORPYRIFOS ON PREDATORY GROUND BEETLES IN GRASSLAND

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ABSTRACT

The use of insecticides on grassland is likely to increase and there is a need to assess the effects these compounds have on non-target fauna, in the field. The present field-scale work assessed the effects on predatory ground beetles (Carabidae) of the chemical used most commonly for the control of leatherjackets chlorpyrifos (Dursban 4). Chlorpyrifos was applied to three fields in March 1986. The number and identity of ground beetles present in these and three untreated fields was monitored using pitfall traps until November of the same year and from June through September 1987. There was no immediate effect of chlorpyrifos on the number of beetles, but their numbers did not increase as rapidly in sprayed fields as on the untreated fields during May and June 1986. By four months after spraying and on all subsequent sampling occasions there was no overall significant difference in their numbers on sprayed v. untreated fields. The effect of chlorpyrifos on carabid populations in the field was therefore concluded to be short-lived and transient.

INTRODUCTION

Grassland is by far the most important crop in the UK. It covers over 65% of the enclosed agricultural area and in addition there are some 5 million ha of open, hill land. The total value of milk, meat and other livestock products exceeds that of all other crops put together.

In comparison with most other crops grassland receives little insecticide - less than 5% of that applied to cereals despite the much smaller area of the latter (Wilkins, 1985). However, farmers now have an increased awareness of pest problems in grass and there is a greater likelihood of insecticides being used. Further, methods of compensating for losses caused by pests, e.g. applying more N fertilizer, feeding more concentrates are becoming ever more expensive. Consequently the alternative of reducing pest losses by pesticide application is more relevant than previously. In time, methods of controlling our major grassland pests which will rely less or not at all on the use of chemicals are likely to be developed (Clements, 1985). But in the meanwhile approved chemicals such as chlorpyrifos will continue to be used and their use will probably increase as farmers' perception of the benefits of using them increases.

In two field scale experiments frequent and careful observation showed that chlorpyrifos applied to grass for the control of leatherjackets had no detectable untoward effect on the birds and mammals at least in the short term. Nor were there any apparent effects on earthworms (Clements & Bale; in press). This is not surprising as the toxicity of the chemical to poikilotherms and oligochaetes is relatively low (Worthing & Walker, 1983). However, it is feasible that populations of certain other groups of non-target fauna may have been affected. Important among these are predatory ground beetles (Carabidae). The present work was done to assess effects of chlorpyrifos on them, in the field, since laboratory or micro-plot work probably does not convey a realistic picture of the effects of a pesticide on such a mobile group of insects.

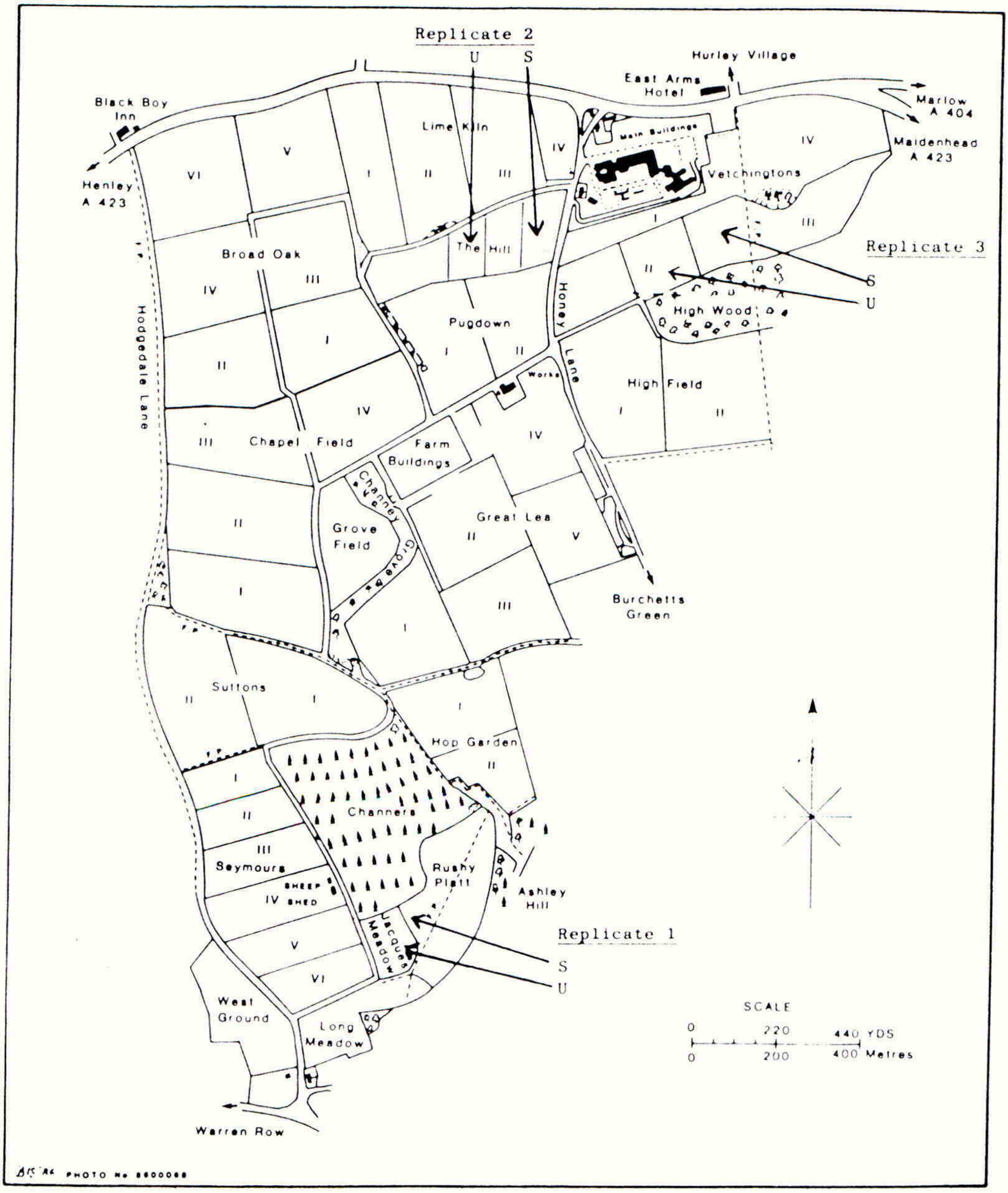
MATERIALS AND METHODS

Three replicate pairs of fields of long-established, ryegrass-dominant swards were selected on the IGAP farm, Hurley. One field in each pair was sprayed with chlorpyrifos (Dursban 4) at the recommended rate of 0.72 kg ai/ha in 300 litres water/ha on 26 March 1986. All fields were rotationally grazed, but were not necessarily grazed on the same days. None of the fields were closed-up for silage or defoliated by machine on any occasion. All fields received N fertilizer in the range 100 to 250 kg/ha/year. The fields in each pair were separated by a physical boundary e.g hedgerow and bank or by a distance of approximately 200m which was intended to greatly reduce or even eliminate the invasion of beetles from one field in each pair to the other. Replicate pairs of fields were separated by a major physical barrier (public road) or large distance (1.5 km, Fig. 1).

The carabid beetle population was sampled in each field using pitfall traps. These consisted of a polypropylene beaker (73mm internal diameter x 108mm deep, PPC No 16 beaker) inserted into a 115mm length of 75mm internal diameter plastic drain pipe, sunk into the ground so that the top of the beaker was slightly (c. 5mm) below the surrounding soil level.

Twelve traps were placed in each field, in four lines of three. The beaker inserts were replaced with a fresh one every week from 26 March - 19 May, 1986. From 20 May to 3 November, 1986 and 15 June to 7 September 1987, 8 such traps, in four lines of two, were placed in each field and the beaker inserts replaced with a fresh one every two weeks. Each beaker was filled to a depth of approximately 50mm with a mixture comprising 10% formalin (40% formaldehyde w/v solution) + 5% glycerol and 85% water. Adult carabid beetles caught were identified to species level and counted.

Fig. 1. Map of IGAP farm, Hurley, showing location of sites used (S - sprayed; U - unsprayed).



RESULTS

On average, over all three replicates, spraying with chlorpyrifos stemmed an increase in the number of carabid adults caught between 20 and 60 days after spraying (Fig. 2). Subsequently the differences were smaller and not significant.

There was considerable variation between replicates in the number and species of beetles caught. For example in the period 120-160 days after spraying there were more carabid beetles in replicates 2 and 3 than replicate 1 (Fig. 3). The species composition of replicate 1 was also different to that of replicates 2 and 3. For example, *Pterostichus cupreus* and *Bembidion lampros* were the most common species in replicate 1, but *Pterostichus madidus* and *Calathus fuscipes* were the commonest in replicates 2 and 3 (Table 1).

TABLE 1

Species composition of carabid beetle adults caught in three insecticide treated and three untreated grass fields.

Species	Proportion (%) of each species caught					
	Replicate 1		Replicate 2		Replicate 3	
	S	U	S	U	S	U
Pterostichus madidus	1.5	1.9	18.9	40.9	17.7	19.2
" cupreus	57.2	42.8	0	0	0	0
" melanarius	20.9	13.8	22.6	28.5	5.4	8.8
Bembidion lampros	68.3	17.9	3.3	6.7	0.8	2.9
" biguttatum	60.0	40.0	0	0	0	0
Loricera pilicornis	43.1	22.8	5.0	16.8	10.4	2.0
Calathus fuscipes	0.2	0	26.2	17.8	35.9	20.0
All others	33.7	17.6	6.0	11.6	5.6	25.5

S - sprayed; U - unsprayed.

DISCUSSION AND CONCLUSIONS

The use of insecticides in grassland is an emotive subject since the crop is regarded by many as one that should not be tampered with because it is considered to be natural or semi-natural. This may be partially true for some less intensively managed areas, but these are unlikely to be treated with insecticide - for simple economic reasons. The areas most likely to be treated are the more intensively managed ones which are already manipulated by man to a large extent, through fertiliser application, reseeding and controlled management. The proportion of these likely to be treated with insecticide is a matter of conjecture, and

Fig. 2 Overall mean number of Carabid adults caught per trap in fields sprayed with chlorpyrifos and unsprayed fields

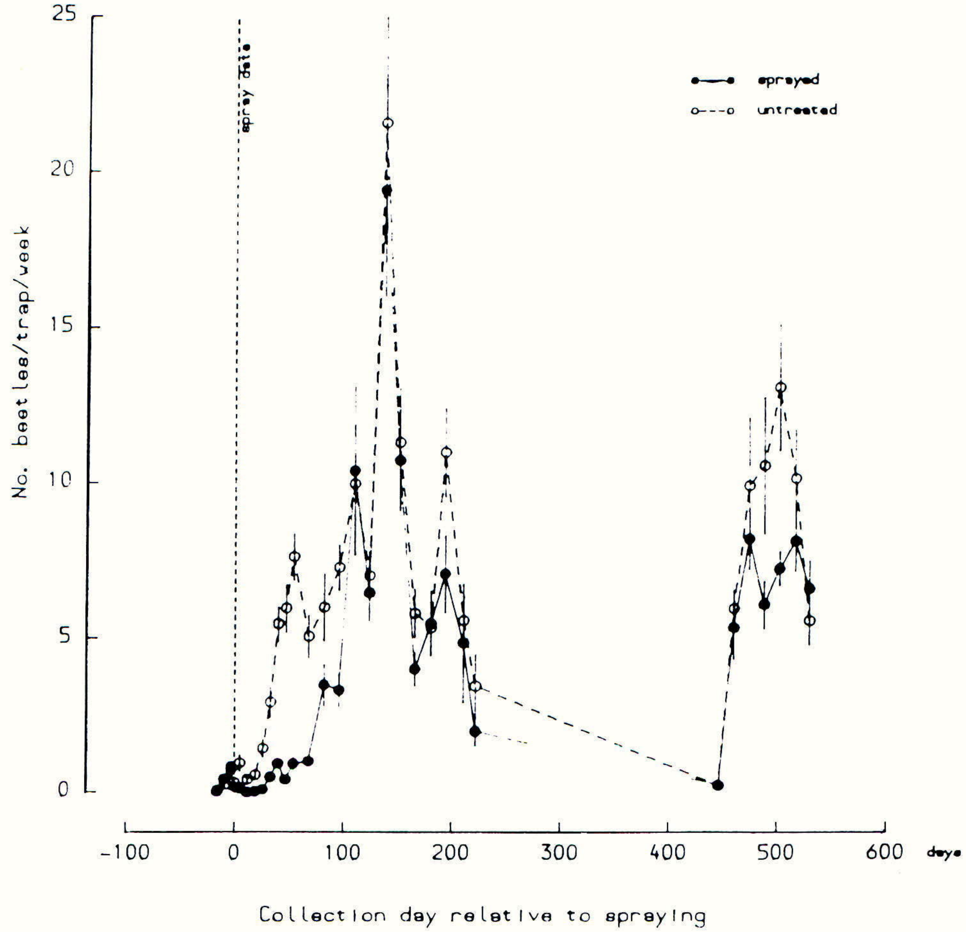
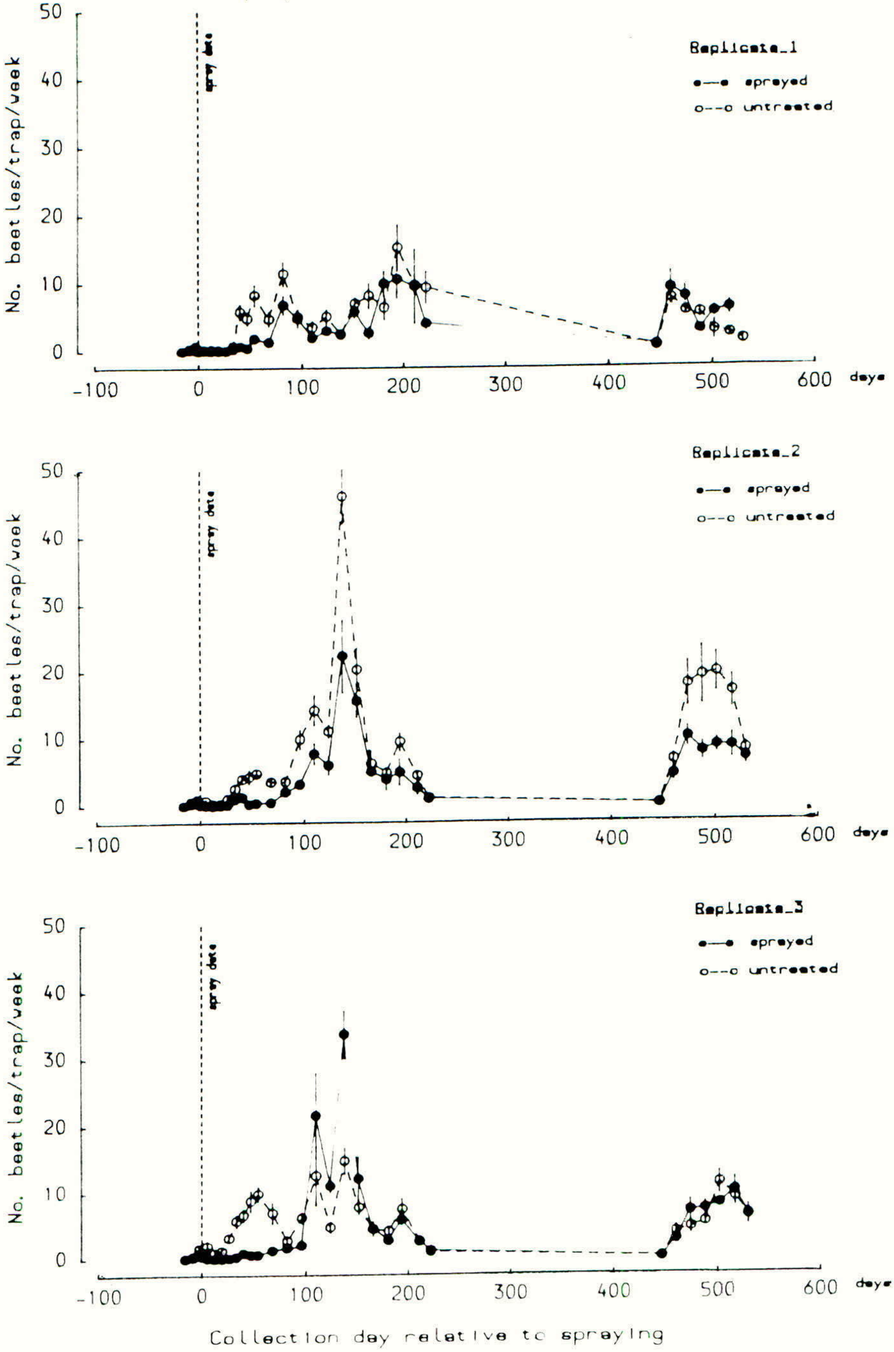


Fig.3 Mean number of Carabid adults caught weekly per trap in fields sprayed with chlorpyrifos and unsprayed fields



although likely to increase, in the view of a recent MAFF panel of experts is unlikely to exceed 500,000 ha/year (unpublished report). Consequently the use of insecticides in grassland will probably be well below the level used in arable crops. Also, since there are now rapid and simple procedures for assessing leatherjacket populations in individual fields, farmers should be able to identify easily those pastures which would benefit from insecticide and treat those only. This contrasts with the situation in many arable crops, where very large, consolidated areas may be treated with pesticide, greatly reducing the opportunity for ground beetles to re-invade. Further, grass fields are usually small, seldom exceeding 5 ha, and are therefore probably more easily re-colonised than the large fields associated with, say, cereals.

Pitfall traps as used in the current work are simple and easy to use. But they are regarded by some e.g. Briggs (1961) as being of little value for the direct estimation of absolute populations. However, in the present work it was more important to be able to compare populations in fields with similar vegetation and management on one farm and for this purpose pitfall traps seem adequate.

In this experiment the effects on carabids were small and transient. The carabid population was unaffected at first, but did not increase as rapidly on sprayed compared with untreated fields during the period 6 - 20 weeks following spraying. That the pesticide application had a delayed impact on the number of beetles caught is curious. Possibly it was because the chlorpyrifos application had no significant direct effect on the beetles but killed some of their prey and it was some time before the effects of starvation were manifest.

The subsequent recovery of the population is also worthy of comment. The majority of carabids, and certainly those making-up the bulk of the populations sampled, have only one generation per year. Consequently it is unlikely that their numbers recovered as a result of reproduction. Since carabids are so active it is more likely that their numbers recovered as a result of invasion from surrounding fields. This, also reduces (or masks) the long-term effect on carabids of the use of chlorpyrifos.

Data from large-scale replicated field experiments to assess the side effects of pesticides can be difficult to interpret (Sotherton, Moreby & Langley, 1987). Variations between replicates can be great. Practically, it is almost impossible to find a large enough uniform area that is ideal for this kind of work. In grassland there is scope for considerable variation between fields, because of the infite number of combinations of management variables possible. In practice, probably no two fields would be exactly comparable in terms of aspect, soil type, botanical composition, age of sward, frequency and timing of defoliation, class of animal used, and fertilizer regime. Laboratory tests may indicate the relative innate toxications of pesticides, but they do not always predict their impact in field situations (Edwards & Thompson, 1975). A useful preliminary method to assess the impact of pesticides on mobile surface-dwelling insects such as carabids in the field is to erect barriers e.g. vertical polythene sheeting around large plots. This prevents the escape of carabids from the plots, or supplementation of their population by invasion. However, it is a false situation, since in practice there is probably considerable egress and ingress of carabids from and into fields, which needs to be taken into account, as in the present work. Despite the difficulties in interpreting results using this method it does seem that chlorpyrifos has little effect in the long-term on carabids in grassland. There was no evidence in this work of a large reduction in their number initially. Their slow build-up was probably augmented by invasion from adjacent areas. Provided that very large areas, comprising several farms, are not sprayed simultaneously there would appear to be little long-term risk.

Further, since there were large differences between untreated replicates in the number of beetles caught it would appear that normal farm management practices and field location have a greater effect on carabids than chlorpyrifos, although this requires further work to quantify the relative importance of these effects.

ACKNOWLEDGEMENTS

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REFERENCES

Briggs, J.B. (1961) A comparison of pitfall trapping and soil sampling in assessing populations of two species of ground beetles (Col. Carabidae) Report East Malling Research Station 1960, 108-12.

Clements, R.O.; Bale, J.S. (in press) The short-term effects on birds and mammals of the use of chlorpyrifos to control leatherjackets in grassland. Annals of Applied Biology 112.

Clements, R.O.; Bentley, B.R. (1985) Pest damage to establishing grassland and the feasibility of control. In: Weeds, Pests and Diseases of Grassland and Herbage Legumes J. Brockman (Ed). British Grassland Society Occasional Symposium No. 18, 165-172.

Edwards, R.A.; Thompson, A. (1975) Some effects of insecticides on predatory beetles. Annals of Applied Biology 80, 132-135.

Sotherton, N.W.; Moreby, S.J.; Langley, M.G. (1987) The effects of the foliar fungicide pyrazophos on beneficial arthropods in barley fields. Annals of Applied Biology, 111, 75-87.

Wilkins, R.J. (1985) Concluding Remarks. In: Weeds, Pests and Diseases of Grassland and Herbage Legumes J. Brockman (Ed). British Grassland Society Occasional Symposium No. 18, 262-266.

THE USE OF MULTIVARIATE ORDINATION TECHNIQUES TO ASSESS THE EFFECTS OF CHLORPYRIFOS ON GROUND BEETLE AND SPIDER COMMUNITIES IN GRASSLAND

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ABSTRACT

Spider and ground beetle community data from sites subjected to different regimes of chlorpyrifos application were ordinated using Principal Co-ordinates Analysis to determine the extent to which pesticide was a factor affecting the communities present. The potential use of these techniques in assessing the effects of pesticides on non-target invertebrate communities is investigated.

INTRODUCTION

Whilst the effects of pesticides on their target organisms are easy to demonstrate, predicting their effects on surrounding non-target groups is usually much more difficult, where there are often many other species besides the pest to consider. This is especially the case with invertebrates, where even in comparatively simple agro-ecosystems the non-target species may occupy several trophic levels and the number of taxa may outnumber the pests by 2 or 3 orders of magnitude. Whilst it is theoretically possible to evaluate the toxicological effects of pesticides on all of these non-target species individually, these studies are of limited value in predicting their effects at the ecosystem level, where there are many other factors which may override or interact with pesticide usage in influencing community structure. In these circumstances techniques of summarisation are required which can be used to describe whole communities in simple terms. One approach that has proved valuable in investigating the structure and functioning of complex invertebrate faunas has been the use of multivariate ordination techniques (Eyre et al. 1987, Rushton et al. 1987, Luff et al. 1988). Ordination techniques can be used to summarise differences in the species composition of such communities, representing the differences on axes which are easier to comprehend than the original data matrix. A major advantage of such summarisation is that it is often possible to relate each of the axes, in declining orders of significance from the first, to the major environmental variables determining the differences in species composition. It would be true to say that this has been the aim of most applications of ordination techniques to biological data of this type. Whilst ordination has been used extensively in this way in the past (see review in Gauch 1982) there have been few applications of these techniques to the effects of pesticides on communities of non-target organisms. The aim of this paper is to evaluate the extent to which one technique, Principal Co-ordinates Analysis (Gower 1966) can be used to investigate the effects of pesticide usage on non-target invertebrates under normal agricultural conditions. Spider and ground beetle community data from improved upland pasture systems subjected to periodic spraying with Chlorpyrifos are analysed using these techniques and the potential use of this form of analysis in studies of the effects of pesticides on non-target organisms is discussed.

MATERIALS AND METHODS

Field work

The sample area was located on sheep grazed, improved upland pasture at the Ministry of Agriculture Experimental Husbandry Farm at Redesdale, Otterburn, Northumberland (O.S. Grid reference NY 8396). Sample sites were selected from pastures subjected to different frequencies of "Chlorpyrifos" application in a management regime of progressive, rotational pasture renewal. All swards were sown with a mixture of ryegrass (Lolium perenne L.), clover (Trifolium repens L.) and timothy grass (Phleum pratensis L.) but three different methods of preparing the seed bed were utilised. These were, firstly, direct drilling into the pre-existing unimproved pasture; secondly, sward destruction with herbicides, surface rota-tilling to 5 cm, followed by direct drilling and thirdly, sward destruction as above followed by a year of root crops prior to reseeding. The frequency with which Chlorpyrifos was applied to pastures over the years following reseeding was determined by the population density of Tipula larvae present in autumn of each year. Pastures with in excess of 800,000 individuals ha^{-1} were sprayed in the spring after the population estimate was made.

Initially, five sites were sampled in 1986. In 1987 a further eight sites were sampled in order to increase the range of pesticide treatments analysed: of these, four were bi-replicate samples of two of the 1986 sample sites. Data from one of the sites collected by the same technique in 1985 were also included to bring the total of sites sampled to 14. The pesticide treatment and reseeding histories of each of the sites are given in Table 1. Spider and ground beetle communities on each site were sampled using pitfall trapping. Nine pitfall traps consisting of polypropylene pots of 8.5 cm diameter, part filled with ethylene glycol were used on each site. The traps were left for one month after which they were emptied and refilled with fresh ethylene glycol solution. The contents of all nine traps were pooled each month and stored in 70% alcohol prior to sorting and identification. Each site was sampled five times from May to November in any one year.

Data analysis

Ground beetle and spider species lists were produced for each of the 14 sites. The presence or absence of individual species were then used as site attribute data to create a between site similarity matrix and subsequently, a between site distance matrix for each group, following the method of Williamson (1978).

This was undertaken by multiplying the matrix of presence/absence data, where rows were sites and species were columns, by its transpose (i.e. rows became species and columns became sites) to produce a site-by-site similarity matrix. Entries in this matrix represented the number of species common to each between site comparison. These

TABLE 1
Details of pesticide treatment and pasture improvement history of smaple sites. + = sprayed with Chlorpyrifos, - = untreated

Site	Year reseeded	Method	Year treated with Chlorpyrifos									Year sampled
			79	80	81	82	83	84	85	86	87	
1	1976	Root crop	+	-	-	-	-	-	+			1985
2	1976		+	-	-	-	-	-	+	+		1986
3	1976	followed	+	-	-	-	-	-	+	+	-	1987
4	1976		+	-	-	-	-	-	+	+	-	1987
5	1978	by	+	-	-	-	-	-	-	-		1986
6	1978		+	-	-	-	-	-	-	-	-	1987
7	1978	direct	+	-	-	-	-	-	-	-	-	1987
8	1980			+	-	-	+	-	-	+		1986
9	1981	drilling			+	-	+	-	+	+		1986
10	1983						+	-	+	+		1986
11	1982	Direct drll					-	-	-	+	-	1987
12	1982	into sward					-	-	-	+	-	1987
13	1980	Rota-tillered			-	-	-	-	-	-	-	1987
14	1980	+d. drill			-	-	-	-	-	-	-	1987

TABLE 2
Total number of species and animals caught from the sample sites at Redesdale E.H.F.

Site number	Spiders		Ground Beetles	
	Species	Individuals	Species	Individuals
1	11	101	11	23
2	12	199	9	24
3	25	603	12	71
4	14	416	9	48
5	15	428	9	42
6	15	857	10	171
7	18	946	11	129
8	15	268	13	47
9	22	425	12	41
10	20	221	6	39
12	20	441	15	96
13	15	238	9	118
14	13	246	9	100
Totals	47	5389	35	949

between site similarities were then converted to measures of distance in space using the first Minkowski metric where:

Distance between S1 and S2 = Number of species in S1 + Number in S2
-2 x Number common to both

This between sites distance matrix was then ordinated with Principal Co-ordinates Analysis (P.Co.A.) (Gower 1966) so that the sites could be "mapped" in two dimensions. This analysis proceeds by undertaking an Eigenanalysis (spectral decomposition) of a modified form of the distance matrix (the details of the modifications are not necessary to understand the principle so they are not explained further here). The Eigenanalysis effectively identifies the major axes of variation through, in this instance, the original 14 dimensions of the distance matrix and produces scores for each site on axes of declining significance from the first. In order to assess the extent to which pesticide use was a major factor determining the between site distances, and hence species composition of the sites, the site scores for each axis of the ordination were then related to their known pesticide treatment histories.

RESULTS

Total numbers of individuals and species of each group caught over the course of the study are shown in Table 2. There was no clear relationship between the pesticide treatment history of the sites and either species number or the total number of individuals caught of either group; but the complexity of the data does serve to demonstrate the need for a multivariate approach in their analysis.

Plots of the sites scores for the first two axes of the Principal Co-ordinates Analysis of the spider and ground beetle incidence data are shown in Figure 1A and 1B respectively. Numbers alongside each point indicate the site number as in Table 1. Considering the spiders first (Fig. 1A), all of the sites sampled in 1987 (numbers 3,4,6,7, 11,12,13 and 14) had axis-1 scores less than zero, with the direct drilled 1982 reseeds having the lowest scores of all. None of these sites had received pesticide treatment for at least a year prior to sampling and two (numbers 13 and 14) had received none at all. The site with the highest axis-1 score was number 9, which had been treated more times than any of the other pastures sampled. Whilst it was not possible to relate axis scores directly to the time since last treatment with Chlorpyrifos because sites 13 and 14 had never received treatment at all, site axis-1 scores were significantly correlated with the percentage of years since reseeding that each site had been treated ($r = 0.6757$ $p<0.05$). The second axis of the ordination did not appear to be related to any of the measured management characteristics. The results of the ground beetle ordination (Fig. 1B) are essentially similar to those of the spider ordination except that the second axis rather than the first appeared to be related to the frequency with which sites were treated with Chlorpyrifos. As with the first axis for the spider ordination, the sites at the extreme ends of this axis were those which had not received treatment for at least a year and the the one (number 9) which had been treated for four out of the six previous years. Site axis-2 scores were significantly correlated with the percentage of years since reseeding that sites had been treated with the pesticide ($r=-0.6018$ $P<0.05$).

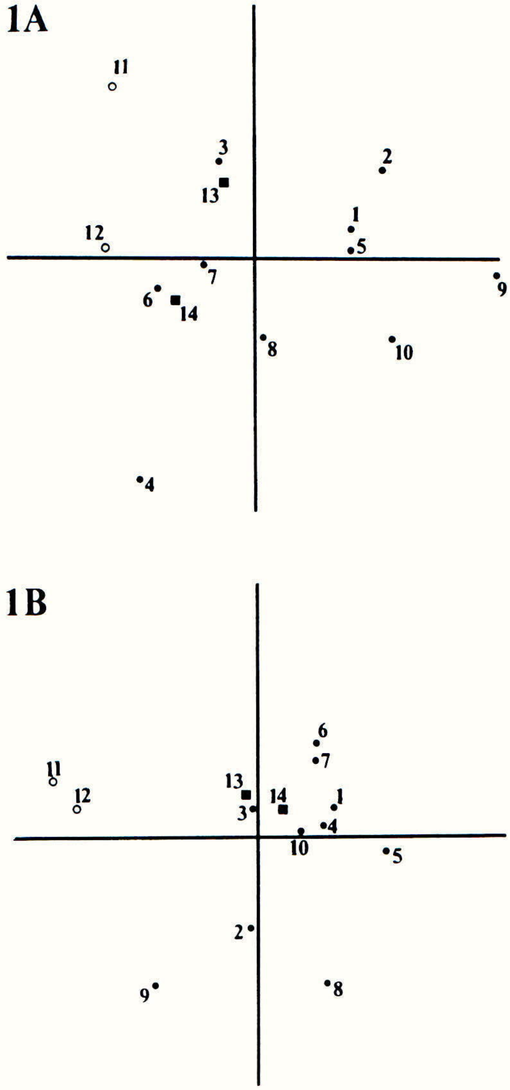

Fig. 1. First two axes of the Principal Co-ordinates Analysis of the presence and absence data of species of spiders (Fig. 1A) and ground beetles (Fig. 1B) from 14 sample sites at the Redesdale E.H.F., Northumberland.
Method of pasture improvement: ● = root crop followed by direct drilling; ○ = direct drill into sward; ■ = rota-tillered and direct drilled.

DISCUSSION

In the case of the spider data the first axis of the Principal Co-ordinates ordination appeared to be related to the frequency of pesticide usage suggesting that this may be an important factor influencing the spider communities on the sites. In the case of the ground beetles it was the second axis that appeared to be related to the frequency of pesticide usage. Whilst the first axis of a Principal Co-ordinates ordination represents the major axis of variation through the data matrix, interpreting the second axis can be more problematical. The mathematics of the analysis dictate that axes subsequent to the first are required to be orthogonal to each other, which whilst ensuring that the axes are uncorrelated, does not guarantee their independence. This problem is most acute when there are large differences in species composition across the range of sites sampled and particularly so when there are some sites which have no species in common. In this instance the scores for the second axis may only be quadratic representation of the first (the "horseshoe effect" of Digby and Kempton 1987). In this study the variation in species composition of the spider and ground beetle communities sampled was not large. It is thus likely that the second axes can be interpreted as having some biological meaning, suggesting that the frequency of Chlorpyrifos use also influenced the ground beetle communities present on the sample sites. The effects of the pesticide on these non-target organisms could not have been demonstrated so readily without the use of multivariate techniques.

These results indicate that Principal Co-ordinates Analysis can be a useful tool for illustrating the effects of pesticide use on whole invertebrate communities but what other ordination techniques are available and to what extent could these be used in studies of this type? The answer to this question depends to a great extent on the type of data collected and the size of the data matrix. In the present study we used pitfall trapping to sample the spider and ground beetle faunas. Whilst Merrett (1983) and Luff (1975) have demonstrated that pitfall trapping may give a reasonable indication of the range of species of spider and ground beetle present even in habitats with quite complex vegetation, there is no way that this sampling can be used to assess the size of populations of individual species. There are several techniques available for the ordination of incidence data derived from sampling of this type. Where the data set contains information on only a small number of sites, all of which have at least some species in common, then the "horseshoe effect" described above is minimised and Principal Co-ordinates Analysis can be used. Where variation across the data set is larger as for instance when some sites may have no species in common at all, Gauch (1982) recommends the use of Detrended Correspondence Analysis (DECORANA, Hill 1979) and Williamson (1978) a modification of Principal Co-ordinates Analysis he called STEPACROSS. Both of these methods attempted to overcome the "Horseshoe effect" ensuring that second and subsequent axes of the ordination are not related to those preceding. The use of Detrended Correspondence Analysis in community ecology has been extensive (see review in Gauch 1982) largely because it is readily available as a computer package. Although the mathematical basis for the evasion of the "horseshoe effect" is more sound in STEPACROSS than in DECORANA this technique is not generally available and consequently has been largely unused.

Where estimates of the population densities of individual species are available, these techniques may also be used to investigate between-site differences in species abundances as well as composition. As with the ordination of incidence data the technique chosen will also depend on the range of species composition in the data matrix (Gauch 1982). The use of all of these techniques in the assessment of the effects of pesticides on non-target organisms awaits thorough investigation. The purpose of this paper will have been served if the potential for using any of these techniques in studies of this type becomes recognised.

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REFERENCES

Eyre, M. D.; Rushton, S. P., Luff, M. L.; Ball, S. G.; Foster, G. N.; Topping, C. J. (1986) The use of invertebrate community data in environmental assessment. Agricultural and Environmental Research Group, The University, Newcastle upon Tyne.

Gauch, H. G. (1982) Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge.

Gower, J. C. (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika, 53, 325-338.

Hill, M. O. (1979) DECORANA- A FORTRAN program for detrended correspondence analysis and reciprocal averaging. Ecology and Systematics, Cornell University, Ithaca, New York.

Luff, M. L. (1975) Some features influencing the efficiency of pitall traps. Oecologia (Berlin), 19, 345-357.

Luff, M. L.; Eyre, M. D.; Rushton, S. P. (1988) Classification and ordination of habitats of ground beetles (Coleoptera: Carabidae) in north-east England. J. Biogeography, (in press).

Merrett, P. (1983) Spiders collected by pitfall trapping and vacuum sampling in four sites of Dorset heathland representing four different growth stages of heather. Bulletin British arachnological Society, 6, 14-22.

Rushton, S. P.; Topping, C. J.; Eyre, M. D. (1987) The habitat preferences of grassland spiders as identified using detrended correspondence analysis, Bulletin British arachnological Society, 7, 165-170.

Williamson, M. (1978) The ordination of incidence data. Journal of Ecology, 66, 911-920.

CRITERIA FOR THE DESIGN, EXECUTION AND ANALYSIS OF TERRESTRIAL NON-TARGET INVERTEBRATE FIELD TESTS

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ABSTRACT

Draft guidelines for the design, execution and analysis of terrestrial non-target invertebrate field tests are developed. These address problems of integrating ecological and statistical considerations in site selection, plot size, replication and layout and indicate important questions that should be asked prior to undertaking such studies. The potential of these guidelines for the development of Good Laboratory Practice standard operating procedures is considered.

INTRODUCTION

A large proportion of studies concerning the environmental effects of pesticides on non-target invertebrates have arisen from fears concerning the spectrum of activity of a pesticide or from laboratory studies that have indicated unacceptable toxicity to this group. In some cases, this concern has focussed upon the potential impact of pesticides on the beneficial capacity of the arthropod community and resultant effects on pest population density or in others, on invertebrate densities and vertebrate survival.

Frequently, conventional field experimental designs have been undertaken without due regard for the taxonomic and ecological diversity of the non-target invertebrate community or variation in its spatial and temporal dynamics (Jepson in press). In particular, the lack of rational scientific criteria for the selection of sites and design of experiments has often resulted in inconclusive results with considerable problems of analysis and interpretation.

This paper outlines a framework for the planning, execution and analysis of terrestrial field studies, highlighting in particular aspects of these tests which may lead to difficulties in interpretation.

PLANNING

There are at least five stages in the planning of terrestrial studies following the decision to undertake field work. This initial decision should be highly dependent upon the nature of the data required and the degree to which the mechanisms of any observed effects need to be resolved. The relative benefits of laboratory based, semi-field and field based approaches are poorly exploited at present (Jepson 1987).

Insect diversity, abundance and the selection of experimental sites

Given the wide temporal and spatial variation in non-target invertebrate distribution, particular difficulties of interpretation may be expected when comparing results for the effects of one compound on more than one site. For example, in two recent studies of the foliar fungicide pyrazophos (Sotherton et al. 1987, Wratten et al. 1988) differences in the abundance and diversity of arthropods in the two sites may explain differences in the number of significant effects found.

This problem may be overcome to a certain extent by focussing on species at risk from within the arthropod community (Jepson 1988) or by pooling invertebrate groups or guilds with common ecological characteristics (Sotherton et al. 1987). General pooling of taxonomic groups should be avoided since this will combine genera or species with different ecological characteristics.

The trade-off between plot size and replication

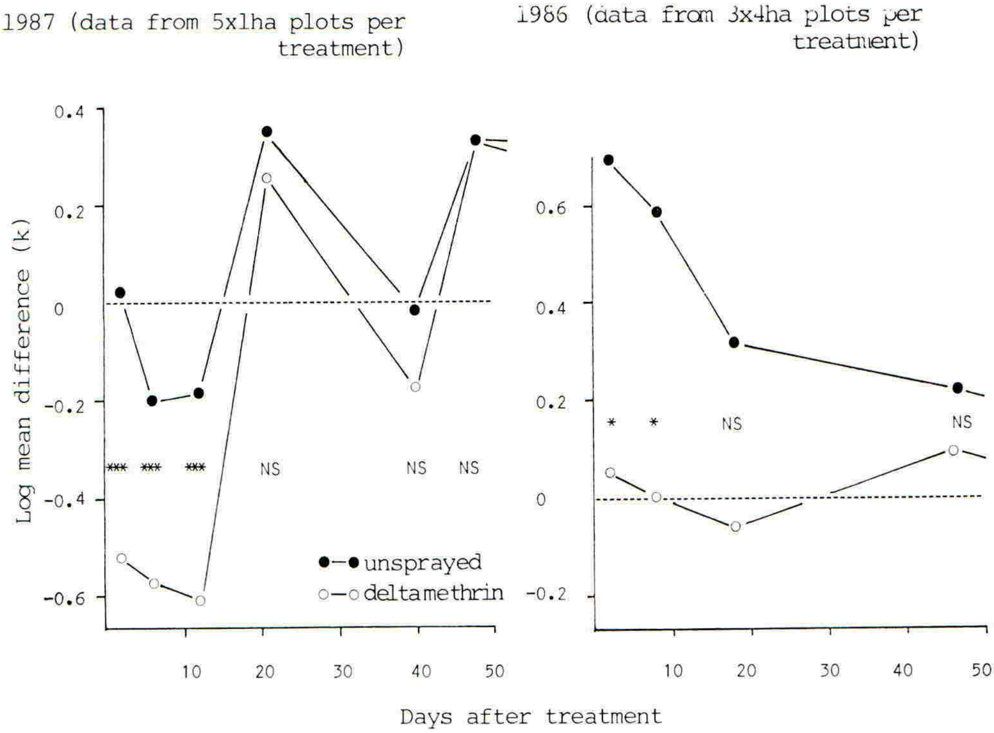
An upper limit on experimental scale will normally be imposed by constraints of land availability and resources. Plot size will however have a profound influence on the relative duration of effects on different arthropod groups because of variation in their rate and timing of dispersal. This factor may explain differences in the duration of effects on epigeal invertebrates found by Sotherton et al. (1987) and Wratten et al. (1988). It is clear that ecological factors should play an important part in determining optimum plot size that can be achieved within any particular experimental design. For example, there is likely to be a lower limit on plot-size beyond which significant effects will no longer be detectable.

The statistical requirement for replication will result in pressure for smaller plot size and there will therefore be a compromise between the need for statistical rigour and ecological factors when determining the scale of an experiment. For example, Pullen (unpublished data) has shown that statistically significant effects on epigeal invertebrates, following the autumn application of synthetic pyrethroids to winter cereals, were more readily detected when there was sufficient replication within the experimental design. There was however a compromise between replication and plot size which resulted in a decline in the duration of these effects in the higher replication study (Fig. 1).

Experimental design and plot layout

Statistical and ecological criteria should be used to establish the most appropriate experimental design and plot layout. Latin square designs are likely to be the most appropriate if, for example, the re-invasion of plots by arthropods is uni-directional. This might be the case if for example, treatment is carried out at a time of year when crop colonisation by invertebrates is taking place from adjacent non-crop, overwintering habitats (Coombes & Sotherton 1986). The use of the within-field latin square design is however, likely to impose a restriction on plot-size if the requirements for replication and comparison treatments are met. Within randomised block designs, individual blocks may be placed in separate fields permitting larger plot sizes. This design may be appropriate if invertebrate re-invasion is omni-directional, as would be the case for highly mobile groups or mid-summer, post-dispersal epigeal species.

Neither of these designs however represents strictly realistic conditions of chemical usage since large unsprayed control areas act as refuges for invertebrates following spray application and reservoirs from which re-invasion may take place once toxic residues have ameliorated.



1987 (data from 5x1ha plots per treatment)

1986 (data from 3x4ha plots per treatment)

Fig. 1. Mean changes in pitfall trap catch (relative to pre-treatment levels) of the carabid beetle Bembidion obtusum on plots of winter cereals sprayed with or deltamethrin or remaining unsprayed, north Hampshire, autumn 1986 & 1987. Levels of significance (* = P<0.05; *** = P<0.001) express changes in the size of the difference of pre- and post-treatment numbers between treatments).

Sampling techniques

Little regard is given at present to the biases imposed by the use of particular sampling methods. The main reason for this is the failure to define the taxonomic groups that may be at risk of long term side effects; it has instead become the tradition to use a selection of techniques to provide general monitoring data for some groups and not others. If general monitoring is genuinely required, then all the important invertebrate guilds should be sampled, employing techniques appropriate to ground and plant active species, diurnal and nocturnal groups and aerial dispersers (Table 1).

Where more refined questions are being asked or species at risk have been defined, some attempt should be made to provide absolute estimates of abundance. In this case, particular attention should be paid to the number of traps and sampling positions necessary and the sampling efficiency of the technique to be employed. Sampling techniques which discriminate between those arthropods surviving the original treatment, those that have emerged within the plot following treatment and those from untreated areas have rarely if ever been considered despite problems of interpretation that may arise if these categories are not separated.

TABLE 1

A comparison of the relative value of the most used invertebrate sampling techniques in studies of pesticide side-effects in cereals. More specialised techniques are available for particular groups such as parasitoids and spiders (these are excluded because of the expertise required). ***, appropriate techniques which can be calibrated; **, appropriate techniques but some doubts over efficiency; * will capture, but ineffecient; - of no value. (Brackets indicate logistical problems such as the need for night sampling).

	Pitfall trapping	D-vac (vacuum insect collector)	Water trap	Plant searching	Ground searching (random quadrat)
Activity related (+) or potentially absolute (o)?	+	o	+	o	o
Published technique for determining sampling efficiency (Y/N)?	Y	Y	N	Y	Y
Relative value for sampling different invertebrate groups					
Ground active					
nocturnal	**	(***)	-	-	*
diurnal	**	***	-	-	**
Plant active					
nocturnal	*	(***)	-	(***)	-
diurnal	*	***	-	***	-
Aerial disperser	-	*	**	-	-

The role of laboratory and semi-field studies

Laboratory and semi-field based studies of pesticide side-effects on non-target invertebrates have an important role to play in discriminating between direct toxicity and indirect effects and in providing quantitative data (Jepson 1987).

For example, Table 2 gives the results of a series of tests designed to determine the toxicity of two pesticides to coccinellids under normal conditions of field use (Langley 1985). The insects were released into cages and exposed to tractor applied sprays for a fixed period.

This technique permitted a rapid (less than 10 days) evaluation of potential side-effects on a highly dispersive species which was quantified in terms of mortality (which would be impossible under normal field conditions). Replication also permitted detailed statistical analyses. This type of experiment could be used cost effectively in many circumstances to support equivocal data from field studies or to supplement the range of insects tested. The collection of treated organisms permits sub-lethal effects on behaviour and fitness to be recorded, as well as lethal effects.

Conventional laboratory testing commonly fails to contribute to field studies because of the difficulty in simulating realistic rates and routes of exposure to pesticides. Further advances in this form of testing are required before its role can be properly evaluated.

TABLE 2

Percentage mortality of *Coccinella septempunctata* to two pesticides and a water spray control applied to spring barley (GS 58; May/June 1986). Insects were confined within cages for 48 hours following spraying then returned to the laboratory for mortality assessments at 48 and 72 hours. Analysis on arc sin transformation of percentage mortality data. Numbers followed by the same letter do not differ significantly at the 5% level.

		Control (water)	pyrazophos 600g a.i. ha^{-1}	dimethoate 340g a.i. ha^{-1}
Trial 1	48 hours	28.0a	37.4a	38.9a
	72 hours	28.0a	75.4b	85.2b
Trial 2	48 hours	4.1a	82.2b	78.1b
	72 hours	8.2a	92.0b	95.3b
Trial 3	48 hours	3.9a	97.5b	96.4b
	72 hours	6.9a	98.9b	98.8b
Trial 4	48 hours	0.9a	96.3b	70.6c
	72 hours	3.5a	98.8b	84.7c
Mean	48 hours	9.2	78.4	71.0
	72 hours	11.7	91.3	91.0

EXECUTION

The most important aim of semi-field and field experimental execution is to simulate the level and route of exposure to pesticides that non-target invertebrates will encounter in the real world with the product under commercial use. The importance of pesticide application on an appropriate spatial scale has been dealt with above. However, within the constraints of a particular plot size it is still necessary to ensure that the correct distribution of pesticide is attained within the crop canopy. This can be achieved by adjusting rate, volume and application parameters such as ground velocity and nozzle selection. It is also important to consider possible interactions between the test chemicals and other chemical inputs that are required to correctly simulate commercial practice.

Another important feature of experimental execution is the rigour of data collection and the degree to which the original protocols are correctly followed. Within the industrial context, and increasingly during registration procedures, these are encouraged by the application of Good Laboratory Practice (GLP). One advantage of GLP control of field procedures is supervision of stages where weaknesses and errors can arise. This includes aspects of experimental planning as well as execution. For example, criteria may be set for determining appropriate plot size and experimental layout and also to establish the efficiency of different sampling techniques. Quality assurance procedures and the achival storage of data then encourage rigorous analysis and a high degree of comparability between studies.

The present protocols proposed as a basic skeleton for terrestrial field effects studies lack sufficient detail in areas such as planning and analysis for submission to GLP monitoring (Jepson in press). They also contain inherent weaknesses since their flexibility can lead to experimental designs which may obscure harmful effects on important invertebrate groups (no criteria for determining appropriate plot size, design layout or statistical analysis).

ANALYSIS

The requirement for rigorous statistical analysis should have an overriding influence on decisions at the planning stage. An established analytical framework is yet to develop, however, for terrestrial field effects studies on non-target invertebrates. This is partly a result of the general failure to identify organisms at risk or to rank key beneficial species and isolate those which are to be subjected to statistical analysis. It is also however a result of the difficulties in establishing clear criteria for experimental design in the light of spatial and temporal variability in non-target invertebrate density. One example of this is the level of variation in insect numbers encountered between plots prior to treatment. This source of variation was excluded by Sotherton et al. 1987 by the use of mean log-differences within treatments for one date prior to pesticide application and several dates following it. The log difference values were then entered into a conventional analysis of variance to detect differences between treatments.

At least three important areas still require the development of statistical testing methods. Firstly the analysis of time series data concerning the recovery and re-invasion of non-target invertebrate communities following treatment. In ecological terms, the duration of a chemical effect is likely to be more important that the initial level of that effect. It is therefore unfortunate that the spatial scale and duration of most studies do not permit this phenomenon to be recorded in detail. We consider the development of field experimental and analytical methods in this area to be an urgent priority. Secondly, similar arguments apply to tests which examine indirect effects of pesticide application on non-target invertebrates such as resurgence of insect pest populations or experimental methods which measure the rate of consumption of artificial prey. In general, indirect phenomena such as resurgence may have so many contributory causes as to render them inappropriate for integration within this type of study. Finally, integrated experimental and analytical procedures are required to overcome the problem of detecting small but significant changes in non-target invertebrate populations that exhibit wide natural fluctuations in population density.

INTERPRETATION

There are still considerable problems with the interpretation of terrestrial field studies. These particularly concern the relative importance of the level of the initial effects and the duration of recovery and recolonisation by arthropods with differing life histories (Jepson 1988). Considerable advances may result from the implementation of more rational procedures for selecting species for investigation and determining plot size and layout.

Conclusions concerning the relative importance of direct and indirect effects are still being made using inappropriate field-derived data. Semi-field techniques may provide cost-effective means by which the necessary supplementary data may be obtained to isolate mortality effects from prey depletion and repellency.

Hazard criteria cannot be established until a more rational approach is taken to these studies, especially with regard to establishing appropriate time scales for observations and the integration of data concerning the duration of effects. General questions concerning indicators of environmental quality should be addressed as well as those which apply to effects on economically important beneficial insects. At present it is possible to design experiments which conform to general protocols but which obscure potentially important long term side-effects. The following section gives draft guidelines for the design, execution and analysis of terrestrial field effects studies of non-target invertebrates within arable crops.

GUIDELINES FOR THE PLANNING EXECUTION AND ANALYSIS OF TERRESTRIAL SIDE-EFFECTS STUDIES ON NON-TARGET INVERTEBRATES

Planning

Site selection

1. Are species at risk known/are they present?
2. Have the effects of field boundary type, soil type etc., on habitat quality been taken into consideration?
3. Does the range of insect diversity and abundance fall within the range necessary to adequately monitor effects on general invertebrate populations.

Plot size and replication

1. Have ecological considerations such as the dispersal rate of important species been taken into account in determining plot size?
2. Have the requirements of the statistical analysis been considered in determining the level of replication?
3. Has the relative importance of initial effect and duration of effect been established in determining plot size and replication?
4. If plot size is restricted, have barriers or cage techniques been considered?

Experimental design and plot layout

1. Has the possibility of untreated areas acting as reservoirs for the re-invasion of treated plots been considered in designing the experiment?
2. Do you know the source of any re-invasion? Does the experimental design take this into account?

Sampling techniques

1. Are sampling techniques available for the groups or species at risk? Has their efficiency been established and can absolute population estimates be obtained?
2. If species at risk have not been identified, have the requirements for general population sampling been met?
3. Has the spatial and temporal heterogeneity of invertebrate abundance been taken into account in establishing the number of sampling points and frequency of sampling?

Laboratory and semi-field tests

1. Are controlled dose or exposure studies required to distinguish between direct mortality and indirect effects such as prey depletion or repellency?
2. Can the rates, routes and condition of field exposure be simulated in laboratory and semi-field conditions?

Execution

Operational factors

1. Does spray application simulate the mode of expected use realistically? Have factors such as chemical rate, application volume, ground speed and nozzle selection been taken into account?
2. Can the distribution profile of pesticide through the crop canopy be measured and compared to standard data?
3. Could other chemical inputs also affect the species being investigated? If so can they be replaced?

Crop factors

1. Does the crop conform to standards of husbandry appropriate to commercial practice and provide an equivalent habitat for non-target invertebrates?

Good Laboratory Practice

1. Have standard operative procedures for each stage of the planning, execution and analysis been prepared?
2. Have monitoring procedures for site selection, sprayer calibration, sampling data analysis been prepared?

Analysis and interpretation

1. Can pre-treatment variability in numbers of insects per plot be excluded from the final analysis?
2. Has the problem of detecting small effects on species with large temporal and spatial density fluctuations been considered?

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REFERENCES

Coombes, D.S.; Sotherton, N.W. (1986) The dispersal and distribution of polyphagous predatory Coleoptera in cereals. Annals of Applied Biology 108, 461-474.

Jepson, P.C. (1987) An experimental rationale for the quantitative evaluation of pesticide side-effects on beneficial insects in cereal crops. Bulletin of the International Organisation of Biological Control 1987/X/1 "Intergrated Control of Cereal Pests", pp 206-215.

Jepson, P.C. (1988) Ecological characteristics and the susceptibility of non-target invertebrates to long term pesticide side effects. This volume.

Jepson, P.C. (in press) Experimental options for developing selective pesticide useage tactics within cereals IPM. In: "Integrated Crop Protection in Cereals". R. Cavalloro and K.D. Sunderland (Eds.). (C.E.C.) Balkema.

Langley, M.G. (1985) The effects of a foliar fungicide on beneficial arthropods found in cereal crops. Unpublished MSc thesis. Southampton University.

Sotherton, N.W.; Moreby, S.J.; Langley, M.G. (1987) The effects of the foliar fungicide pyrazophos on beneficial arthropods in barley fields. Annals of Applied Biology 111, 75-87.

Wratten, S.D.; Mead-Briggs, M.; Vickerman, G.P.; Jepson, P.C. (1988) Effects of the fungicide pyrazophos on predatory insects on winter barley. This volume.

1988 BCPC MONO. No. 40 ENVIRONMENTAL EFFECTS OF PESTICIDES

ECOLOGICAL CHARACTERISTICS AND THE SUSCEPTIBILITY OF NON TARGET INVERTEBRATES TO LONG TERM PESTICIDE SIDE EFFECTS

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ABSTRACT

Criteria for evaluating the hazard posed by pesticides and the risk of non-target invertebrates suffering long-term side-effects are developed using basic information concerning life history. The trend for ecological factors to become more important than operational or toxicological ones in determining recovery rate following exposure is examined. The risk of organisms suffering long-term side-effects is related to their pattern of resource exploitation, dispersal rate and reproductive rate and correlated with life history strategies at different positions on the r-K spectrum. The consequences of this for the scale and duration of experimental studies is discussed.

INTRODUCTION

In order to obtain a quantitative expression of hazard, or the degree to which a pesticide will harm the non-target-invertebrate community, the level of risk (or the probability of being harmed) to individual species must be measured. This paper uses ecological criteria to define these two factors, assuming long-term effects to be those that persist into the following crop or season at statistically significant levels. (Short term, or within crop or season effects are not dealt with directly).

The use of ecological characteristics to describe organisms at risk is proposed as an alternative to the use of simple taxonomic criteria or economic importance. Taxonomic criteria are inappropriate given the diversity of the non-target-invertebrate community and the wide variation in abundance of individual species in space and time. It is impossible therefore to select particular groups that could be taken to represent the whole community. The selection or ranking of species by beneficial capacity or economic value is also unrealistic given the wide taxonomic and ecological diversity within the beneficial community (Jepson, in press). In addition the idea that the potential economic impact of pesticides (via resurgence and secondary pest outbreaks) can be predicted from their toxic effects on a small number of beneficial species arises from the mistaken assumption that predators and prey are in a simple coupled system. In the real world, a predatory complex of many species exerts a variable effect on any particular pest species.

This paper takes a novel approach by separating the non-target invertebrate community into groups with similar ecological characteristics. These groupings have arisen from the assumption that exposure to pesticides and the potential for recovery following this are likely to be affected by factors such as the proportion of life history

TABLE 1
Factors affecting the level and duration of pesticide impact on non-target invertebrates

BIOLOGICAL FACTORS	OPERATIONAL FACTORS
EXPOSURE TO PESTICIDES	
At the time of spraying	At the time of spraying
- proportion of population in sprayed area	- application volume
- degree of protection by crop canopy or soil refuges	- nozzle parameters and droplet spectrum
- droplet capture efficiency	- application frequency
Following spraying	Following spraying
- residual exposure: distribution pattern and diel activity cycle	- persistence and breakdown of a.i.
- dietary exposure: availability of contaminated prey	- formulation
	- environmental influences on bio-availability
SUSCEPTIBILITY	
- genetic, structural and physiological factors, mediating uptake metabolism and toxic effect	- intrinsic toxicity of a.i. - application rate
- environmental factors mediating toxic effect	
RECOVERY/REINVASION	
Direct ecological factors	
- mobility/dispersal/timing	- persistence and breakdown in environment
- reproductive rate/voltinism	
- isolation/proximity to non crop reservoirs	
Indirect ecological factors	
- degree of oligophagy/polyphagy	- spectrum of activity and toxic effects on alternative prey items in substrate
- extent of depletion of preferred prey	
Sub-lethal effects	
- repellency	
- behavioural activation	

that a species spends within the crop environment and the degree to which immigration from non-crop areas contributes to field populations each season. The approach taken below is to review the biological and operational factors that mediate pesticide effects on non-target species and then to examine differences in ecological characteristics that may explain the range of effects detected in the field. The groupings which arise may then be used to aid interpretation of experimental results or to select groups for investigation during field studies (Sotherton et al., 1988).

BIOLOGICAL AND OPERATIONAL FACTORS MEDIATING THE LEVEL OF SIDE-EFFECTS

Table 1 summarises the factors that are likely to affect the level of pesticide effects on different species and the rate of recolonisation of the treated area. The importance of exposure and the temporal and spatial factors influencing it, are deliberately emphasised. Failure to give this factor sufficient priority has had an important effect on the design and interpretation of laboratory and field tests (Jepson, 1987). At the time of spraying, the canopy can provide substantial protection from direct spray exposure for epigeal organisms, especially in its later stages of development (Jepson et al., 1987). Initial exposure is also affected by the method of spray application, timing and frequency; the manipulation of these factors may provide a route to the more selective use of pesticides (Jepson, in press). Exposure following spray application is a function of pesticide bioavailability (Graham-Bryce, 1987) and invertebrate behaviour, distribution and diet. These factors have yet to be investigated in the arable crop situation.

Susceptibility of different species to the pesticide is of primary importance, given a certain level of exposure at the time of application. Its importance following application is likely to decline however, in relation to ecological factors such as dispersal rate which govern the rate and extent of reinvasion. This latter process will also be affected by the extent of pesticide effects on resource availability to certain non-target invertebrates.

This summary deliberately attempts to realign the focus of terrestrial non-target invertebrate testing methods towards the process of recolonisation and recovery following exposure to pesticides. This has implications for both the scale and duration of field tests and also on the relative importance of semi-field testing methods which may quantify the direct toxic effects of pesticides on selected species (Sotherton et al., 1988).

INVERTEBRATE GUILDS AND THE RISK OF LONG TERM EFFECTS

The invertebrate community exploiting resources available within arable crops such as winter cereals can be subdivided into guilds which group species with similar ecological characteristics. For example, the predatory arthropod community (feeding upon or parasitising aphid pests) may be divided into guilds of aphid specialists such as coccinellids, syrphids and parasitic hymenoptera which are highly dispersive, colonising infested crops following pest invasion, predatory groups such as staphylinids, which disperse into the crop from non-crop areas but which have a broader diet range and groups such as carabids that complete all or part of their life-cycle within the crop and tend to be polyphagous feeders. Table 2 examines hypothetically, the extent to which the different biological and operational factors mediating side-effects (Table 1) will affect each of these guilds following spray application. It reveals that there will be a degree of separation between them in the relative importance of different factors and the rate at which they will recolonise the crop. In ecological terms, this separation is a result of differences in the pattern of resource utilisation (ie. exploitation of temporarily available prey on the crop or of a range of alternative prey items that inhabit the crop environment on a longer term basis), the level and extent of dispersal and reproductive rate.

In the real world, only a sub-set of organisms within each of these guilds will be exposed to pesticides and those organisms will exhibit a wide range of susceptibilities to the chemical concerned. Field experimental data will not therefore tend to reveal these trends on initial inspection. This only serves to emphasise the importance of defining guilds or other groupings at risk of long term effects, so that the effects on these species can be examined in detail. The table also reminds those planning experimental studies that toxicological and operational factors will tend to dominate the initial phase of effect but that differences in recolonisation and recovery rate will be most affected by ecological factors. The extent to which current experimental designs provide data on reinvasion and recovery by groups at risk is open to question.

A GENERAL MODEL OF RISK BASED ON LIFE HISTORY STRATEGIES

The ecological characteristics used above to classify the invertebrate guilds in winter cereals constitute important components of the life history strategies of the organisms concerned. These stratagies are the product of selection pressure within environments with varying degrees of temporal stability and patterns of resource availability. These pressures have tended to select organisms that exploit temporary resources and which have high dispersal capacities and reproductive rates and more sedentary organisms with low reproductive rates that exploit the resources within more stable habitats. These two extremes of population dynamics and life history are encompassed in the postulate of r and K selection (MacArthur and Wilson, 1967).

Table 3 is an attempt to represent the functional guilds of invertebrates inhabiting winter cereals according to their relative positions on the r-K spectrum of life history strategies. This exercise is intended to illustrate the probable importance of life history parameters in determining the rate and extent of recovery after pesticide exposure, it also forms a convenient way in which information on non-target invertebrate side-effects from other crops and environments may be interpreted. The table indicates those pesticide usage tactics which involve the highest risk of long-term side-effects (ie. delayed recovery) by organisms with different life histories. The additional component of frequency of use of pesticides has been included to represent the full range of options within the real world. The extent to which this matrix of possibilities supports or aids interpretation of specific side-effects field studies will be considered elsewhere however, it does indicate that

TABLE 2
Constraints on the recovery of the different functional guilds, within the beneficial arthropod community of winter cereals, present at the time of application of a broad spectrum pesticide

INVERTEBRATE GUILD	TIME INTERVAL SINCE EXPOSURE TO PESTICIDE			
	IMMED.FOLLOWING SPRAY APPLICATION	WITHIN SAME CROPPING PERIOD	THE FOLLOWING CROPPING PERIOD	MORE THAN ONE CROPPING PERIOD AHEAD
DISPERSIVE, PEST-SPECIFIC PARASITOIDS AND PREDATORS eg. Coccinellids Syrphids most Hymenopteran parasites	Initial effect for all groups dependent upon exposure and uptake of pesticide and susceptibility of organism to specific pesticide. Ecological factors other than position at the time of spraying unimportant	Potential for rapid reinvasion from outside crop, subject to availability of prey and toxicity of chemical residues	No persistent effects of previous application since pests inhabiting new crop form the resource to be exploited	Ditto
DISPERSIVE POLYPHAGOUS PREDATORS eg. Staphylinids some spiders and some parasitoids		If dispersal phase completed, little potential for recovery/ other groups as above	Some persistent effects possible via alternative food availability. Groups with soil active phase may be affected by residues	Persistent effects unlikely
FIELD RESIDENT POLYPHAGOUS PREDATORS eg. Carabids, some spiders		Little potential for recovery by wholly resident field species. Those entering from field boundary affected by residues and food availability	Recovery affected by rate of dispersal, and reproductive rate mediated by alternative food supply. Residues may also be important	Recovery may still be incomplete for slow dispersers or species sensitive to food availability. Scale of application important

strategic decisions in chemical pest control may have a significant effect on the extent of harm to different non-target invertebrate species.

This analysis looks beyond small-scale, within-season field experiments and examines the consequences of full scale commercial use of one or more pesticides. On this spatial scale and over long time intervals, the capacity of different species to recolonise treated areas is likely to be the most important component of pesticide side-effects. This is especially the case in an agricultural environment where the toxicity of individual products is declining but the general rate and frequency of use of insecticides is increasing.

THE CONSEQUENCES FOR FIELD TESTING METHODS

It is clearly unrealistic to examine a dynamic effect on the basis of one pesticide application, given the importance of spray timing in relation to the phenology of non-target invertebrates. It is also unsound to extrapolate effects detected within one season and on a small scale to side-effects that may arise on the scale of commercial use. Field experiments on these limited temporal and spatial scales must be seen as discriminating for some potential effects and not others ie. initial indices of toxicity to those organisms present in the crop at the time of spraying and not the duration of the recovery phase. If the most significant form of side-effects is seen to be delayed recovery by certain invertebrate groups, then a new methodology must be developed. This will inevitably not be able to simulate commercial use, especially in parts of the world where the scale and intensity of spraying are measured on a regional basis.

Tests on more than one site and with sufficient attention paid to plot size may however, be an effective first step. As a novel alternative, integrated laboratory and semi-field methods could examine the toxicity of pesticides to potential organisms at risk and the persistence of toxic residues on foliage and soil. They could also examine the spectrum of activity against the dietary items of non-target invertebrates such as microarthropods. Mathematical modelling and/or systems analysis would be required to examine the consequences of these effects on a realistic scale.

THE CONSEQUENCES FOR DEFINING SIGNIFICANT HAZARD

The most important feature of this ecological approach is that it views agricultural crops as a habitat, providing resources which are exploited by non-target invertebrates. Some resources are short-lived (eg. crop pests) and the invertebrates that exploit them are dispersive, and tend to have a high reproductive rate; in other words their life history strategies approach the 'r' end of the r-K continuum. Other invertebrates, resident within the field exploit longer term resources and their life history strategies approach 'K', in the r-K continuum. Changes in resource availability may elicit emigration away from the field, especially in the case of dispersive species. Changes may also cause intergenerational effects such as reduced fecundity or more subtly, increased susceptibility to environmental perturbations once populations fall below critical population densities. These latter effects may be

TABLE 3 Theoretical framework linking the probability of suffering long-term pesticide side-effects with life history strategy, operational factors causing these effects are given assuming commercial scale application in more than one season

RELATIVE POSITION OF ORGANISM IN r-K SPECTRUM			
r	INTERMEDIATE	K	RISK OF SUSTAINING LONG-TERM EFFECT
DISPERSIVE, WITH LONG POTENTIAL PERIOD OF CROP COLONISATION RAPID REPRODUCTIVE RATE, PROBABLY A DIET SPECIALIST	ENTERING CROP FROM NON-CROP AREAS WITH ONE OR MORE PHASES OF DISPERSAL. LESS RAPID REPRODUCTION, PROBABLY LESS SPECIALISED DIET	COMPLETING ALL OR PART OF LIFE CYCLE WITHIN CROP. UNIVOLTINE OR BIVOLTINE, POLYPHAGOUS	
Coincident with time of a broad spectrum persistent spray application used frequently and on a large scale	Species with one phase of dispersal sensitive within season. Also intermediate between r and K.	Use of toxic product at sensitive phase of life cycle or frequent use of low toxicity product. Treatments which affect diet. Combined effects of more than one product	HIGH RISK
Similar to above, but compound of limited persistence or scale and intensity of use reduced	Intermediate	Exposure to products applied before emergence or colonisation, especially non-persistent compounds. Limited effects on diet	INTERMEDIATE RISK
Compounds used outside period of colonisation or use of selective products	Intermediate	Selective pesticide compounds or application strategies. Reduced usage via IPM	LOW RISK

expected with invertebrates that have low reproductive rates and limited capacities for dispersal.

In this context, an ecological definition of significant effects will tend to look beyond initial impact to the following season to examine whether or not the quality of the field environment as a habitat for non-target invertebrates has changed. Thus changes in the resource base may be seen as being equally important in terms of consequences for long-term population dynamics as other effects on a field scale, and differences in life histories of the organisms concerned as being the dominant factors on a larger scale.

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REFERENCES

Graham Bryce, I.J. (1987) Chemical Methods in Burn, A.J.; Coaker, T.H.; Jepson, P.C. Integrated Pest Management. Academic Press. pp 113-160.

Jepson, P.C. (1987). An experimental rationale for the quantitative evaluation of pesticide side-effects on beneficial insects in cereal crops. Bulletin of the International Organisation of Biological Control 1987/X/1 "Integrated Control of Cereal Pests ISBN 92-9067-006-1 pp 206-215.

Jepson, P.C.; Cuthbertson, P.S.; Thacker, J.R.; Bowie, M.H. (1987) A computerised droplet size analysis system and the measurement of non-target invertebrate exposure to pesticides. Aspects of Applied Biology 14, 97-112.

Jepson, P.C. in press. Experimental options for developing selective usage tactics within cereal IPM. In. Integrated Crop Protection in Cereals Eds. Cavalloro, R. & Sunderland, K.D. (C.E.C.) Balkema.

MacArthur, R.M.; Wilson, E.O. (1967) The theory of island biogeography. Princetown University Press.

Sotherton, N.W.; Jepson, P.C.; Pullen, A.J. (1988) Criteria for the design, execution and analysis of terrestrial non-target field tests. This volume.

DISCUSSION

R. Clements: I am fortunate to be working in grassland as many of the difficulties of doing this type of work really don't apply. For example, Dr Jepson quite rightly said that, in cereals, it is unrealistic to examine a dynamic effect of one pesticide application. I can see that, in cereals where a number of applications will be made in the year, it really must be very difficult to interpret observed effects. In grassland, of course, the situation is much simpler. We only have one application made every 5 or 10 years. Also, in our work only 3 small fields within a large farm were sprayed. This is a realistic representation of what normally happens. It is a very different situation to that in cereals and some other arable crops. We are fortunate in being able to work in a real life situation without worrying about scaling up effects from a one or two hectare plot to what might happen a 300 hectare field.

P. Jepson: This is a confusing area. Firstly, when I say it is unsound to look at a dynamic effect on the basis of one treatment, I am not concerned about commercial realism. I am talking about the way in which a highly fluctuating dynamic situation can respond to single applications. It would be a rare event to get a significant effect. You need to apply several times in order to investigate the range of effects you can get. The point is the nature of the numerical data we obtain and the biological variation, not the number of applications in cereals. Secondly, we are also trying to equate the long-term effects which are detected to the scale of commercial use of products. One problem that Dr Sotherton and I have highlighted is that even Boxworth is on a small scale to us. The commercial scale of use of a product, not just in cereals but in any crop, can be up to hundreds of thousands of hectares per annum in a small region. We are trying to consider those larger scale effects. It may be true that a sub-unit of grassland treatment is 2-3 hectares but, if chlorpyrifos treatment becomes widespread, you may, in an extreme case, have a patchwork of treatment over a whole region of the UK in any one year. That, to me, is a very different type of phenomenon which cannot actually be tackled by the research methodology that we have to-day. This is why Dr Sotherton and I suggest that modelling approaches and semi-field experiments should be used so that the mechanism and timing can be elucidated for the type of effects we find.

S. Rushton, Newcastle University: The mosaic system of chlorpyrifos usage in grassland in the UK presents a perfect system for multivariate analysis.

R. Brown: Dr Sotherton, you said that, however big the scale of your experiments, you always had the problem of being surrounded by unsprayed areas which caused re-invasion. Is the presence of unsprayed areas telling you something about the habitat? What should we be doing to identify the patchwork of spraying?

N. Sotherton, Game Conservancy: I don't think we need to look at the patchwork of spraying, but we do need to calibrate the rate at which animals are re-invading. That is a big gap in our current work. Only when that gap has been filled can we realistically interpret our findings.

T. Lewis: Would Dr Sotherton like to say how you can investigate that? Who is going to work on rate of re-invasion and on what scale? How many people? Are you talking about winged or wingless creatures?

N. Sotherton: I think we need to look at a range of species with a range of dispersabilities from, for example, collembola to hoverflies. In terms of who is going to work on it, I think the answer is anybody who can generate the money to do it. I think it is an urgent priority and that all our current work is flawed by the lack of that information.

P. Jepson: Dr Lewis is highlighting the complexity of investigating such a phenomenon. However, there are ways of tackling it. Simon Duffield, for example, is treating plots of different sizes, from a fraction of a hectare up to tens of hectares, and looking at the rate at which organisms recolonise those habitats and then attempting to extrapolate that to a much larger scale.

R. Brown: I was intrigued by Dr Sotherton's suggestion that introduction of Good Laboratory Practice to much of our research might help. In industry GLP makes our job quite difficult in some cases. Would he like to expand on that?

N. Sotherton: I wouldn't, except to say that if standards are slack it is one way experimental practices could be tightened up.