SESSION 6B

ENVIRONMENTAL FATE AND EFFECTS OF PESTICIDES – THE VALUE AND USE OF FIELD STUDIES COMPARED TO LABORATORY BASED MODEL SYSTEMS

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INVITED PAPERS 6B-1 to 6B-4

THE ROLE OF MESOCOSM STUDIES IN PESTICIDE REGISTRATION

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ABSTRACT

A variety of mesocosms have been used to study the effects of pesticides on freshwater environments. They provide a bridge between the laboratory and real-world environments and are particularly useful for investigating biological effects of pesticides under conditions of real-world exposure. For exposure assessments a high degree of environmental realism is necessary but there is little or no advantage in replicating mesocosms for this purpose. For study of biological effects there are two basic experimental designs, analysis of variance (ANOVA) and regression. An unreplicated regression design has clear advantages over ANOVA for field testing of a new pesticide, or in determining biological effects of a wide range of overspray or run-off concentrations.

INTRODUCTION

The term mesocosm was proposed by Odum (Odum, 1984) to describe bounded and partially enclosed outdoor experimental units that closely simulate the natural environment, particularly the aquatic environment. A variety of systems have been used to study the fate and effects of chemicals in freshwater environments, including small, 1 m^3 stainless steel enclosures, small, concrete ponds, lake limnocorrals and large, earth-lined ponds. In their design the aim is to provide some degree of environmental realism, thus allowing studies of ecological effects that cannot be studied in the laboratory. At the same time the aim is to provide a number of experimental units that are similar in their biological structure and function, thus allowing replication of treatments and statistical verification of cause-and-effect.

The large systems generally provide a high degree of environmental realism but are relatively expensive, in terms of both capital and operating costs, and therefore it is difficult to provide a sufficient number of replicates. The smaller systems do not provide such a high degree of environmental realism but they are easier to replicate. The size and the complexity of the mesocosms and the degree of replication have a considerable effect on the cost and the resources that are required for any given study. For example, in several recent mesocosm studies carried out to meet the pesticide regulatory requirements of the US Environmental Protection Agency (EPA) large earth-lined ponds were used and several treatment levels were replicated. Such multidisciplinary studies are logistically complex, labour intensive and very expensive, each costing approximately $2 million (Hill *et al.*, 1990). Costs of this magnitude, if not prohibitive, must represent a significant proportion of the total budget available for environmental risk assessment for a given pesticide. It is therefore pertinent to consider whether the information obtained from such regulatory studies justifies their high cost or whether alternative strategies and experimental designs can achieve the same objectives at a lower cost. In this paper the objectives of mesocosm studies will be discussed in relation to pesticide registration and some factors affecting their design and cost-effectiveness will be reviewed.

OBJECTIVES OF MESOCOSM STUDIES

In a regulatory context the overall objective of a mesocosm study may be stated quite simply as "to resolve uncertainties arising from a preliminary risk assessment". It is generally accepted that environmental risk assessment requires the use of tiered schemes of testing involving a hierarchy of chemical tests, toxicity tests and estimates of chemical release rates into the environment. A mesocosm study must not be seen in isolation from all of the other studies, both laboratory and field-based, that are carried out with a new pesticide. A decision on whether or not a mesocosm study is required will be based on data for physicochemical properties, toxicity, persistence and estimated exposure concentrations. If these are very much less than concentrations that are estimated to be safe to aquatic life, then a mesocosm study will not be required. However, if there is some degree of risk, or uncertainty, then further studies are indicated to investigate the potential risks or uncertainties in more detail.

Uncertainties arising from a preliminary risk assessment may be many and varied. They may concern the transport and degradation of the pesticide, its spectrum of toxicity, its potential for indirect or secondary effects on populations, communities and ecosystems or any combination of these factors. For any given pesticide there will be specific questions that will need to be addressed, depending on whether the uncertainties are concerned with estimating exposure of the organism, bioaccumulation, toxicity or other effects. Depending on the nature of the question, or questions, that need to be addressed a variety of test systems, approaches and protocols may be appropriate. The decision on whether a mesocosm study is required, or on what kind of mesocosm study should be carried out, needs to be reviewed on a case-by-case basis. However, a recent proposal by the EPA to establish a guideline (Touart, 1988) for mesocosm studies does not appear to permit such a flexible response to the investigation of uncertainties inherent in risk assessment procedures. By contrast, in this paper it is suggested that several alternative protocols and test systems are appropriate and would be more cost-effective.

EXPOSURE ASSESSMENT

As emphasised in the previous section the design of a mesocosm study is dependent on its specific objectives, which should be clearly and explicitly stated. Generalisations such as "to evaluate the fate and effects of the pesticide on aquatic ecosystems" should be avoided. More specific hypotheses, based on a preliminary risk assessment, should address specific areas of uncertainty.

It is the general consensus of opinion of those involved in aquatic toxicology and risk assessment that there is more uncertainty in estimating exposure concentrations on the basis of laboratory data than in estimating ecological effects on the basis of single-species toxicity tests. In the words of Kenneth Macek (Macek, 1988), "The shift from aquaria to microcosms to field studies is not concerned with toxicity; it is concerned with the real variable in hazard assessment, the exposure assessment". Toxicity can be measured in the laboratory and the results of laboratory tests can be extrapolated to the field without any great difficulty, **provided the exposure of the organism can be predicted**. Herein lies the major difficulty in extrapolating from the laboratory to the field. In laboratory toxicity tests pesticide concentrations are maintained at a relatively constant level by regular replenishment of solutions or by the use of flow-through techniques. In the natural environment pesticides are subject to a variety of dilution, dispersion and degradation processes and these can have a profound effect on the concentration to which the organism is exposed and the duration of that exposure.

If exposure assessment is the most important objective of a mesocosm study, mesocosms should be designed to provide a high degree of environmental realism. In small, e.g. 1 m^3, enclosures dispersion and degradation processes may be affected by the relatively large ratio of surface area to volume compared with a natural body of water. Solomon & Liber (1988) showed that the walls of 20 m^3 enclosures had a significant effect on the rate of dissipation of methoxychlor while Lungle (1988) also demonstrated that adsorption of chlorpyrifos to the walls of littoral enclosures was a significant sink for the pesticide. Unrealistically high dissipation rates may therefore occur in small enclosures for pesticides that have a tendency to bind to organic matter, i.e. those that have a high octanol-water partition coefficient, K_{ow}. Conversely, unrealistically low dissipation rates may occur for pesticides that are subject to evaporation or phototransformation. The rate of evaporation of pesticides from water surfaces is critically dependent on air movement across the water surface (Wolff & van der Heijde, 1982) and clearly this will be severely restricted by the walls of small enclosures. Biodegradation is primarily associated with plants and sediments in the natural environment and for pesticides that are readily biodegradable the rate of transport from the water column to the sediment determines the overall rate of loss from the water column (Crossland & Bennett, 1984). In small enclosures the rate of mixing of the water column and hence the rate of transport to the sediment may be substantially different from that in larger bodies of water. Phototransformation is critically dependent on the incidence and duration of natural sunlight, particularly the UV part of the solar spectrum (Zepp & Cline, 1977). Any shading effect of the walls of the enclosure may therefore have a considerable effect on rates of phototransformation. For all of the reasons mentioned above a relatively large earth-lined pond that closely simulates the natural environment is preferable to a small, walled enclosure in order to assess exposure.

Replication of earth-lined ponds is not necessary for exposure assessment of most pesticides. Environmental parameters that affect dispersion and degradation of pesticides are physical and chemical in nature and there should be very little variation in these parameters between mesocosms within an experimental block. Parameters such as windspeed, water temperature, depth of water, organic matter content of sediment, duration and intensity of sunlight can have an important influence on the overall rate of loss of pesticide. The influence of such parameters on the rates of transport and degradation of pesticides have been studied and mathematical models can be used to extrapolate from a particular mesocosm study to other kinds of aquatic environments. Relevant environmental parameters should therefore be monitored but they should not vary significantly within a properly managed set of mesocosms. There is a considerable body of experimental evidence to support this view.

In studies with a PCB congener (2,5,4'-trichlorobiphenyl; 3-CB) evaporation and sorption were identified as the key processes involved in loss from the water column (Crossland *et al.*, 1987) of three 50 m^3 earth-lined ponds. It was calculated that evaporation accounted for 86 – 87% loss and sorption onto sediment for 10% loss from the water column after 28 days. At various times after treatment of the three ponds water samples were collected and analysed for 3-CB. The results of these analyses are given in Table 1. Four hours after treatment there was some variation in concentrations of 3-CB from different ponds, probably because of imperfect mixing of the chemical in the water column at this time. However, on every occasion thereafter, there was very little variation between ponds. This study, with a compound that is subject to evaporation and sorption but not to degradation in the aquatic environment, shows that the rates of dispersion and partitioning between water, air and sediment are not likely to vary between mesocosms.

TABLE 1. Concentrations of 3-CB in pond water

Time (days)	Concentration of 3-CB ($\mu g\ l^{-1}$) Pond 1	Pond 2	Pond 3	Mean (and SD)
0	<0.1	<0.1	<0.1	
0.2 (4 h)	10.9	13.4	12.3	12.2 (1.0)
1	8.0	8.5	7.7	8.1 (0.3)
4	3.0	3.0	2.9	3.0 (0.05)
7	1.7	1.8	1.8	1.8 (0.05)
14	0.7	0.8	0.8	0.8 (0.05)
28	0.2	0.2	0.3	0.2 (0.05)

In studies with methyl parathion (MEP), also carried out in 50 m^3 earth-lined ponds, sorption and biodegradation were identified as the key loss processes (Crossland and Bennett, 1984). Again, there was very little variation in concentration-time profiles for MEP between mesocosms. It was subsequently demonstrated (Crossland *et al.*, 1986) that the rate of biodegradation in sediment (4.0 d^{-1}) greatly exceeded the rate of transport to the sediment (0.02 – 0.05 d^{-1}). Thus the rate of transport to the sediment was the key process that affected the overall rate of loss from the pond water.

Rather more variation between mesocosms may be expected when degradation rather than transport processes predominate, e.g. for compounds with a low value for K_{ow} that are subject to degradation in the water column. In studies with pentachlorophenol, PCP, (Crossland & Wolff, 1985) and 3,4-dichloroaniline (Wolff & Crossland, 1985) phototransformation was identified as the key loss process. There was considerable variation in the light absorbance of water from different ponds and variable light attenuation of pond water accounted for a two to three fold variation in the rate of loss of these compounds from the pond water. Experimentally-determined half-lives and rate constants for PCP in three outdoor ponds are given in Table 2.

TABLE 2. Rate constants and half-lives for PCP in outdoor ponds

Time (d)	Pond 1 Rate-constant (d^{-1})	Pond 1 Half-life (d)	Pond 2 Rate-constant (d^{-1})	Pond 2 Half-life (d)	Pond 3 Rate-constant (d^{-1})	Pond 3 Half-life (d)
1 – 7	0.26	2.7	0.23	3.1	0.18	3.8
8 – 14	0.27	2.6	0.24	2.9	0.21	3.2
15 – 16	0.34	2.0	0.25	2.8	0.30	2.3
28 – 30	0.23	3.1	0.15	4.7	0.21	3.4
34 – 37	0.32	2.1	0.32	2.1	0.30	2.3
Mean	0.27	2.5	0.24	2.9	0.22	3.2
Range	0.23-0.34	2.0-3.1	0.15-0.32	2.1-4.7	0.18-0.30	2.3-3.8

In this instance variation between ponds could be attributed to variation in the clarity of the water, depending on whether the dominant vegetation was unicellular algae, in which case light absorbance was high; or whether the dominant vegetation was macrophytic, i.e. filamentous algae and vascular plants, in which case light absorbance was low. Robinson-Wilson *et al.* (1981) have also reported relatively rapid loss of PCP from ponds containing macrophytes compared with those containing unicellular algae. On the other hand Solomon & Liber (1988), in studies with tetra- and pentachlorophenol, found very little difference in concentrations or in rates of dissipation between replicate plastic enclosures situated in a lake. Presumably there was much less variation in the nature of the aquatic vegetation of these enclosures than was the case in the experiments in earth-lined ponds. Solomon & Liber (1988) also noted that differences in the rates of dissipation between replicate enclosures were low in studies with methoxychlor, 2,4-D, hexazinone, triclopyr and chlorpyrifos.

It follows that the most important objective of a mesocosm study, i.e. exposure assessment, can be achieved using only a single replicate. This approach has the advantage not only of considerable savings in labour and capital but also that available resources can be directed towards a more intensive, temporal study of the fate of a pesticide in different matrices, e.g. water, vegetation, sediment and fish. In practice, it is generally considered best to use several mesocosms to investigate several rates of treatment and to obtain information on biological effects, using a dose-response unreplicated experimental design, described in a later section.

TREATMENT RATES

The question of choice of treatment rates in mesocosm studies is obviously important but it has received little attention until recently. In previous studies the field treatment rate has often been used to represent a "worst case" scenario, i.e. the accidental overspraying of the whole of the surface area of a waterbody with a treatment rate recommended for normal agricultural use. One mesocosm might be treated in this way and others at one-tenth and one-hundredth of the recommended field rate, to represent lesser degrees of contamination. A more realistic approach than this has recently been proposed by Hill *et al.* (1990), with reference to spray drift deposition of pyrethroid insecticides. From a study of aerial spraying practices in the U.S.A., and from published data for rates of spray drift deposition, it was calculated that the range of downwind deposition onto a 100 m wide body of water, such as a farm pond, is 0.3 – 2.5% of field rate, with an average of 1.2%. The littoral nearshore inevitably receives the greatest amount, up to 10% of the nominal field rate. These data were substantiated by similar results obtained in monitoring field applications of pyrethroids adjacent to farm ponds. On the basis of this review the authors proposed that in studies designed to assess the risks of pyrethroid contamination of farm ponds in the U.S.A. 1% of the maximum field rate should be applied to mesocosms. Higher and lower rates should be applied to allow interpretation to different use patterns and rates.

ASSESSMENT OF BIOLOGICAL EFFECTS

The discussion so far has centred on exposure assessment and choice of treatment rates. However, an important objective of mesocosm studies is to study toxicity and biological effects, **under conditions of real-world exposure**. For this purpose it is generally agreed that some degree of replication of treatments is necessary to distinguish natural variation between mesocosms from the effects of treatments. This general principle has been well established in agricultural research in relation to the evaluation of the effects of fertilisers on crop yields and pesticides on weeds and insect pests. It is virtually a *sine qua non* in the design of biological field experiments. However, the degree of replication that is

required is dependent on the variability of the data that are being collected. In agricultural experimentation this can generally be estimated on the basis of past experience. Furthermore, the experimental objective can often be stated quite explicitly, e.g. "to evaluate the effect of a herbicide on the density of a particular species of weed". The situation in mesocosm studies is far more complex. Advantages that are claimed for mesocosm studies are that it is possible to study toxicity to a far wider range of species than is possible in the laboratory, it is possible to study effects at several levels of biological organisation, e.g. at the population, community and ecosystem levels, and that it is possible to study secondary effects of the treatments, e.g. predator-prey interactions, algal blooms and effects on dissolved oxygen concentrations. While this is true, the design of mesocosm experiments that simultaneously investigate a multitude of biological effects is fraught with difficulties. The degree of replication required to investigate effects on zooplankton is different from that required to investigate effects on benthic macroinvertebrates or fish. Rather few replicates will be required to investigate effects on an abundant species that is more or less randomly distributed. On the other hand a large number of replicates will be required to investigate effects on less common species, particularly if their distribution is aggregated. Investigation of secondary effects requires greater replication than investigation of primary effects (Hurlbert, 1975) because they generally occur on a longer time-scale, with increasing variability between mesocosms.

It is clear that in order to obtain statistically valid information from mesocosms for a wide range of biological variables, a large number of replicates would be required and the costs and the effort involved would be very considerable. Alternative approaches are clearly desirable and in the rest of this paper some of these alternatives will be considered.

An alternative approach, which has been found to be cost-effective and acceptable to some of the European regulatory authorities, has involved a combination of laboratory tests, mesocosm studies and environmental monitoring. Planning of the mesocosm studies has followed this general guideline:

1. The literature is reviewed to obtain all relevant data on fate and effects.
2. Where data are inadequate or missing appropriate laboratory tests are carried out.
3. The key processes involved in dispersion and degradation are identified and the rates of these processes are estimated.
4. Appropriate mathematical models are used to estimate the rates of these processes in the mesocosms.
5. Concentration-time profiles for the chemical in water and sediment are predicted from the models and thus the exposure of the aquatic organisms.
6. The estimates of exposure are compared with the results of single-species toxicity tests to predict biological effects in the mesocosms.
7. The mesocosm study is designed in the light of all available information.
8. The mesocosm study is carried out, paying particular attention to the monitoring of those environmental variables that might affect the key loss processes and the most susceptible organisms.
9. Predictions based on laboratory tests are compared with experimental results from the mesocosms. If there are discrepancies between predictions and experimental results further laboratory tests are carried out to elucidate the reasons for such differences.
10. If the environmental risk is judged to be acceptable, environmental monitoring of the pesticide is carried out under conditions of normal agricultural use to substantiate the findings of the previous work in a variety of field situations.

Typically, only three or four earth-lined ponds have been used for this type of mesocosm experiment. Examples of such experiments have been described previously (Crossland, 1982, 1988). Two or three treatment levels were chosen and a wide range of biological parameters were measured. Generally, the primary biological responses are of an "all-or-nothing" nature so that it is very easy to differentiate between those species that are affected and those that are not, without the need for replication. An example of the kind of response measured in such an experiment is shown in Fig. 1.

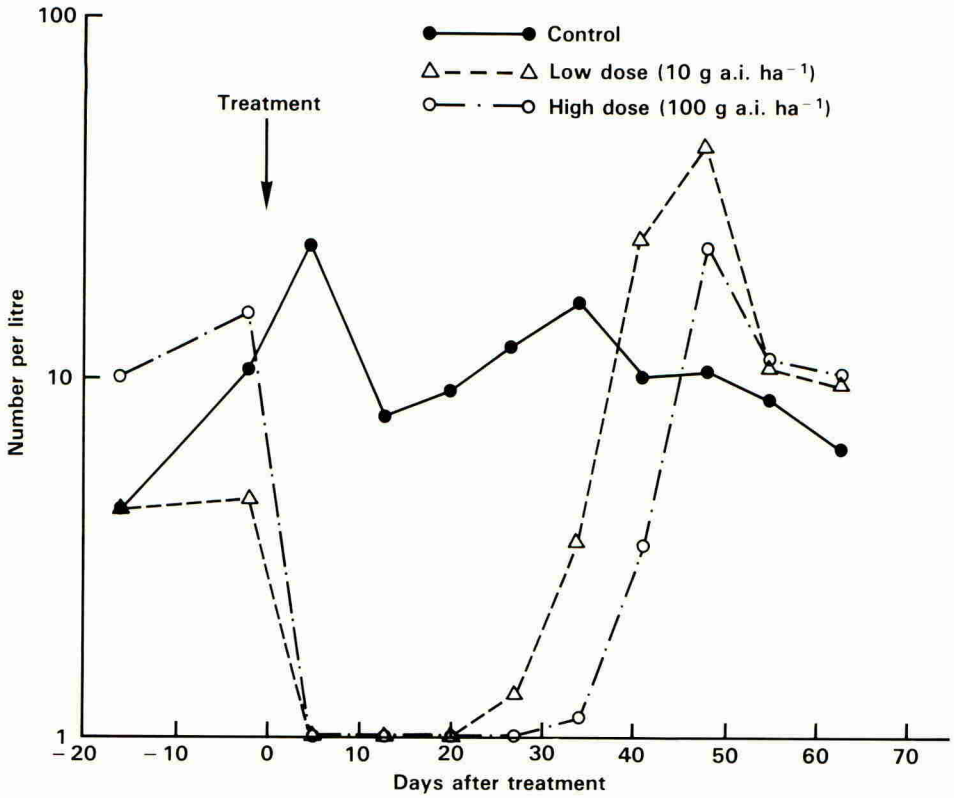

FIG 1. Effect of fenpropathrin on *Daphnia* spp.

In this experiment the pyrethroid insecticide fenpropathrin was sprayed over the surfaces of two ponds, one at a "low" dose, equivalent to 10 g a.i. ha^{-1}, the other at a "high" dose, equivalent to 100 g a.i. ha^{-1}. A third pond remained untreated and served as a control. The most abundant zooplankters were *Daphnia* spp. (mainly *D. longispina*), *Diaptomus* sp., *Cyclops* sp. and the nauplii of *Diaptomus* and *Cyclops*. Populations of *Daphnia* spp. were severely affected by both treatments. In the low dose treatment, populations were reduced to zero for a period of three weeks and then recovered rapidly to pre-treatment levels four to six weeks after treatment. In the high dose treatment, populations were reduced to zero for four weeks and then recovered to pre-treatment levels from five to seven weeks after treatment. In both treatments the recovering *Daphnia* populations overshot an equilibrium density level before decreasing to pre-treatment levels. There was 100% mortality of caged fish in the

pond treated with 100 g ha^{-1} but no mortality of fish in the pond treated with 10 g ha^{-1} of fenpropathrin. Qualitative, rather than quantitative, results of this kind can be very informative during the early stages of environmental risk assessment for new pesticides. They can be obtained for a wide variety of species and biological responses, without the need for replication. It may not be possible to detect small effects or subtle biological responses in such unreplicated tests, nor is it possible to link cause-and-effects with any degree of statistical probability. On the other hand any major effects of the treatment will generally be easily detectable. It should, for example, be possible to assess which of the common species of zooplankters, aquatic insects, other invertebrates and fish are most at risk. It is also possible to obtain information on bioaccumulation and depuration using caged fish. In the light of data obtained from such an unreplicated study it might be decided (a) that no further data are required, i.e. that there are no unacceptable environmental risks (b) that the environmental risks are acceptable, subject perhaps to environmental monitoring of the perceived risks under various conditions of field use (c) that further laboratory or field assessments are required, e.g. using small, replicated mesocosms as described later or (d) that the environmental risks are unacceptable.

An appropriate size of mesocosm will depend on the organisms to be studied, e.g. zooplankton, benthic macroinvertebrates or fish and on the environment to be simulated, e.g. farm pond or small lake. Its configuration, e.g. whether earth-lined pond or plastic enclosure will depend on whether organisms in the water column are of most interest or whether the benthos is also of importance. A variety of mesocosms are available and their design and construction has been reviewed by Solomon & Liber (1988). The choice of appropriate system will depend on the objectives of the study.

Replicated experiments are most appropriate when the objectives can be defined somewhat precisely, in terms of a clearly defined group of organisms or specific biological responses. For example, if the zooplankton are, on the basis of laboratory toxicity tests, considered to be the most susceptible group of aquatic organisms, it may be appropriate to carry out a mesocosm test using small 1 m^3 plastic enclosures of the kind described by Stephenson & Kane (1984). The planktonic communities contained within such enclosures will behave, at least for a period of a few weeks, much as they do in a larger body of water and satisfactory replication of communities can be achieved. Perturbations of these communities by pesticides can be investigated using either ANOVA or regression designs. Liber *et al* (1990) used 100 m^3 plastic enclosures, or limnocorrals, to investigate the advantages and disadvantages of these two basic types of design. Two experiments were carried out in a 10.3 ha lake in Canada using 2,3,4,6-tetrachlorophenol (TeCP) to investigate its effect on the abundance of zooplankton. The first experiment was based on blocked analysis of ANOVA procedures and used three treatments (0, 0.75 and 1.50 mg l^{-1} TeCP) with three replicates of each. The second experiment was based on regression procedures and used eight treatments ranging from 0 to 7.3 mg l^{-1} of TeCP, with no replication. Both of the TeCP treatments in the 'ANOVA' experiment had statistically significant effects on zooplankton abundance and it was possible to draw firm conclusions with respect to differences in community structure and recovery, which were some of the original purposes of the experiment. However, it was not possible to extrapolate to what could be regarded as a "safe level", e.g. a No Observed Effect Concentration (NOEC) for zooplankton populations, a common drawback of the ANOVA design. In the "regression" experiment there was no effect at concentrations of 0.1 and 0.25 mg l^{-1} but effects of varying degrees of severity were observed at all five concentrations from 0.5 to 7.3 mg l^{-1} (Fig. 2). Estimates of the EC_{50} under field conditions were obtained for individual common species and these corresponded reasonably well with estimates of the EC_{50} obtained in the laboratory and from the literature. Estimates of the NOEC for various species and for the total zooplankton abundance were obtained by linear regression analysis. Estimates of the upper and lower 95% confidence limits of the NOEC were also obtained. For 9 out of 14 species that were assessed the NOEC was greater than or

equal to the estimated EC_{50}, suggesting that an impact could not be identified as significant until the population density had been reduced by at least 50%.

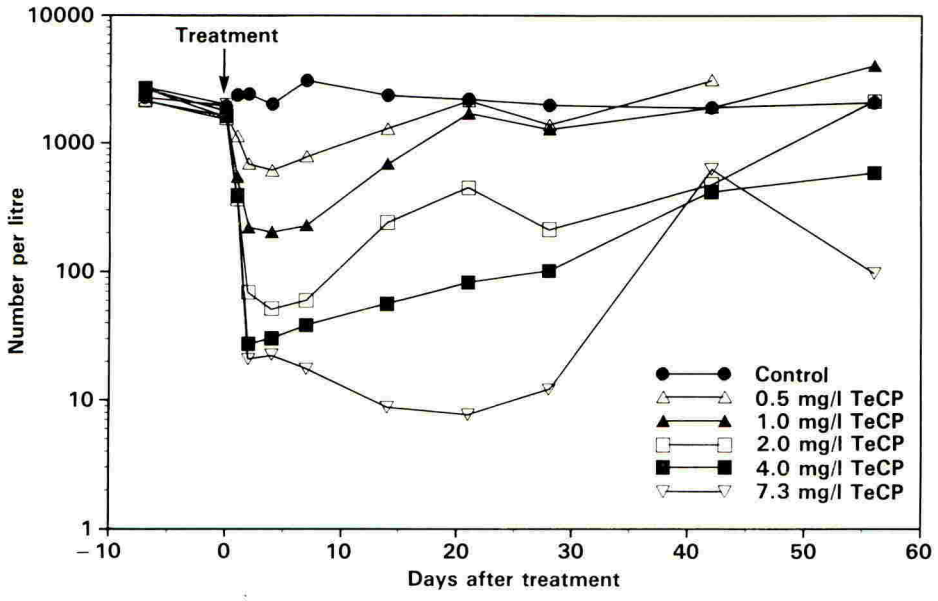

FIG 2. Effect of 2,3,4,6-tetrachlorophenol on zooplankton (Liber *et al.*, 1990)

It was concluded that the regression design would probably be best in the initial field testing of a product, or in determining the response to a wide range of unpredictable overspray, surface run-off or effluent concentrations. Kennedy *et al* (1989) also consider that the regression design has clear advantages over the ANOVA design in mesocosm studies used for evaluating the effects of insecticides on freshwater environments. Graney *et al* (1989) discussed several alternative experimental designs for mesocosm tests and strongly recommended the use of a regression type design in mesocosm tests. There now appears to be a consensus of scientific opinion that favours this rather than the ANOVA design. In conclusion, it seems unlikely that any single protocol, guideline or type of mesocosm is appropriate for the study of different questions that may arise during risk assessment procedures for new pesticides. The objectives and therefore the design of such studies need to be defined on a case-by-case basis.

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PROSPECTIVE GROUND WATER STUDIES - LOGISTICS AND DESIGN

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ABSTRACT

Prospective ground water studies are now an integral part of the registration requirements for the USA. Such studies are triggered for new active ingredients believed to have the potential to leach to ground water. Whilst the protocol for such studies is clear, there are many design and logistical problems which need to be overcome in order to produce a study from which a meaningful assessment of leaching potential can be made.

INTRODUCTION

In 1987 the Environmental Protection Agency (EPA) put forward draft guidelines for ground water studies (Eiden et al., 1988). These studies were incorporated into the existing environmental study requirements for pesticide registration in the USA. Three types of ground water study were outlined:

(i) Small-scale prospective,
(ii) Small-scale retrospective, and
(iii) Large-scale retrospective.

The retrospective studies focused on products already established in the market, while the prospective study was introduced to evaluate new active ingredients. This paper outlines the triggers, aims, design and logistics relating to the prospective ground water requirement.

TRIGGERS

For the development of new active ingredients, EPA has developed a tiered approach to environmental studies (Barrett et al., 1989). Laboratory studies aimed at determining the physico-chemical properties of a new compound (hydrolysis, photolysis, adsorption to soil), together with soil metabolism data provide the first tier of studies.

Having generated data on the fate of the compound in laboratory soil studies, the parent compound and significant metabolites are then monitored under field conditions in soil dissipation studies. These second tier studies are designed to determine the rate of decline of the parent compound, together with the formation and decline of metabolites. Soil samples are required to be taken to a maximum depth of 90 cm, in order to monitor the movement of the chemical and its metabolites.

The third tier, prospective ground water studies are triggered if detectable residues of the parent compound, or metabolites are determined at 90 cm in second tier studies.

Prospective ground water studies may also be required for existing products, if a new use is proposed in vulnerable hydrogeological areas, or if the potential for leaching is suspected, but the existing data is not sufficient to make an accurate evaluation.

AIMS OF PROSPECTIVE STUDIES

Prospective ground water studies are designed to track the movement of the test chemical and significant metabolites from the point of application, the soil surface, through the vadose zone, by soil and soil-solution sampling, and ultimately into ground water, by the sampling of monitoring wells. The studies are conducted under extreme worst case conditions and sampled for a minimum period of two years, following application. Site selection is based on permeable soils, in areas of high rainfall, overlying shallow unconfined aquifers.

SITE SELECTION

A single large field plot should be selected, typically 1-2 hectares in size. The area should be flat and not subject to run-off during heavy rainfall. The soils should be uniform and permeable, sands and loamy sands, through to the water table and free of any retentive clay layers. The whole trial area should be characterised down to the water table, and should have no prior history of the test chemical usage.

In practice site selection is a complex, time consuming and expensive task. To successfully find a site that fulfils all of the required criteria is reasonably straight forward, however, the researcher should always try to ensure that the site selected is capable of supporting the target crop. Areas of sandy soils with high rainfall are typically only marginal agricultural land, having very poor moisture and nutrient holding capacities. Prospective studies of this sort should be carried out under worst-case, but still agriculturally viable, conditions.

The first phase of site selection can be carried out by reviewing soil maps, compiled by the United States Department of Agriculture (USDA) Soil Conservation Service (SCS), or by using the Data Base Analyzer and Parameter Estimator (DBAPE) complied by the EPA (Carsel et al., 1989). Either approach will lead to specific areas which broadly conform to the correct soil series. However, only by touring the sites will the general topography of the areas become apparent, and only by hand auguring will the true extent of the soil profile and depth to ground water be revealed. Typically, the SCS mapping will only cover soil sampling to approximately 2 metres and the site will need to be characterised down to the water table.

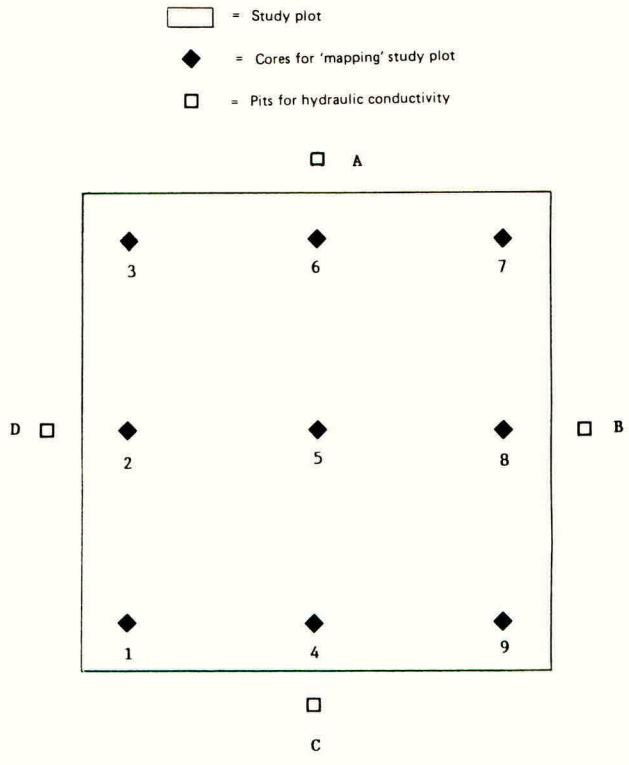
Site selection is usually a process of elimination, some being too extreme, while others may have retentive clay layers at depth. When two or three seemingly acceptable sites remain, a full grid-point characterisation of the sites can be carried out. Soil should be sampled from grid positions on the test area and the soil characterised as a function of depth. Sub-samples, taken at this time, can then be laboratory analysed for their sand, silt, clay content, and also

chemically, for background interferences.

Having selected a single site, the expensive task of the determination of soil permeability can be undertaken. Vertical hydraulic conductivity determinations can be carried out directly, in the field, using a permeameter, or more usually, by taking 'undisturbed soil cores' back to the laboratory for testing. Great care has to be taken when generating the cores for testing, smearing of the core ends would restrict water flow and give unrealistic values for vertical conductivity. Cores are usually taken in one or two foot increments down to the water table and if the coring positions are located along the edges of the test plot the information can be coupled with the grid mapping, thus providing additional information on the soil characteristics across the test plot (Figure 1).

Laboratory determinations are carried out by saturating the soil cores and measuring the rate of percolating water. Measurements are made using either constant or falling-head permeameters depending upon the soil type. (Taylor, D.W., 1948; Bowles, J.E, 1978; American Society for Testing and Materials, 1982). Typically, vertical hydraulic conductivities of 0.01 inches per hour or greater are typical of loamy sand, or sands.

FIGURE 1 : Soil Sampling Positions for Plot Characterisation

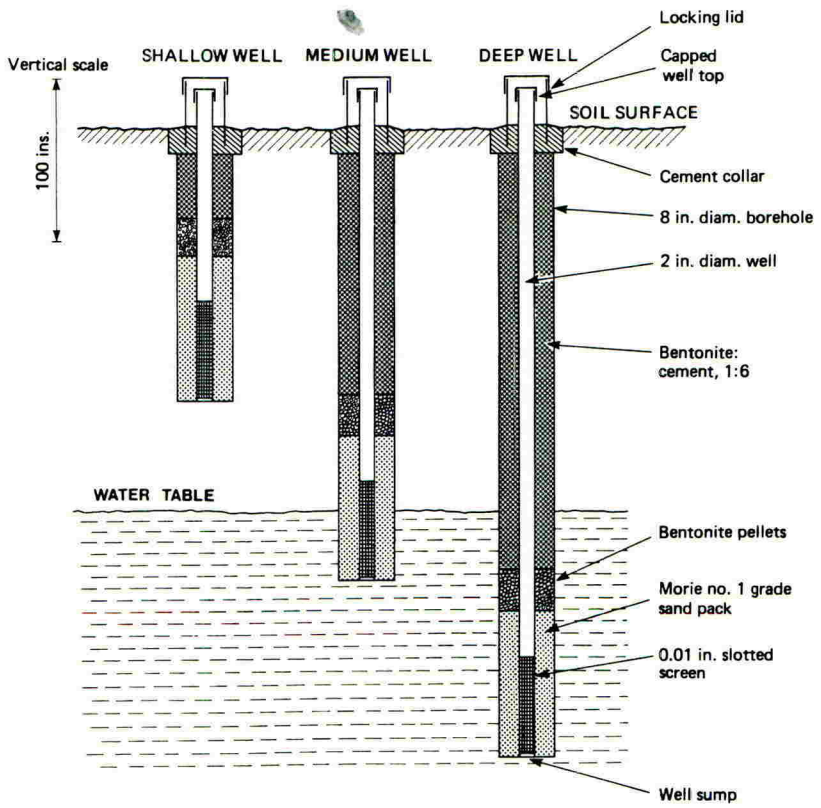


WELL INSTALLATION AND HYDROGEOLOGY

The depth to ground water, flow direction and flux of the aquifer are all parameters which must be determined in order to characterise the aquifer. The use of experienced staff is vital if sampling and monitoring wells are to be successfully established, and the necessary parameters recorded.

Well installation is performed by drilling a borehole to the required depth and inserting a length of slotted-screen pipe. The well materials can be stainless steel, Teflon or PVC, depending upon compatibility with the test chemical. A sand or gravel pack is placed in the borehole, around the well screen and a bentonite seal placed above the pack. The remaining borehole void is then filled with a cement/bentonite grout to land surface where a lockable inspection lid is installed (Figure 2). Prior to sampling or pump testing, the wells should be developed by pumping water continuously until clean water is obtained. This process removes fines created during the drilling process and helps to consolidate the gravel pack.

FIGURE 2 : Sampling Well Structure and Cluster Design



Surveying a network of wells to a common datum point and measuring well depths relative to this datum will provide the information on flow direction. Aquifer flux/transmissivity is determined by pump testing the wells. Several techniques can be employed, but all are aimed at measuring the rate of flow of water into a well under steady state conditions (Canter et al, 1987). The cost of well installation may be reduced by using the necessary sampling wells on the plot as part of the network of wells covering the surrounding study area.

The requirement is to sample ground water form at least three wells on the test plot, at monthly intervals after application and that these wells should skim the water table. In practice it is unlikely that the researcher will have detailed knowledge of the annual fluctuations in ground water levels and to take account of this it may be necessary to install three well clusters, each cluster containing a shallow, medium and deep well, so that an upper ground water sample can always be sampled (Figure 2). Prior to commencing the study it is wise to position a sampling well off, and 'down stream', of the test plot. This well may then be of use if residues are detected in ground water and plume movement observations are necessary.

IRRIGATION

Prospective ground water studies, being conducted under 'worst case' conditions, will require the use of irrigation to supplement the annual rainfall. Obviously the drier the season the more irrigation required and irrigating a 1-2 hectare plot is not a trivial exercise. Both volume and mode of application are important factors in the design of an irrigation system. Application of large volumes of irrigation water are necessary even to maintain minimal precipitation over these large test plots. Even on a modest plot size of 1 hectare, 50,000 litres of water would be required to provide a supplementary 5 mm of 'rain'. With such large volumes required the irrigation water cannot be drawn from the test aquifer without vastly distorting the flow patterns. It is therefore necessary to sink a separate irrigation well which draws ground water from a deeper aquifer than the test aquifer. To install such a well, with sufficient capacity, and to be able to deliver the water to the plot at the required rate, is a major engineering exercise. A variety of application systems are available ranging from simple gun irrigators to linear or radial tracking systems. However, the guns are prone to pooling water, give poor coverage and can cause run-off on occasions. Linear tracking systems offer the best method of application, although the cost of such systems is high.

SAMPLING

The study design requires tracking of the test compound from the point of application through the soil and unsaturated zone and ultimately to ground water.

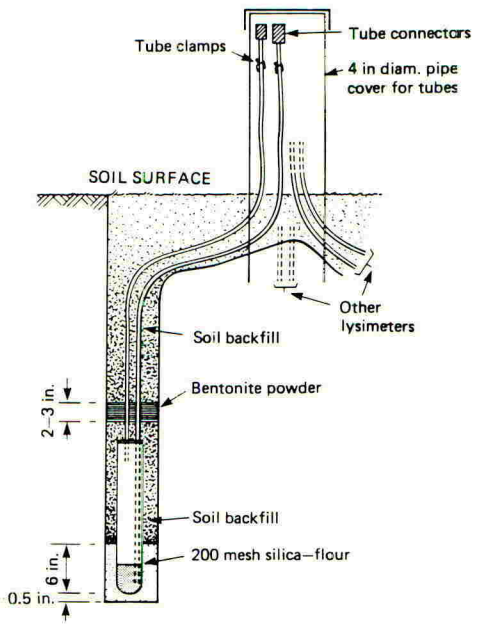
Soil

Full details of the required sampling plan is laid out in the draft guidelines for this study (Eiden et al, 1988), although the aim is to use a progressive sampling plan which tracks the movement of the compound through the soil to the lowest practical depth of sampling. Below this depth suction lysimeter units are used to continue the monitoring. When taking soil samples from field leaching studies it is vital that no cross-contamination of samples occurs. Even using zero-contamination techniques, great care should be taken to ensure that the high surface residues do not contaminate lower depths. At least in the early stages of the study, when surface residues are very high, samples from depth should be taken only after removing the upper soil layers. Obviously sampling at depth through the top soil layer could result in surface residues being pushed down the soil profile, during sampling, and resulting in artifactual residues in deep samples. Care should be taken to store, transport, prepare and analyse the surface and deep soil samples separately to ensure no cross contamination.

Soil-Pore Water

Soil moisture from the vadose zone is abstracted using suction lysimeter units. These are installed by hand auguring a narrow borehole to the required depth and seating the porous ceramic cup of the unit in a silica flour pack. The unit is then sealed with bentonite and the soil back-filled and consolidated to ground level (Figure 3).

FIGURE 3 : Installation of Suction Lysimeter Units



Samples are taken by evacuating the units and slowly drawing moisture into the lysimeter, the sample being collected by releasing the vacuum and applying a positive pressure sufficient to drive the collected water up to the surface where it can be collected in a sampling bottle. In some instances analysis of the soil pore water provides a very sensitive analysis technique, up to an order of magnitude below detection limits typical in soil. In practice however, the units are difficult to use and the data difficult to interpret. Depending upon the sensitivity of the analytical method, it may be necessary to collect up to a litre of soil pore water to perform an analysis. On sandy soil with poor moisture retention it would be impossible to collect such a volume and it is often required to pool samples from replicate lysimeters, to provide sufficient material for analysis. Collection times also vary, depending upon the soil moisture, and it is not uncommon to only collect 2 or 3 ml after two weeks of sampling.

Data interpretation is also problematic. A residue in a suction lysimeter that was correctly installed will only provide a qualitative assessment of the actual soil residue. Assumptions as to the actual sampling radius of the unit, the soil moisture at the sampling depth and adsorption coefficient for the compound at the sampling depth all prevent a quantitative assessment. Given the uncertainties of these units, many researchers now advocate more detailed soil analysis, to provide vadose zone information, believing that the additional time and resource require to develop exceptionally sensitive soil methods, preferable to suction lysimeter data.

Ground Water

Ground water is abstracted from the sampling wells by the use of submersible or surface mounted pumps, or simple bailers, although in each case the sampling device and test chemical should be checked for compatibility. Samples can be collected by either simple bottle, or cartridge collection, provided the stability of the analyte has been established. Prior to taking a sample the well should be purged until the pH, conductivity and temperature of the well sample has stabilised. This procedure, while time consuming, ensures that the sample being collected is an aquifer sample and not a static well sample, but also ensures that the samples taken are clean and free of fines which aids the final laboratory determination.

Great care should be taken when sampling in windy conditions. Air-borne surface soil particles can easily contaminate ground water samples, and it is wise in such instances to irrigate the plot immediately prior to sampling to reduce the risk of dust. It is also good practice to store and transport ground water samples separately from soil samples, which would obviously contain residues far above those expected in ground water.

MANPOWER AND FACILITIES

Precise details of the manpower requirements for this type of study are obviously highly dependent upon circumstance. However, it should be recognised that these studies cannot be carried out anywhere. Their location is specified by soil type, rainfall and aquifer characteristics, and given these stringent requirements it is highly likely that such studies will be carried out in remote areas far away from the researcher's base facilities. In these circumstances it is necessary to provide either a dedicated field-based team and support, or to provide a mobile 'fly-in' team. Given the duration of these studies to provide either level of resource is not easy. Requirements for supplemental irrigation, monthly groundwater sampling and perhaps continual lysimeter sampling require that manpower must always be available.

It is unlikely that a researcher will posses all the necessary skills required to perform these studies and in such cases it is wise to employ consultants to provide specialist expertise (hydrogeologist, soil scientist, engineer, chemist, agronomist etc.).

In the field a base/workstation is essential, although in remote areas this may be difficult to achieve. The facility should enable staff to prepare and clean equipment for sampling events and to store samples prior to shipment to the analytical facility. If possible it is wise to divide such a building into high and low level work areas, thus reducing further the risk of cross contamination. Similar precautions should also be taken at the analytical laboratory.

CONCLUSIONS

Studies aimed at determining the potential for pesticides to contaminate ground water are now an integral part of United States pesticide registration process. Prospective ground water studies form the last tier of studies that may be required to complete this evaluation. The studies are expensive, logistically difficult and, for many researchers, a new area of environmental science. With care and planning many of the challenging problems associated with these studies can be overcome and if the preliminary work is thorough, the final interpretation of results will be more straight forward.

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INTERACTIONS BETWEEN THE BIOLOGY OF ORGANISMS AND THE FATE AND EFFECTS OF PESTICIDES

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ABSTRACT

In order for a chemical to affect the functioning of an organism, it must be available to that organism. This implies both that it is physically present in the environment surrounding the organism and that it will be taken up and retained by the organism long enough to cause an effect. A great deal of testing and research is done on chemicals which will not, in practice, be available to the organisms tested. Both physical factors (adsorption of chemical to soil particles for example) and biological factors (the physiological state of the organism, season etc.) determine both whether the chemical will be taken up and whether it will cause adverse effects. This presentation will examine the knowledge in this area and suggest further areas to be developed in the future. Both laboratory results and results obtained in the field will be considered together with the question of whether current model systems give information relevant to the field.

INTRODUCTION

This contribution will deal specifically with pesticide effects on populations of terrestrial vertebrates, though the principles should be applicable to other systems.

Prediction of the effects of pesticides on birds and wild mammals currently rests on the results of laboratory tests with severe and well documented limitations. Much of this testing is carried out as a routine requirement of regulatory authorities, national and international, and little thought is given to the usefulness or relevance of the tests in answering the main question which should be posed - namely will the compound have adverse effects in the field. There is also little distinction made between effects which kill individuals and effects which reduce populations. It is easy to conclude that laboratory test results are of little or no relevance and that only field observation will yield meaningful answers. Unfortunately, field studies also pose problems of expense and design. Limited observation in the field with small numbers of individuals of few species under local exposure can also fail to identify potential hazard under conditions different from those of the test site.

Both means of determining effects need to be related to exposure to come to a meaningful risk assessment for the compound. Laboratory tests are frequently run at exposure doses considerably higher than those likely to be experienced in the field. Field tests are often performed with little indication of the exposure actually experienced by the organisms observed. Accurate estimation of exposure in the field is difficult for many compounds used in agriculture.

We will review the limitations of present methodology and suggest means of improving both laboratory and field testing for effects of chemicals on birds and mammals. This will be done with predictive testing for regulatory purposes in mind and hence concentrate on simple tests which can be done at reasonable cost as routine.

TEST ORGANISMS

In the laboratory, testing is limited to those species which can either be bred in captivity or caught in large numbers in the wild (pest species). Frequently this limitation imposes test organisms in the laboratory which are physiologically or behaviourally different from the majority of related organisms in the field. In the case of birds, the test species are most often precocial (with young feeding independantly from hatching) whilst most wild birds feed helpless young. In the case of mammals, there is little or no testing on wild species in the laboratory. Prediction of hazard is based on laboratory strains of rodents. These are physiologically markedly different from wild mammals and have lost many of the responses of their wild counterparts to environmental variables. Seasonality in breeding has disappeared with inbreeding of laboratory strains. All laboratory populations become different from their wild ancestry in a short time. Body weight increases and variability in response to environmental variables increases. Field studies have the advantage of working with a range of species which are living under natural conditions and responding to environmental variables in a natural way. There is, however, a disadvantage in that the number of individuals is small (except in very large scale observation which cannot be done routinely) and the number of species is large. Statistical analysis of results is complex and seldom satisfactory. Very detailed observation founded on knowledge of the field biology of many species is necessary to design the field experiment and observe the relevant variables.

Species differences, in many cases, are likely to be differences of degree rather than fundamental biological differences. Species within the same group have the same basic physiological and behavioural repertoire with differences between species and populations being differences in emphasis on different aspects of the repertoire. Whilst laboratory mammals are physiologically fundamentally different from their wild relations, this is probably not so for laboratory birds.

Small scale field studies observe the relevant species but give limited information on subtle effects; large scale field studies are expensive and often inconclusive because of uncontrolled variables. Laboratory studies use unrealistic species under unrealistic conditions but give reliable results statistically. A clearer understanding of the underlying physiological and behavioural responses of wild species is needed to allow extrapolation between laboratory studies and the field situation on a biologically meaningful, rather than a mathematically meaningful basis.

MORTALITY

The size of field populations of organisms are, obviously, determined by the balance between birth rate and mortality. In general, larger species survive longer as individuals and produce fewer young per year than smaller species. Mortality is high in all species. Population size is subject to large variation from year to year for many species. Much data is available to

chronicle these changes, but there is less indication of the underlying causes. In species with stable populations, survival will, by definition, be restricted on average to one pair of offspring for each pair of breeding adults. In many cases, this means that a large proportion of individuals, young and adults, fail to survive to succeeding years.

Even highly acutely toxic chemicals may, therefore, kill large numbers of individuals without affecting populations. Many of the poisoned individuals would have died anyway and their premature death from poisoning improves the chances of other individuals' survival. Estimation of percentage survival of a test population of birds or mammals (or more commonly estimation of dose required to kill a standard percentage of the population) does not, therefore, give a measure of long-term effects on population size in the field. Rather, at best, it estimates the likelihood of short-term incidents or kills occuring in the field. This is an important measure to have; it is not desirable to kill any individuals of non-target organisms if this can be avoided. It does, however, have little biological relevance to the organism as a species. Further problems arise with laboratory measurement using death as an endpoint. If some species in the field are more sensitive than the laboratory test species, they may die at much lower exposure. Exposure of the test species is either to a single dose or to a continuous uniform dose. Laboratory mortality studies tend to overestimate likely field effects because exposure is almost always exaggerated.

In the field, a high acute dose may be experienced, but longer-term, ingestion of uniformly contaminated food is less likely. In field tests, low mortality observed may be due to low toxicity or low exposure. Even where there is a simple measure to indicate exposure (such as inhibition of blood esterases by organophosphorus compounds) it is not easy to sample large enough numbers of animals with sufficient frequency to get an estimate of real exposure of even a local population. In the case of other chemicals, it may be impossible to get even a rough estimate of intake in the field, beacuse of lack of a measure which will not kill the animal. Exposure of other species or the same species under other circumstances may lead to higher mortality. Without a clear understanding of the factors affecting availability of the particular chemical to the observed species, it is not possible to generalise from the particular field trial. Field observation may, therefore, underestimate effects on other species or in other circumstances.

In both cases, the inaccurracy of the result derives largely from the exposure, either from it being unrealistic, unmeasured or unmeasurable. Better estimation of likely exposure in the field would render many acute toxicity tests unnecessary. Indeed many now conducted serve little or no purpose since it is clear that the doses given to the test animals are thousands of times higher than the maximum possible exposure dose. Some estimate of exposure can be made using physicochemical properties of chemicals together with information on persistence and fate in the environment. The window of availability of non-persistent chemicals can be determined by these means. It is difficult to see how laboratory feeding experiments could be made more realistic with exposure equivalent to that in the field. Laboratory animals are usually much better fed than individuals in the wild and have higher stored food reserves which can ameliorate the effects of chemicals. The pattern of feeding throughout the day differs between captive and wild animals. A choice given in laboratory tests between contaminated and uncontaminated food may also help to make the hazard assessment more realistic, though there are examples of the apparent ability to

discriminate between dosed and undosed food suggested by laboratory observation not occuring in the field.

Much effort has been put into reduction of variability in test populations of organisms for use in laboratory testing. There is justification for this in laboratory studies on rodents and other experimental mammals which are aimed at assessment of human health effects of chemicals. In contrast, the use of environmentally relevant species in the laboratory should aim to retain the variability of populations in the wild. It is this variability which specifically allows field populations to deal with xenobiotics. Little variability in field populations is likely to increase the chance of both large scale mortality and sub-lethal effects reducing the population.

Field trials can, therefore, shift perception away from the likely overestimate of hazard produced by laboratory tests, but the shift may be too far in the other direction. Mortality tests can indicate the likelihood of field "incidents" but give little information on true ecotoxicological effects of chemicals. Exposure data are needed to make real use of effects observations. Feeding habits of animals in the wild and their uptake of chemical contaminants warrants more study. Comparative information from laboratory mortality tests is useful in assessing field results or determining the need for field trials. Limit tests (working up from zero to minimum effective dose) would, in most cases, be as useful as full toxicity curves.

REPRODUCTION

Populations of organisms can be affected by direct mortality (which must be high and regular to have effect at the population level) or by reduction in the rate of reproduction. Standard tests on bird reproduction in all regulatory schemes reflect the importance placed on this second component of possible ecctoxicological effects.

Current test methods implicitly assume a simple model for the control of reproduction by environmental variables. Birds are kept on short days for the initial period of the test and transfered to long days at a later stage. The test, therefore, assumes that light is the principle, if not the only, environmental variable used by wild birds to time their reproductive cycles. This follows the approach taken by physiologists studying bird reproduction in the laboratory but is in clear contradiction to most observations made by ecologists in the field.

It is certainly true that birds (and wild mammals which are not included in testing of this kind) use light as an indicator of season and that an inbuilt biological clock interacts with light cycles to time the development of the reproductive system preparatory to breeding. However, it is also true that fine tuning of the onset of breeding uses other environmental and biological variables (food availability, temperature, weather, availability of nesting sites, social interaction etc.). Considerably less experimental work in the laboratory has been done in this area and most results have been obtained in the field. Field results are usually correlations between an environmental variable and reproductive success and it is difficult to establish cause and effect relationships. These reactions to variables other than light are clearly important in the wild and warrant further study in the laboratory.

There is only one documented case of a chemical affecting reproductive success in the

field sufficiently to cause decline in populations of animals - DDT. Even here, complications of exposure to a wide variety of organochlorine pesticides, including the more acutely toxic cyclodienes, has complicated interpretation of results (Newton 1979). The field effect of DDT has coloured both the development of test methods (the standard OECD bird reproduction test is designed as an attempt to identify new DDTs) and perceptions of how chemicals might affect populations through reproductive effects in the future. It is generally assumed that the effect of DDT on bird reproduction relied upon its persistence and biomagnification. This is misleading since the real effect of DDT, as with all chemicals, is a result of coincidence in time between a sensitive stage of the annual cycle (egg laying) and a sufficiently high exposure to adversely affect the process. In the case of DDT, the sufficiently high exposure came about by release of material stored in fat into the bloodstream at the ciritical time of eggshell deposition. For future chemicals affecting reproduction, availability to the organism at the critical phase of the reproductive cycle could arise from application time of the compound or from storage and availability externally in the environment. There need be no internal storage or accumulation of the chemical.

In the many published bird reproduction tests, the effects seen in the laboratory test have seldom been observed in the field. Many of the adverse effects reported seem to be explicable in terms of indirect effects of the chemicals on food consumption of the test birds. Whilst the test encourages measurement of food consumption at two different stages of the test (short day period and long day period), there is no indication of how to interpret effects on food consumption. Birds are extremely sensitive to reduction in food availability and changes in food quality, though the work done has been restricted to chickens and other commercially important birds.

We have done experimental work on the effects of environmental variables on food consumption of wild bird species and on the way in which food availability is perceived by birds. Adverse weather conditions do change the feeding behaviour of birds. Caged starlings held outside will reduce their activity and food consumption when it is raining or severely cold, even where the individual bird is sheltered from direct effect of the weather. Perch hopping activity is reduced and energy conserved in times of adverse weather. This is in direct contradiction to the response of laboratory rodents, for example, where inbreeding and controlled conditions have led to the loss of natural response to cold and where increased food consumption is used to maintain body temperature. Temperature fluctuation in caged birds kept indoor also leads to variations in food intake. As with outdoor caging, reduced temperature causes reduced activity and reduced food intake. Higher temperatures following periods of cold increase food consumption again, often to levels higher than average. At constant temperature, food consumption is related to photoperiod. Longer daylengths cause the bird to increase food intake, at least over the limited range between the 8 hours and 16 hours of light a day so far studied. This relationship mirrors changes in the rate of gonadal growth. The amount of food eaten per day under conditions of constant temperature shows very little variation from day to day suggesting a very accurate control of food intake by birds (figure 1). A similar accurate control of food intake is typical of wild mammals (Silverstone 1976).

It is also clear from laboratory experiments that birds do not perceive food availability in simple terms. They do not monitor absolute intake of food nor rely simply on estimation of

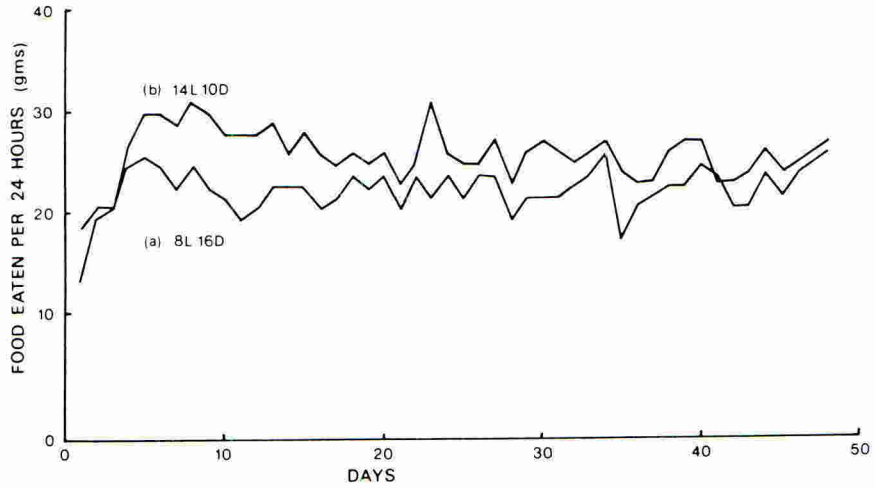

Figure 1. Means of food eaten per day over a 48 day period on short days (8L:16D) or long days (14L:10D) by starlings (*Sturnus vulgaris*)

body condition (that is stored fat or protein). No physiological mechanism by which this could be done has been suggested for either birds or mammals. In a series of simple experiments, we have shown that starlings can develop or fail to develop their gonads to reproductive maturity with identical food intake and body conditions between responding and non-responding individuals. Starlings were held on either short days (8 hours of light and 16 hours of darkness; 8L:16D) or long days (14L:10D) normally sufficient to cause full gonadal development within a short period. The birds on long days were given food for the full light period or for only 6 hours of their light day, starting from dawn (figure 2). Response of the birds is shown diagramatically in the figure. Those on long days with *ad lib* food showed full gonadal growth. Those on short days showed no gonadal growth. The group with restricted food period produced individuals with full growth and individuals with no growth (about 50% of each). There were no intermediate responses suggesting a simple physiological switch. Food consumption was identical for all long day groups; birds ate exactly the same amount of food irrespective of time available for eating. Responding birds ate the same amount of food as non-responding birds. Analysis of bodies at the end of the experiment showed insignificant differences in body fat content and body protein content (fat-free dry weight of pectoral muscle) between groups with no trend towards higher stored resources in responding birds.

There is, therefore, the beginnings of laboratory corroboration of field observations on the importance of food availability to breeding, though the mechanisms by which this is achieved remain obscure. Mechanisms are also obscure in field studies. One suggestion is that time spent to achieve food targets is used by birds to determine whether to commit resources to breeding. In the case of pigeons differences between sub-populations in onset of breeding could be explained in terms of time free for courtship behaviour after food needs were satisfied. The information from laboratory experiments, together with the field observations must be incorporated into testing of chemicals.

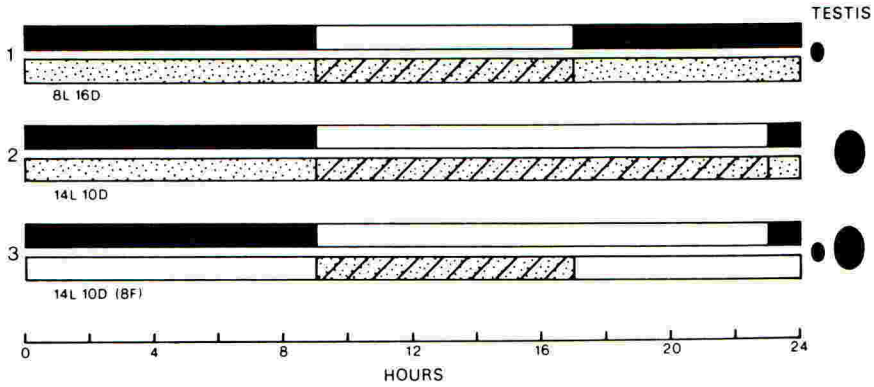

Figure 2. The upper bar in each case shows the light treatment during 24 hours. Lower bars show times of food availability (dotted) with time of feeding (hatched). Testicular response is to the right (to scale).

The lack of real field effect of a range of chemicals other than organochlorines on reproduction of birds, preclude a review of variation in action. It is, however, clear that many laboratory suggestions of effect have not been manifest in the real world. It is, therefore, difficult to justify the expense and time, together with the experimental animals used, devoted to a reproductive test which appears not to address important factors related to breeding of organisms in the field. It is also difficult to justify a standard test which is based largely on experience with one class of chemicals, the organochlorines.

It remains important to predict future adverse effects of chemicals on bird reproduction. The way forward would seem to be the identification of the critical factors used by birds to begin breeding and the physiological systems by which successful breeding is achieved. The latter is well studied in birds and the detailed physiological studies on breeding need to be incorporated into test design. A test, or series of tests, could then be devised to establish effects of chemicals on these critical points, both environmental and physiological, which determine reproductive success in the field. Test systems need to be approached from the direction of susceptibility of biological systems rather than from the direction of effects of previously identified dangerous chemicals.

Field trials have a part to play in the investigation of reproductive effects. However, the only trials reported, which have given reliable results (eg. Busby et al. 1990), have been large scale studies which are unsuitable for routine use. Identification of critical factors and their investigation in the laboratory should help in both the design and the running of field trials aimed at specific effects, rather than attempting to be all embracing.

Current laboratory testing for reproductive effects on birds is based on an oversimple model of reproductive cycles. Incorporation of research on environmental variables other than light should both clarify results of laboratory tests and allow the development of simpler test procedures over shorter time scales aimed at critical points in the reproductive process.

Insufficient field evidence of reproductive effects of chemicals on birds is available to approach the problem from this direction. Better design of laboratory studies of chemical effects would allow better design of field experimentation and each should complement the other.

SUB-POPULATIONS

Little work has been done in the laboratory on different exposure and different effects on sub-populations of organisms. Laboratory tests have tended to dose all test animals evenly and to ignore social interactions between individuals by separate caging of test birds. In the field, there is clear and observable sub-division of the population into subgroups. Only a small proportion of a field population will breed successfully and most studies have shown that a very small number of individuals succeed in passing on genetic material to succeeding generations.

The effects of chemicals will depend on both availability of the compound to the organisms and interaction between the compound taken into the body and the physiological and biochemical processes going on at the time of exposure. The effect will be greatest, from the point of view of the biology of the species, if breeding individuals obtain more chemical and are more susceptible than their non-breeding conspecifics. No attempt is currently made to assess either diversity in uptake or diversity in effect between these different sub-populations. This is a further area where field ecological information needs to be incorporated into laboratory testing.

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BENEFICIAL ARTHROPOD TOXICITY ASSESSMENTS WITH THREE INSECTICIDES IN LABORATORY, SEMI-FIELD AND FIELD STUDIES.

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ABSTRACT

A range of tests were conducted of the effects lambda-cyhalothrin, dimethoate and pirimicarb, applied at rates recommended for aphid control, on two aphid predators, a carabid beetle and a spider. The results were evaluated in the light of the known profiles of these compounds from the field. All the tests reflected the profiles qualitatively but quantitatively some tests were more severe than others. Most severe was the track-sprayer contact and soil residual test, followed by track-sprayer contact, field spray, track-sprayer soil residual and lastly microapplicator contact tests. An examination of the residues of lambda-cyhalothrin in dosed carabid beetles showed that they declined by 29% in 24h in the field and by 86% at 15°C in a CT room. Despite this, all the non-field methods used still produced a higher deposit on the animals than the field spray. The track-sprayer contact and soil residual test would appear to be the most suitable for a Tier I study as it gives the right results qualitatively but tends to be more severe quantitatively, therefore representing a worst case.

INTRODUCTION

Currently, there are many protocols for assessing the effects of agrochemicals on beneficial and non-target arthropods. However, with the possible exception of honey bees (Felton, Oomen & Stevenson 1986), there is no proposed way in which the results gained from one tier of testing can be reliably interpreted in relation those of the next. Many European countries are now raising the importance of beneficial arthropod studies in the registration process and are considering labelling products according to their risks to beneficials. It is therefore important that the relationship between the results obtained at different tiers of testing is well understood and that the framework for interpretation of the results is agreed, as has recently been done for terrestrial vertebrates (Somerville & Walker 1990).

With beneficial arthropods, there are arguments for making the Tier I test either in the laboratory with a microapplicator, in a greenhouse with a track sprayer or in field micro-plots. In order to investigate the effect of test protocol on the outcome of such a Tier I experiment, the lethal and sub-lethal effects of three aphicides, lambda-cyhalothrin, dimethoate and pirimicarb on two aphid predators (a carabid beetle and a lycosid spider) were compared using microapplicator contact dosing,

track-sprayer residual soil, contact, residual soil and contact exposure of arthropods and a field spray over micro-plots. In each test, assessments were also made of the insecticide residues picked up by the test animals. The results from this study are compared to the known profiles of these compounds obtained in full field trials over a number of years.

MATERIALS AND METHODS

Test chemicals

Lambda-cyhalothrin

As a 5% EC formulation, prepared from fully characterised technical material, found to actually contain 5.52% lambda-cyhalothrin on analysis (August 1989). Application rate 7.5 g AI/ha in 200 l of water in the track sprayer, 300 l in the field.

Pirimicarb

As a 50% SG formulation, prepared from fully characterised technical material, found to actually contain 49.3% pirimicarb on analysis (August 1989). Application rate 140 g AI/ha in 200 l of water in the track sprayer, 300 l in the field.

Dimethoate

As an EC formulation, nominally containing 400 g/l, found to actually contain 379 g/l on analysis (May 1989). The Application rate was 400 g AI/ha in 200 l of water in the track sprayer, 300 l in the field.

Test animals

Test animals were held under test conditions of 15^{o}C, 16/8 h light/dark and 70 % r.h. in a constant temperature (CT) room. They were held on sieved Jealotts Hill soil, a fine sandy loam (New Jersey scale) with 3.3% organic matter, in plastic coffee-cup cages. These cages consist of a cut-down 4 cm plastic coffee cup base (inner container) filled with soil and with a drainage hole. This sits in a full sized 9 x 7 cm coffee cup also with a drainage hole and a mesh lid. The soil was moistened by standing on soaked capillary matting after spraying, and subsequently daily with a wash-bottle.

Healthy adult carabid beetles (Pterostichus melanarius) were collected in pitfall traps at Jealotts Hill between June and August 1989, this ensured that they were all newly emerged adults of the summer 1989 cohort. Prior to testing, they were held in boxes of well rotted pig manure, being largely maintained on its concomitant fauna and their diet supplemented by dead blowfly larvae. Healthy lycosid spiders were collected at least 24 h before a test with an aspirator. They were held individually in plastic coffee cup cages and fed on dead Drosophila pupae. A majority of those tested were Pardosa spp, further taxonomic resolution being impossible on live specimens.

Test procedure

Test solutions were prepared in deionised water with "AGRAL" as a

wetter (500 mg/l). Controls were deionised water with the same concentration of "AGRAL". All application solutions were analysed for concentration and the volume-output of the track-sprayer checked by re-weighing filter papers after spraying and by deposition cards in the field plots. Actual application rates were very close to nominal ones.

For each application method, two replicate samples of animals were dosed separately for residue analysis. Additionally, samples were either (1) frozen to -18°C directly after dosing, (2) held under field conditions for 24h first or (3) held under laboratory conditions for 24h first, before freezing.

Microapplicator

For the microapplicator tests, the application rate in ng AI/arthropod was calculated from the application rate in g AI/ha and a manual assessment of the two-dimensional dorsal silhouette of a sample of the test animals, these are rates given in Table 1.

TABLE 1. Rates of dosing test animals with the microapplicator

Compound	Dose applied in ng AI/arthropod	
	Carabids	Lycosids
Lambda-cyhalothrin	56	18
Pirimicarb	1040	336
Dimethoate	2590	840

Dosing consisted of three replicates of 10 animals for each species. Animals were anaesthetised with carbon dioxide and the dose applied to the dorsal body surface with a "Burkard" microapplicator fitted with a syringe and a 25 gauge needle. After treatment, arthropods were returned to fresh holding cages and assessed for six days.

Track-sprayer

Applications were made to soil only (soil residual), animals only (contact) and animals and soil combined (soil residual and contact), each representative of a type of test under evaluation. Three replicate runs of ten animals were conducted with each species. The track-sprayer was fitted with a Tee Jet 8001E nozzle, 20 cm above the target. Spraying was conducted at 200 l/ha, 40 psi. After treatment, arthropods were returned to fresh holding cages and assessed for six days.

Field Plot

Enclosures of $1m^2$ were placed in spring wheat (sown 28 March 1989). There were three replicate test runs, each of two enclosures (a & b) per beetle or spider per treatment. To distinguish them from animals resident in the plots, beetles were marked by scratching the elytra and spiders with "TIPEX" typewriter correction fluid. After marking, they were held for 24h and the healthy ones released into the enclosures 48h before spraying. Ten beetles or 10 spiders were released into each respective (a) enclosure. In each (b) enclosure, beetles and spiders were placed on sieved test soil

in plastic dishes and returned to their holding cages and then to the laboratory for six days of observations within 2h of spraying. Spraying runs were conducted on 15 and 23 June and 14 August 1989. The sprayer had three 50cm-spaced Lurmark 02-F110 flat fan (orange) nozzles and was operated at 35cm above the crop, at 40 psi with a forward speed of 0.8 m/s. Mortality in the plots was assessed by operating pitfall traps from 7 days after spraying to re-capture survivors in the first run, but this was reduced to 24h for runs 2 and 3 as hyperactive animals were not noted beyond 24h.

Assessment procedure

Assessments of the symptomology of test animals in holding cages were made at 30 min (where possible), at 1, 2, and 3h and daily for 6 days. At the final assessment, any animals that had buried into the soil were dug up and assessed; symptomology assessment categories are shown in Table 2.

TABLE 2. Symptomology assessment categories.

Category	Description
A	Healthy: alive and active, normal behaviour
B	Uncoordinated: poor co-ordination of movement
C	Considerably affected: lying on back with twitching legs, does not walk when blown on
D	Dead: no response when blown on
X	Buried

Residue analysis

Samples of 1g of beetle were macerated in 60 ml of 50% acetone:hexane. The entire solution was filtered under vacuum and the residue was washed with further 50% acetone:hexane. The filtrate was washed twice with 50 ml of NaCl solution and the hexane layer passed through a sodium sulphate bed to remove any remaining water. The hexane was then evaporated to low volume and loaded onto a florosil adsorption column. After elution with 35 ml of 30% dichloromethane:hexane and using a prewash of 5% dichloromethane:hexane, the eluate was evaporated to dryness and the samples taken up in 1 ml of hexane and analysed using gas-liquid chromatography with electron capture detection.

RESULTS

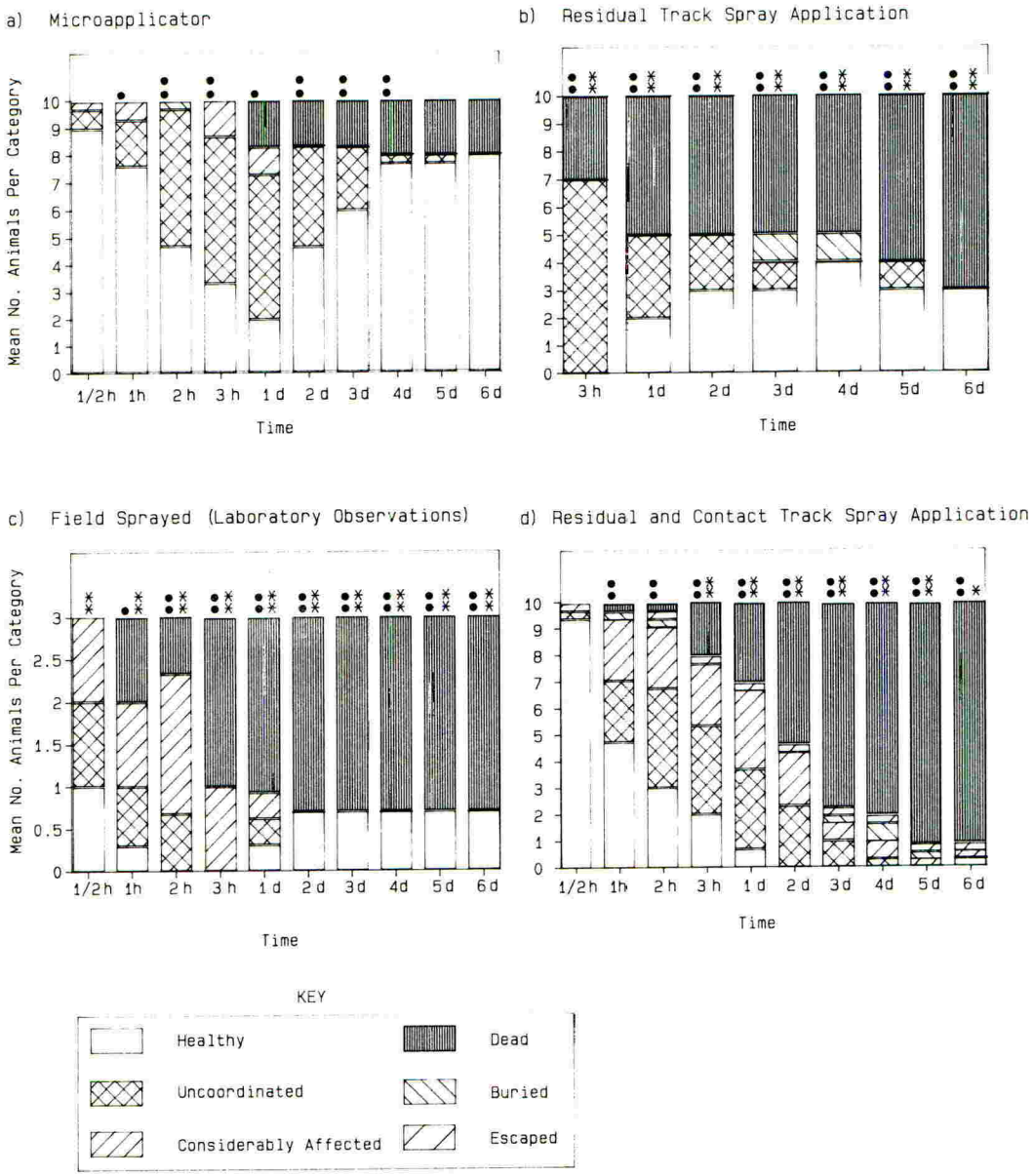
The percentage of dead and sublethally affected animals at six days are shown in Table 3. To illustrate the range of sub-lethal effects, Figure 1 shows all effect-categories for lycosid spiders and lambda-cyhalothrin using four of the five different methods. The residues of lambda-cyhalothrin in carabid beetles following dosing via the different methods is shown in Table 4 and the stability data in Table 5.

Table 3. The percentage of dead and sub-lethally affected animals at six days after treatment via different methods.

Treatment	Beetles		Spiders	
	% Dead	% Affected Lethally and Sub-lethally	% Dead	% Affected Lethally and Sub-lethally
LAMBDA-CYHALOTHRIN				
Microapplicator contact	7	7	20**	20**
Track-sprayer soil residual	20	20	70	70
Field spray	22	22	78	78
Track sprayer contact	10	20	100	100
Track spray soil residual and contact	20	20	93	97
DIMETHOATE				
Microapplicator contact	17	17	0	0
Track-sprayer soil residual	10	10	0	0
Field spray	33	56	22	22
Track-sprayer contact	40	70	0	0
Track-sprayer soil residual and contact	80	87	27	27
PIRIMICARB				
Microapplicator contact	3	3	0	0
Track-sprayer soil residual	0	0	0	0
Field spray	0	0	0	0
Track-sprayer contact	0	0	0	0
Track-sprayer soil residual and contact	0	0	3	3
CONTROL				
Microapplicator contact	0	0	0	0
Track-sprayer soil residual	0	0	0	0
Field spray	0	0	11	11
Track-sprayer contact	0	0	0	0
Track-sprayer soil residual and contact	0	3	0	0

** Significantly different from field spray of the same chemical (1%)
There were no significant differences at 5%

Figure 1 : Lycosid Mortality and Sublethal Effects After Lambda-cyhalothrin Applications Using Four Different Dosing Methods



• Significantly different From Control At 5% Level (No's Affected)
•• Significantly different From Control At 1% Level (No's Affected)

* Significantly different From Control At 5% Level (No's Dead)
** Significantly different From Control At 1% Level (No's Dead)

Table 4. The residues (µg/g) of lambda-cyhalothrin in carabid beetles dosed via different methods.

Method	Mean	95% CL
Microapplicator contact	0.04**	0.02**
Track-sprayer soil residual	0.16**	0.08**
Field spray	0.01	0.00
Track-sprayer contact	0.21**	0.11**
Track-sprayer soil residual and contact	0.09**	0.03**

** Significantly different from field application (1%)
There were no significant differences at 5%

Table 5. The stability of lambda-cyhalothrin residues (µg/g) after freezing beetles immediately following microapplicator dosing or after 24h in field or laboratory holding conditions.

Holding regime	Mean	95% CL
Frozen immediately	0.21	0.25
Frozen after 24h in CT room	0.03	0.05
Frozen after 24h in the field	0.15	0.20

DISCUSSION

Overall, the tests could be ranked in the severity of effects they produced, from most to least severe, as track-sprayer soil residual and contact, track-sprayer contact, field application, track-sprayer soil residual and microapplicator contact. However, due to the variability in the field results, there were few significant contrasts that could be made. None of the effects in the carabid beetle laboratory tests were significantly different from the field applications six days after dosing. However, with lycosid spiders, the microapplicator results significantly underestimated the field toxicity with one compound. This could be due to poor estimation of the surface area or poor chemical absorption from large 1 ul drops, which could also roll off more easily than smaller spray-mist drops.

The exposure of the animals was investigated through residue analysis and to date, the analysis for lambda-cyhalothrin in beetles only has been completed. The stability study showed that compared to animals frozen

immediately after dosing, those held in the CT room lost 86% in 24h whereas those held in the field lost 29%. However, residues recovered from all the laboratory dosed animals were higher than those from the field, suggesting that the laboratory exposure based on full application rates must be considerably greater than that actually experienced by these animals in the field where they largely inhabit the understory of the crop. This supports the analysis of Jepson et al (1990) who predict that exposure to fresh residues of the pyrethroid deltamethrin on the flag leaf will lead to 60-70% mortality in the carabid beetle Bembidion lampros whereas exposure to them on the soil under the crop will result in a mortality of around 10%.

All the tests confirmed the qualitative pattern of effects known for these products in the field. Pirimicarb caused no effects on beetles or spiders even when they received the full field dose, as is well known from the field (e.g. Cole & Wilkinson 1984). Lambda-cyhalothrin and dimethoate both affected the spiders and beetles in the laboratory to an extent that matched what is seen in the field (e.g. Brown, White & Everett 1988).

If any of these studies are to be used as a Tier I screening test they need to be repeatable and representative of a worst case in the field. From this experiment it would appear that the most suitable test would be a track-sprayed soil residual and contact one.

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SESSION 6C

ADVANCES IN THE FORMULATION, PACKAGING AND APPLICATION OF PESTICIDES

SESSION ORGANISER DR G. LE PATOUREL

POSTERS 6C-1 to 6C-11

EFFERVESCENT TABLETS OF'TOPAS', INNOVATIVE FUNGICIDE FORMULATION TECHNOLOGY

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ABSTRACT

Effervescent tablets resulted from CIBA-GEIGY's continuous effort to improve the benefit profiles of its agricultural products and represent an innovation in fungicide formulation. New technology was developed to provide growers with an easy and safe to use, and environmentally sound product (no measuring of powders or liquids; easy to open packs with virtually no contamination of the primary packaging). In addition market requirements are met, namely for large tablets with short dissolution, excellent dispersion and fast handling properties. A granulated mixture of potassium carbonate, a water-soluble acidic substance and a water-absorbing, water-insoluble compound serves as an effervescent dispersing system which blended with penconazole, surfactants and inert materials is pressed into effervescent tablets with ≤15% penconazole. The fungicide content meets crop-pathosystem specific and grower's ease-of-use requirements. Tablets disintegrate in water within three minutes, are suitable for dilute and concentrate spraying, and provide excellent powdery mildew control.

INTRODUCTION

To anticipate and meet evolving user's needs with environmentally compatible products, i.e. to improve the benefit profile of products is a declared corporate strategy at CIBA-GEIGY. Effervescent tablets, particularly suited for products which are effective at very low rates, are the latest addition to the CIBA-GEIGY range of novel formulations for agriculture. Tablets are new formulation technology for fungicides, and original ideas were required to develop and launch a marketable product with a benefit profile which in many aspects is superior to currently available problem solutions in the targeted markets.

COMPOSITION AND PRODUCTION

Current Technology

Effervescent tablets are usually composed of (i) the active ingredient (AI), (ii) an effervescent dispersing system and (iii) formulation inerts, such as surfactants, gliding, binding and filling agents (Mohrle 1980).

For the AI it is its solubility, or if water-insoluble, its particle size and melting point which determine its suitability for pressing into tablets; the smaller the particles and the lower the melting point, the more difficult it is to produce effervescent tablets from a particular AI. Requirements for good suspensibility and sprayability of water-insoluble AI go the other way, i.e. the smaller the particle size the better. The challenge of tableting lies in resolving this conflict.

Effervescent systems are composed of ingredients which upon contact with water develop the gas which breaks apart the formulation and disperses the AI. Widely used are CO_2 developing systems containing a metal alkali carbonate with tartaric or citric acid. Surfactants are needed to keep water-insoluble ingredients of tablets in suspension after the preparation of the spray mixture. The choice of surfactants is determined very much by the other ingredients of the tablet. Gliding, binding and filling agents are normally necessary to facilitate the pressing process of tablets, salts of stearic acid and polyglycolethers, lactose or starch are generally used.

There are two general tablet pressing processes: direct compression of powders, and compression of granules.

Effervescent tablets of penconazole

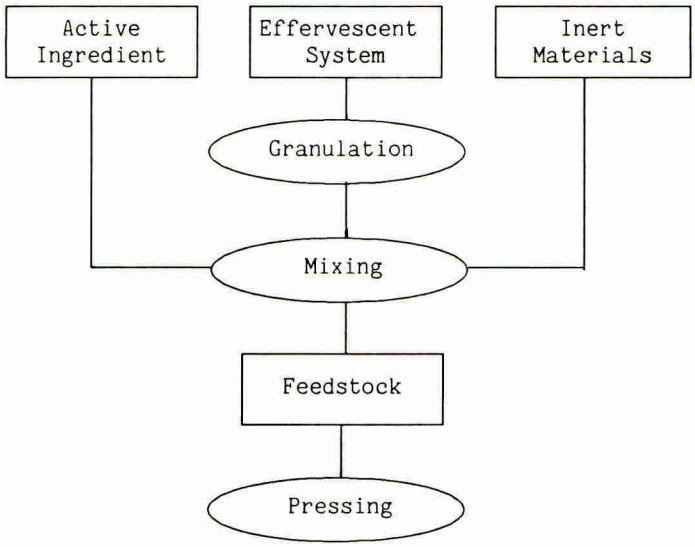
Producing effervescent tablets of the fungicide penconazole was a particular challenge because in addition to dealing with a water-insoluble AI with a low melting point, the following market-imposed requirements had to be met, (i) short dispersion time, (ii) fast and easy dosing, requiring a minimum number of tablets per hectare and thus relatively highly concentrated tablets, (iii) excellent suspensibility and sprayability, and (iv) large sized tablets in order to avoid confusion with pharmaceutical tablets.

Penconazole has a melting point of 60 °C and tends to form agglomerates during storage particularly when finely milled. The high pressure applied during compression of the tablets can also lead to agglomeration of the AI. Agglomerates in tablets are undesirable since they do not disintegrate sufficiently and would cause spraying and biological performance problems. Anticaking agents are employed to prevent the formation of agglomerates, and silicic acid can serve the dual purpose of anticaking agent and milling aid.

The direct compression method proved to be less suitable for penconazole tablets. During tableting problems occurred often with tablets either disintegrating in a top, middle and bottom portion, or having too low a physical strength. This suggested the introduction of a pre-granulation step prior to the pressing process. Pre-granulation of the entire tableting feedstock was not successful, because the resulting tablets did not disintegrate well enough. Pre-granulating the effervescent dispersing system (Fig.1) proved to be most suitable; when pressed into tablets together with the other two components tablets which were both physically strong and yet easily dispersing could be produced. The market requirements for a dispersion time of less than 5 minutes excluded sodium carbonate or bicarbonate as the effervescent dispersing system for penconazole and led to using the much more reactive potassium carbonate. However the higher reactivity of this system required a means to overcome its many handling and stability problems and this was achieved by developing a special process (Zellweger 1990). Potassium carbonate and citric acid together with a finely distributed water-insoluble solid were granulated with the help of an

alcohol. These granules proved very suitable for tableting the large size tablets of penconazole. The water-insoluble solid also absorbs water and was selected from a range of aluminum oxide, silicic acid and clay minerals. The granulation process decreases the reactivity of the components with ambient air humidity without impeding the reactivity of the final tablet with water.

Figure 1: Production flow chart for penconazole tablets



The inert materials, surfactants, solid dispersing, wetting, binding and filling agents used for tableting were selected from the usual range of products well known from wettable powder and tableting technology. Incorporation of a gliding agent also improved the tableting process.

Following the pressing process, tablets are immediately sealed into aluminum sleeves or plastic/aluminum blisters serving the dual purpose of protecting the tablets from humidity and, together with the outer pack, from physical impact.Using this process and given a tablet diameter of 50-76 mm, tablets containing up to 15 % wt/wt penconazole can be produced, the AI content being determined by the specific submarkets and user's needs. The tablet range presently comprises three products (Table 1).

TABLE 1: Properties and use rates of commercial penconazole tablets (TB)

	TB 1.25	TB 2.5	TB 3.75
AI, g	1.25	2.5	3.75
weight, g	30	30	50
diameter, mm	50	50	76
dissolution time, min	5	5	3
dosage rate	1-2 / 100 l	1-2 / 100 l	4-7 / ha

BIOLOGICAL ACTIVITY

The effervescent tablet formulations of penconazole were tested in extensive field trials during the last 2-3 years on grapes, apples, cucumbers, melons, artichokes and beans. The tablets were compared in their activity to penconazole formulated as an emulsifiable concentrate (EC) and a wettable powder (WP). The penconazole rates tested ranged from 1.5 to 5.0 g/100 l and the spray volume was 700-1000 l/ha in grapes or vegetables and 1000-1500 l/ha in apples. Crop tolerance was excellent in all crops tested. Disease control tests concentrated mainly on powdery mildews since penconazole exhibits a particular strength against this disease (Eberle *et al.* 1983). High level and season long control was achieved against ***Uncinula necator*** on grapes at 15-25 g AI/ha, ***Podosphaera leucotricha*** on apples, and ***Erysiphe cichoracearum*** on cucumbers at 2.5 g a.i./100 l, tablets, EC and WP formulations giving equal activity as shown in Tables 2 and 3.

TABLE 2. Activity against ***Uncinula necator*** on grapes. Average of 6 trials, France 1988.

Treatment/Formulation	Dose g AI/ha	% infected leaves	% infected bunches	% bunch surface infected
penconazole tablet	15	4	9	1
penconazole tablet	25	3	4	0
penconazole EC	15	4	7	1
penconazole EC	25	4	3	0
untreated	-	45	72	50.0

TABLE 3. Activity against ***Podosphaera leucotricha*** in apples and ***Erysiphe cichoracearum*** on cucumbers

Treatment/Formulation	Dose g AI/100 l	*P. leucotricha* % infected leaves	*E. cichoracearum* % infected leaf surface
penconazole tablet	2.5	0	11.3
penconazole WP/EC [1)]	2.5	0	11.3
untreated	-	59	50.0
Number of trials		2	3

[1)] WP in apples, EC in cucumbers

Tank mixtures of penconazole tablets with mancozeb provided 96 to 99% control of apple scab (***Venturia inaequalis***) on leaves and fruits and were similar to a corresponding ready made WP formulation.

APPLICATION

Tablets disperse well without agitation in low as well as in high volumes of water; concentrations per 100 l of up to 20 of the TB 3.75 formulation were tested in a variety of commercial equipment and found to have excellent dispersion and sprayability properties making tablets suitable for virtually any ground and aerial spraying equipment.

Tank mix compatibility with a standard range of plant protectants for fruits, grapes and vegetables showed that penconazole tablets are compatible with over 30 commercial plant protectant formulations. It is generally recommended to wait until after the tablets have self-dispersed before adding other poducts to the tank.

General use recommendations for spraying with tablets range between 1 to 2 tablets per 100-150 l in a dilute spray (Table 1).

BENEFITS OF TABLETS

The continuous replacement over the past decade of older types of formulations with more modern ones (Davet 1990) reflects the growing importance of the formulation and presentation of plant protection products as a buying motive for growers, and the determination of industry to improve handling, transportation and storage, and pack disposal properties of agricultural products. Tablets surpass the largely prevailing emulsifiable concentrates and wettable powders for most of those parameters (Table 4).

TABLE 4. Benefit Profile of Tablets vs Alternative Formulations

	Tablet	EC	WP
Handling Properties			
economical (no waste)	+++	++	++
safe (contact, odor)	+++	+	+
clean (contact, odor)	+++	+	+
fast (getting ready to spray)	+++	+++	+
easy (unpacking, dosing, weighing)	+++	++	+
tamper proof	+++	+	+
Transportation & Storage Properties			
non-flammable (solvent free)	+++	o	+++
safe (non-spillable)	+++	+	++
compact (bulk density)	+++	+++	+
durable (shelf life)	+++	++	++
frost hardy	+++	++	+++
Pack Disposal Properties			
empty packs free of product	+++	+	+
environmentally friendly disposal	+++	+	++

As far as handling properties are concerned, tablets are clearly superior to both liquid and powder formulations, with the major benefits deriving from having a pre-measured dose rate which avoids waste and

eliminates the need for weighing or measuring. Combined with the appropriate blister or sleeve packs all possible contact by the user with the product can be avoided. Tablets are thus not only cleaner and safer to handle than those alternatives but working with tablets is also easier and faster, and can be more economical. The fact that tablets rise to the surface of the spray solution shortly before they have completely self-dispersed, is an additional attractive handling feature since it indicates to the grower when the spray solution is ready to be applied. A further possible benefit of tablets is that they are neither easily confused with other products, nor can they readily be adulterated and are thus virtually tamper proof.

The benefits of tablets concerning transportation and storage derive mainly from the absence of any solvents in the TB formulation; tablets thus represent no fire hazard. Tablets also have the advantage of being less sensitive to frost than many liquid products thus minimizing the concerns of shipping and particularly of on farm storage during winter.

Modern requirements for packaging go beyond sustaining the ease of use of the product. Packs which when empty are free of product residue and which can be destroyed easily and without polluting are not only demanded by growers but by the public at large and consequently governmental authorities and legislators. Tablet packagings meet those modern requirements for both, ease of use and environmentally friendly disposal.

Because of these benefits penconazole tablets have the potential to change the way powdery mildew is controlled now. Market acceptance studies in France and Italy have confirmed the attractiveness of penconazole tablets to the trade and to the growers.

ACKNOWLEDGEMENTS

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A TECHNIQUE FOR THE EVALUATION OF NOVEL FORMULATIONS FOR USE AS SPACE SPRAYS AGAINST PUBLIC HEALTH INSECTS

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ABSTRACT

A test technique using portable and disposable polythene cubicles (tents) has been used to evaluate the toxicity of space sprays to three public health insect pests. Novel water-based formulations of cypermethrin and bioresmethrin have been evaluated against the German cockroach (*Blattella germanica)*, Pharaoh's ant (*Monomorium pharaonis*) and resistant and suceptible strains of the common housefly (*Musca domestica*). Dose-response data obtained from these tests shows that 4 ml/m^3 cypermethrin formulation gives complete knockdown of flies and ants, but that there was some survival of cockroaches.

INTRODUCTION

The control of many public health pests is often achieved by the use of insecticides applied as space sprays. The success of space spray treatments will be determined by the species of insect, environmental conditions, insecticide active ingredient and the way in which it is formulated. In order to test candidate space spray formulations it is necessary to be able to control the variables which influence insecticide performance. A number of techniques have been devised over the years. These are usually based on the release of insects into controlled environment rooms and subsequent treatment of the room air space with insecticide. Some of these procedures have been adopted as standards (for example BS4172) within the industry. To prevent test room contamination between treatments thorough cleaning of surfaces is essential. When rapidly degrading insecticides such as pyrethrins are tested normal cleaning practices will be sufficient to ensure that surfaces are satisfactorily decontaminated. However, the testing of more persistent insecticides such as permethrin and cypermethrin has given rise to problems with the retention of deposits requiring elaborate cleaning or lining procedures to prevent contamination. In response to the need to test space spray formulations of photostable pyrethroids at The Central Science Laboratory (CSL) Slough, a test technique based on disposable polythene cubicles has been developed. Use of fresh cubicles for each test ensures that there is no carry over of any toxic deposits between tests.

The tests reported in this paper were carried out to evaluate novel water-based formulations of cypermethrin and bioresmethrin. These microemulsion formulations have been developed by NCH (Europe) in response to a need for effective insecticides which contain little or no organic solvents. In addition microemulsions appear to offer several advantages when

compared with conventional emulsion concentrate type systems:

1. Superior distribution of active ingredient in spray droplets.

2. Excellent stability characteristics, both as a concentrate and after dilution to field application rate.

3. Parity in biological activity, and in a number of cases enhanced activity.

4. Droplet size and hydrophilic-lipophilic balance can be readily controlled using a selection of salts and surfactants to give optimum efficacy. (Lankford, In Press; Scrimshaw *et al.* In Press).

Microemulsions are a clear thermodynamically stable dispersion of two immiscible liquids. The droplet size of the dispersed phase is normally in the range 10-100 nm. They can be considered as two phase systems in which the stabilising monolayer consists of a primary surfactant and a cosurfactant (a mixed film) which is probably penetrated to some extent by molecules of the oil phase.

MATERIALS AND METHODS

Insects

The following insects were used; adult females of the Laboratory susceptible strain of *B. germanica*, females of the Cooper susceptible and Woodhouse resistant strains of *M. domestica* and adult (workers) Pharaoh's ant *M. pharaonis*. The insecticide susceptible strains of *B. germanica* and *M. domestica* have been maintained at CSL for over ten years, and during this time have not been exposed to insecticides. In previous laboratory tests, the Woodhouse strain of *M. domestica* was shown to be resistant to pyrethrins + piperonyl butoxide (1:10 wt/wt) by a factor of x3 at the KD_{50} level. *M. pharaonis* were tested when 0-10 weeks old, *B. germanica* 4-8 weeks old, and *M. domestica* 3-5 days old.

Insecticides

Novel water external microemulsion space spray formulations of 3g bioresmethrin/litre and 1g cypermethrin/litre were assessed. Each formulation was sprayed at 5, 10, 20 and 40 ml/tent (equivalent to 0.49, 0.99, 1.97 and 3.95 ml/m^3). Validation of the concentration of active ingredient in each formulation was carried out using HPLC analysis after the bioassays had been completed. Both formulations were found to be within ±10% of the stated concentration of active ingredient.

Experimental techniques

The test environment consisted of clear polythene (175 micron) cubicles (tents) measuring 2250 x 2000 x 2250 mm high. Access into the tent for spraying and for placement, removal, and assessment of insect bioassays was through a vertical 1500 mm plastic zip located centrally in one face (width 2000 mm) of the cubicle. A clear acetate window 250 x 250 mm positioned on one side of the tent was used for test observations.

An Aerograph(R) airbrush was used to spray the insecticide formulations or water controls into the tents through the zip sealer which was opened at the top just sufficiently to allow the operator's hand and airbrush to pass through. To improve distribution the nozzle was traversed from side to side whilst the airbrush was inclined slightly upwards from horizontal thus ensuring treatment of the upper air space. The insecticides were applied as provided.

Insect bioassays

B. germanica females were removed from cultures to 75 mm x 25 mm dia glass tubes (10/tube) the day prior to testing and left overnight without food at 20°C and 50% r.h. For the assay procedure, *B. germanica* were confined in 100 mm dia glass crystallising dishes (10 insects/dish), the inside rims previously coated with vaseline (yellow/white 1:1 wt/wt) to prevent escapes. Five replicate dishes were used in each tent. A single replicate was placed in each corner, approximately 30 cm from the tent wall, and the fifth replicate was placed in the centre.

M. pharaonis were removed from culture and counted into batches of 25 just prior to testing. Each batch was confined on a 70 mm dia Whatman No 1 filter paper (placed on a glass plate for support) within a 60 mm dia stainless steel ring coated with Fluon(R) (PTFE) to prevent escapes. Four batches of *M. pharaonis* were used in each tent, one placed in each corner approximately 30 cm from the tent wall.

M. domestica were sexed and counted into batches of 100 females the day prior to testing. They were left overnight at 20°C and 50% r.h. in 70 mm x 110 mm dia polystyrene pots with access to 10% sugar solution. One hundred flies were released into each tent immediately prior to spraying.

All tests were carried out at 20°C ± 1°C and 50% ± 5% r.h. For each dose of insecticide there were four replicate tests plus one control sprayed with water at the appropriate volume. Insects were exposed to the insecticide spray for 1 h (which included the spraying time), after which the air within each tent was extracted for a 10 min period. All insects were assessed for knockdown (KD) 1.25 h after spraying had commenced (insects were considered knocked down if they were unable to co-ordinate their locomotory movements and regain a normal stance). After the 1.25 h KD assessment the houseflies and cockroaches were transferred to constant temperature and humidity rooms at 20°C, 50% r.h. and 27°C, 45% r.h. respectively. Assessment counts were then carried out on both species at 24 and 48 h.

Statistical Analysis

Arcsine transformation was used on all knockdown data. Analysis of variance was then carried out on the data to determine variations between doses, time and the dose-time interaction. Further comparisons, where appropriate, were then made using two tailed t-tests on batched totals of 48 h counts only.

RESULTS

Analysis of variance showed that there was a significant difference in knockdown (KD) between doses and between count times for cypermethrin when tested against *B. germanica* and both strains of *M. domestica* ($P<0.01$ in all cases). With bioresmethrin there was no difference in KD for the Cooper susceptible strain between doses or count times, but there was a significant difference in KD for the Woodhouse strain ($P<0.01$) between doses and count times. For *B. germanica* there was only a difference in KD between doses ($P<0.05$).

M. pharaonis

Due to the likelihood of high control mortality after 24 h without food, knockdown assessments were carried out at 1.25 h only; during this period there was no control mortality. All the ants exposed to the three doses of cypermethrin were knocked down (Fig 1). Bioresmethrin was marginally less effective, with 92.5% KD at the lowest dose of 5 ml, but all were knocked down at 10 and 20 ml (Fig 2).

B. germanica

There was no control mortality in any of the tests. Cypermethrin was ineffective against adult female *B. germanica* at 5 and 10 ml/tent (Fig 1). However, an appreciable level of KD was achieved at 20 and 40 ml/tent, where, after 48 h, 73.9% and 99% respectively were knocked down. The difference in KD between these two doses after 48 h was significant ($P<0.01$). There was no knockdown with bioresmethrin at 5 or 10 ml/tent, and at 20 ml knockdown was only 7.0% after 48 h (Fig 2).

M. domestica

Control mortality of both strains was 3.0%.

Cooper susceptible strain

All insects were knocked down after 1.25 h at the lowest dose (5 ml) of cypermethrin. However, 2 flies had recovered by 48 h. At 10 and 20 ml/tent all insects were knocked down after 1.25 h and there was no recovery (Fig 1). Bioresmethrin was similarly effective, 99.5% were knocked down after 48 h at 5 ml, and all were knocked down at 10 and 20 ml (Fig 2).

Woodhouse Resistant strain

Knockdown after exposure to cypermethrin was ≥97.5% at all doses after 1.25 h (Fig 1). However, a proportion at each of the three lower doses recovered to give 48 h KD figures of 71.2%, 72% and 92.5% at 5, 10 and 20 ml/tent respectively. At 40 ml all flies were knocked down after 1.25 h with no subsequent recovery (Fig 1). After 48 h, significantly more flies were knocked down at 40 ml than at 20 ml ($P<0.01$), and similarly at 20 ml compared with 10 ml ($P<0.005$); there was little difference in knockdown (48 h) between doses of 5 and 10 ml.

Recovery from knockdown was also observed after exposure to bioresmethrin (Fig 2). After 1.25 h, knockdown at all doses was >92.0%, but after 48 h some insects had recovered to give 48 h KD figures of 82.2%,

92.0% and 98.7% at 5, 10 and 20 ml/tent respectively. At 40 ml all flies were knocked down after 1.25 h with no subsequent recovery (Fig 2). After 48 h significantly more flies were knocked down at 40 ml than at 20 ml ($P<0.05$) and similarly at 20 ml compared with 10 ml ($P<0.01$) and at 10 ml compared with 5 ml ($P<0.05$).

DISCUSSION

Both microemulsion space spray formulations were effective in knocking down all adult *M. pharaonis* after 1.25 h at 5, 10 and 20 ml/tent (except bioresmethrin at 5ml).

Similarly, all Cooper susceptible *M. domestica* were knocked down by both formulations after 48 h at the same doses. A 2-4 fold increase in the dose of both insecticides was required to KD all the flies from the Woodhouse strain (Fig 1 and 2). Recovery from KD by the Woodhouse strain was observed at 5, 10 and 20 ml/tent. Recovery from KD in strains of houseflies is known to occur with some pyrethroid insecticides and has been reported previously by Scott and Georgiou (1984).

Bioresmethrin was ineffective against adult female *B. germanica* at all doses tested. Some recovery from KD was observed with cypermethrin at 10 and 20 ml, but no recovery was observed at the highest dose of 40 ml (99% KD at 48 h). Although not recorded in the results section a further observation at 72 h indicated that all cockroaches were knocked down. Therefore, future work using this technique, particularly with pyrethroids, may require the period of observation for KD or kill to be extended.

The reliability of the results provided by this test method should enable future work to include the evaluation of insecticide efficacy and selection for insecticide resistance. Dose-response data achieved using this technique may also enable determination of resistance levels to be more representative of field application methods.

ACKNOWLEDGEMENTS

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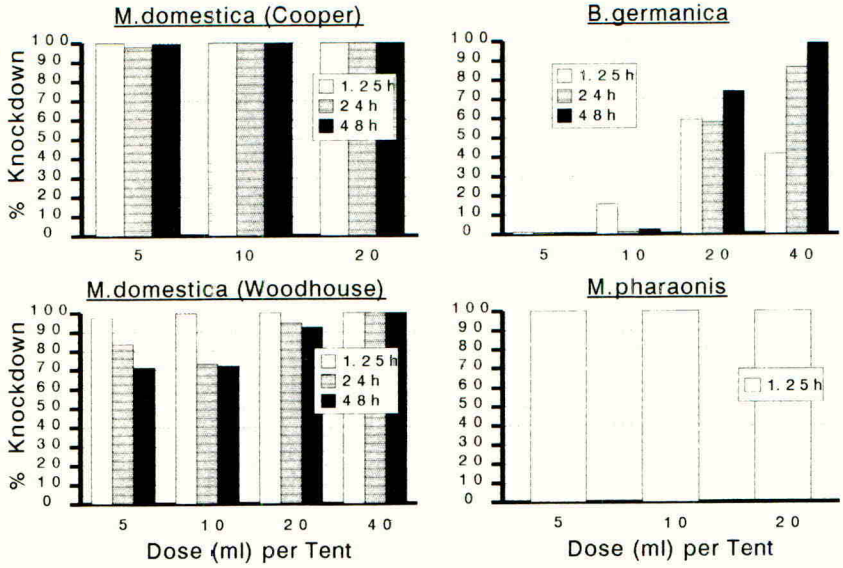

Figure 1. Knockdown (%) of three species of insect pests after exposure to cypermethrin space spray.

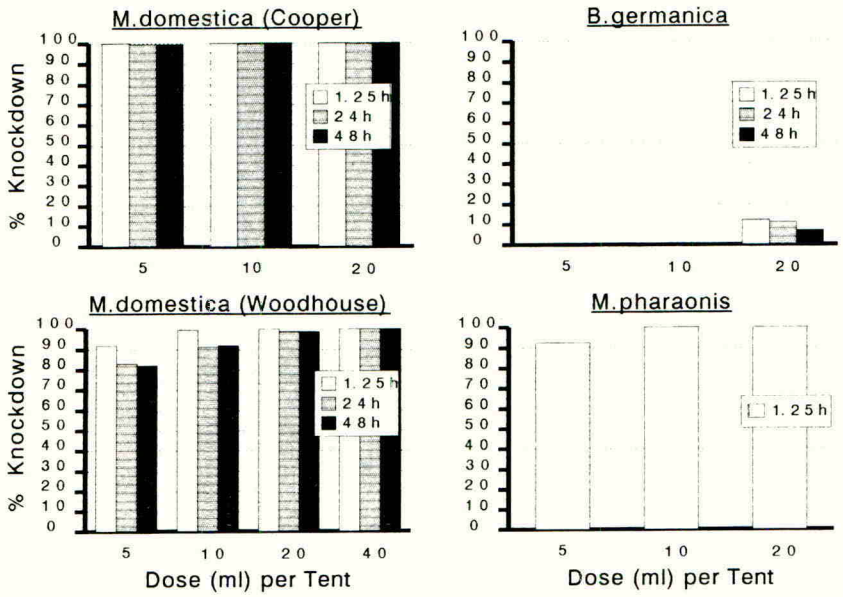

Figure 2. Knockdown (%) of three species of insect pests after exposure to bioresmethrin space spray.

A CLOSED-TRANSFER SYSTEM FOR SAFE HANDLING OF MULTIPLE PACKS OF PESTICIDE

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ABSTRACT

A closed-transfer system has been developed which permits the safe transfer of pesticide products to a sprayer tank. The packs are inserted unopened into a cabinet and pierced by vertical spears. Water under pressure is used to rinse the insides and outsides of the packs. All product and rinsings are transferred to the sprayer tank by venturi or pump. The system will handle most types of products and packs and leaves the packs rinsed clean and punctured. Measurements have shown significant reductions in operator contamination and system and pack residues are well within accepted standards.

INTRODUCTION

Handling concentrated pesticide products and transfering them to the application equipment is known to be the most hazardous part of the spraying operation. The process of opening liquid pesticide containers, sometimes several per tank load, removing inner seals, pouring the contents into the tank which may be at some height and rinsing out the empty containers has been shown by research in several countries to pose the greatest risk of operator contamination. A study by the British Agrochemicals Association in the UK showed that 71% of contamination occured whilst transfering the product and that 87% of that was on the hands (Anon (a) 1983). The introduction of a Code of Practice in the UK (Anon (b) 1990) has reinforced the need to eliminate this hazard by the use of 'engineering controls' to assist the handling and transfer of pesticide products.

Methods to reduce this contamination risk have been sought for the last decade, particularly in California where closed-transfer devices are mandatory for products in certain toxicity categories. In Europe, a number of devices have been developed more suited to the larger number of products and smaller containers generally found. These include suction probes, induction hoppers, pumps and measuring extraction probes (Frost, Miller 1988). Few of these are genuinely closed-transfer devices, most still requiring the operator to open the pesticide container. A group of European agrochemical companies recently commissioned the development of a true closed-transfer device able to measure out and rinse part containers, one container at a time (Lavers 1989).

An important aspect of the handling of pesticide products is the quality and form of the formulation and its packaging. There is a strong trend now to move away from solvent-based liquids towards water-dispersible granules and wettable powders packed in water-soluble film. Clearly any closed-transfer device must be able to handle all forms of product, both liquid and solid based.

The rinsing of pesticide containers of all types is now recognised as being of critical importance. A lead taken by the Netherlands authorities with their covenant (STORL) agreed by suppliers and users (pers. comm.) has established a limit of 0.01% of the original product weight as the maximum acceptable residue to enable the pack to be disposed of by normal means.

Despite all these developments it is certain that for the forseeable future many pesticide products will still be packed in small liquid or powder/granule containers which will need to be opened, rinsed and their contents transferred to the sprayer. The closed-transfer system ('Packman' ™) described in this paper has been designed and developed under farm conditions to:-

* eliminate the need to open packs manually.
* safely extract and transfer product to the sprayer.
* rinse clean the packs and transfer system.
* render the packs unsuitable for further use.
* handle up to 3 or 6 (larger version) packs at once.
* offer alternative use as an induction hopper.
* speed up the transfer and rinsing operation.

It can handle and transfer a wide range of:-

* products: liquids, powders, water-dispersible granules.
* pack types: metal, plastic, cardboard, paper and soluble.
* pack sizes: liquids - 1 to 20 litres.
  solids - 0.5 to 4 kg.

PRINCIPLE

The closed-transfer device described here is shown in Fig.1. It consists of a cabinet large enough to hold up to three unopened packs. The lid is closed and three spears are driven through the packs to pierce two opposite surfaces. The contents

of the packs drain into a sump at the base of the cabinet. The spears are fitted with rotating nozzles which locate inside the packs. Rinse water is introduced under pressure through these in order to clean out the packs. In addition two nozzles are fixed to the cabinet roof to rinse the cabinet and the outsides of the packs. The product and rinse waters are removed from the sump by a venturi.

The device is powered by the sprayer pump. A diverter valve assembly is inserted into the pressure hose from the sprayer pump to its controls assembly. When actuated this diverts water from the pump to the cabinet rinse nozzles and to the venturi at a set pressure. A return hose from the venturi is inserted into the sprayer tank.

In normal operation a two minute rinse time is adequate to extract product and rinse the packs. Better rinsing, especially of powders and viscous liquids, is achieved by pulsing the pack rinse nozzles on/off.

A number of safety features and interlocks are provided. The spears cannot be lowered until the cabinet lid is closed, nor can the lid be opened once the spears are lowered. The lid cannot be closed until the rinse pulse valve is in its open position. The rinse water is automatically turned on as the spears are lowered and similarly stopped as the spears are raised. A clear window is fitted into the lid and the sump discharge hose is clear so that the transfer can be monitored.

OPERATION

In normal use the following steps are undertaken:-

1. Attach clean water supply hose to the sprayer.
   Attach pressure feed hose to diverter valve.
   Attach discharge hose to tank.
2. Start tractor and set sprayer pump to draw in clean water.

3. Raise spears. Open cabinet.
   Insert packs. Close cabinet.
4. Turn diverter. Venturi starts.
5. Lower spears. Rinse starts.
6. Pulse rinse for 2 minutes.
7. Raise spears. Rinse stops.
8. Turn diverter back to sprayer.
9. Open cabinet. Remove packs.

10 Repeat from 3 if required.

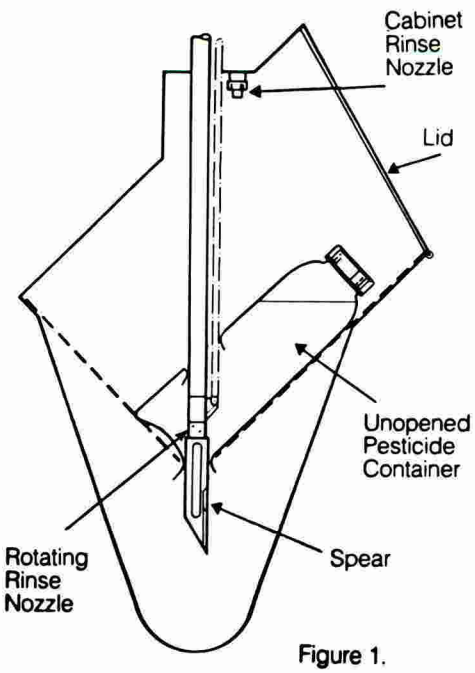

Figure 1.

PERFORMANCE TESTS

The performance of this closed-transfer system was evaluated with respect to a number of parameters (Gilbert 1990):-

- operator contamination during the transfer cycle.
- residual contamination of the cabinet and sump after a transfer cycle had been completed.
- residual contents of the test packs.
- practical tests of a wide range of pesticide products and pack types.

Test products

To evaluate operator and system contamination and pack residues, simulated pesticides were used:-

Liquid - (a) Water + 0.2 ug/ml Lissamine Green
(b) Water + 0.4 ug/ml Sunset Yellow

These were read at (a) 640 nm and (b) 480 nm on a Perkin-Elmer Spectrophotometer using a 4 cm cell with a 'x5'gain.

Solid -	Wettable powder of Waxoline Red O	3.5%
	Reax 45L wetter/dispersant	5.0%
	Precipitated silica	5.0%
	China clay	86.5%

Read at 525 nm on a Perkin-Elmer Spectrophotometer using a 4 cm cell with a 'x5' gain.

Test methods

The simulated pesticide concentrates were packed into the test liquid containers and wettable powder bags. These were transferred to a sprayer tank by operators dressed in items of disposable protective clothing serving as sampling media. Absorbant paper wipes were used to collect other residues which would present indirect contamination hazards. Residues in the emptied containers were extracted by thorough rinsing, both outside then inside using clean solvent (water for Lissamine Green and Sunset Yellow and petroleum spirit for the Waxoline Red O). All sample extracts were stored under suitable cool, dark conditions prior to reading of dye recovery using spectrophotometry. Additionally, all target media were 'spiked' with known amounts of the pesticide simulants and these samples stored under identical conditions to test samples prior to laboratory analysis to validate recovery procedure. Satisfactory (100%) tracer recoveries were found for all combinations of dyestuff and target media.

For both liquids and solids transfer, operator contamination was observed only on gloves and measurement data are for hand contamination only.

Each of the 11 liquid tests used 1 x 1 litre, 1 x 5 litre and 1 x 10 litre containers. In addition, for 8 of the tests the 10 litre container was opened and 1 litre poured into the open cabinet to represent a part-pack operation.

Each of the 10 solid tests used 1 x 1 kg, 1 x 2 kg and 1 x 4 kg bags. During these tests the spear tip was modified to improve removal of pack residues after transfer and results for both designs are given to demonstrate the improvement obtained.

RESULTS

Operator hand contamination.

Liquids - Mean of 0.019 ml dye concentrate (range 0.003 to 0.096 ml)

Solids - mean of 0.0065 grams wettable powder (range 0.0003 to 0.0105 g)

System contamination.

Liquids -	lid	0.325 ml concentrate	(range 0.018-1.838)
	door seal	0.133 ml concentrate	(range 0.015-0.731)
	spears	<0.007 ml concentrate	
	sump	<10 ml concentrate.	
Solids -	lid and door seal	0.0336 g powder	(range 0.0046 to 0.1611)

Pack residues.

Liquids -	1 litre size	- mean <0.0056%	(range 0.0005-0.0175%)
	5 litre size	- mean 0.0043%	(range 0.0003-0.0223%)
	10 litre size	- mean 0.0047%	(range 0.0005-0.0205%)
Solids- spear 1	1 kg size	- mean 0.1243%	(range 0.0058-0.2701%)
	2 kg size	- mean 0.9371%	(range 0.0176-3.6262%)
	4 kg size	- mean 1.6626%	(range 0.1278-4.6793%)
spear 2	1 kg size	- mean 0.0022%	
	2 kg size	- mean 0.0025%	

DISCUSSION

The test results show that the system offers a very significant reduction in operator contamination. The predictive operator exposure model (Martin 1986) can be used to estimate the expected equivalent hand contamination that would arise from manual handling of the 1, 5 and 10 litre containers used in these tests. This shows expected levels of 0.01, 0.2 and 0.5 ml hand contamination respectively by concentrate likely to arise from opening and dispensing contents. This suggests a possible total contamination of 0.71 ml which is 37 times the mean and 7 times the highest value measured in these tests, confirming the safety factor afforded by correct use of this system.

The results of the solids tests are even better, presumably as the part-pack operation was not carried out. The predictive operator exposure model does not include hand contamination data for wettable powder products, but it is accepted that it is likely to be much lower than that from liquid products, confirming that this system is equally safe for solid products.

Residues left in the cabinet were in very dilute form and amounted to less than 0.04% of the liquid product transferred, the solid products being much lower. Mean residues left in the liquid containers were half the Netherlands standard of 0.01%, the highest values ranging only up to 0.022%. For solid products the new spear design 2 brought residues down to well less than this standard.

This closed-transfer system has been shown to provide a very safe, fast and effective method for transferring pesticides from their original packs to the sprayer tank, without having to open the packs, and leaving them rinsed very clean and punctured to prevent further use. The system clearly satisfies the requirement expressed in the UK Code of Practice for 'engineering controls' to be employed instead of solely relying on protective clothing to safeguard operators from the potential hazards of handling concentrated pesticides.

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EVALUATION OF FUNGICIDE APPLICATION METHODS FOR CEREAL DISEASE CONTROL

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ABSTRACT

Nine trials were conducted over three years (1987-89) to compare the efficacy of a fungicide treatment applied through various application systems on winter wheat. The application systems examined included a twin fluid nozzle (Cleanacres Airtec), a swirl nozzle (Delavan WRW Superjet) and the 02-F110 hydraulic flat fan nozzle, all applying sprays at 100 litres/ha; and the 04-F110 200 l/ha hydraulic nozzle used alone and in conjunction with the Ciba-Geigy Crop Tilter. A standard fungicide treatment of propiconazole or prochloraz plus fenpropidin or fenpropimorph was applied through each system at full and half dose at, or shortly after full flag leaf emergence. Disease was assessed at the milky-ripe growth stage and yield measurements were taken.

The standard 200 l/ha medium quality spray proved a reliable method of disease control. Disease control was occasionally impaired when sprays were applied through twin fluid nozzles, swirl nozzles or hydraulic nozzles at 100 l/ha, particularly when fungicide doses were reduced.

INTRODUCTION

Recent years have seen several developments in spray application technology designed to improve the efficiency of pesticide application. Some systems have attempted to reduce spray drift, either by modifying the droplet spectra, reducing boom height above the crop, or by incorporating air with the spray. In many cases, improved deposition of the spray on the target is also claimed. Because these systems often offer advantages in more timely pesticide application by incorporating increased work rates through the use of lower spray volumes (typically 100 litres/ha) with the availability of more spraying days by the ability to spray at higher wind speeds, the inference is frequently drawn that pesticide dose rate may be reduced without loss of efficacy.

This report gives details of experiments carried out on cereals over three years (1987-89) to evaluate fungicide application through various spray application systems. It forms part of a collaborative project funded by the Home-Grown Cereals Authority between ADAS and AFRC and complements work reported earlier on herbicide efficacy, spray drift studies, physical measurements of sprays and spray deposits (Rutherford et al., 1989).

MATERIALS AND METHODS

The application systems

The spray application systems studied were commercially available systems selected to provide contrasting values for drop size and velocity. The 04-F110 hydraulic nozzle producing a medium quality spray and delivering 200 litres/ha was chosen as the standard system with which to compare others. A similar nozzle inclined rearward at 20° to the vertical as part of the Ciba-Geigy 'Crop Tilter' system was included as was another 110° flat fan hydraulic nozzle, the 02-F110 which produced a fine quality spray at 100 litres/ha. The other systems compared were the Cleanacres Airtec twin fluid nozzle and the Delavan WRW Superjet swirl nozzle.

The Airtec twin fluid nozzle was operated at two settings of air and liquid pressures, originally selected to produce both medium and fine spray qualities. However, subsequent measurement of droplet sizes showed that these sprays conformed to 'very coarse' and 'coarse' spray qualities as classified by the system developed by the British Crop Protection Council (BCPC) (Doble et al., 1985). The characteristics of the spray application systems studied are summarised in Table 1.

TABLE 1 Application systems studied

Nozzle	Application volume l/ha	BCPC Spray Category	Pressure kPa	Flow rate l/min
Hydraulic flat fan (Lurmark 04-F110)	200	medium	300	1.60
Hydraulic inclined flat fan (Lurmark 04-F110)+'Crop Tilter'	200	medium	300	1.60
Hydraulic flat fan (Lurmark 02-F110)	100	fine	300	0.80
Twin fluid (Airtec)	100	nominally medium	200L 70A	0.53
Twin fluid (Airtec)	100	nominally fine	300L 140A	0.53
Hydraulic swirl (Delavan WRW Superjet)	100	medium	275	0.76

L = liquid A = air

Each of the application systems was mounted on a half boom section of a 12 m boom Frazier Agribuggy and could be selected independently to spray a 5 m wide plot on one or other side of the tramlines.

Fungicide Efficacy

Trials were conducted on winter wheat on three sites - Bridgets Experimental Husbandry Farm (EHF) Hampshire, Rosemaund EHF, Hereford and Worcester and a commercial farm in Dorset in 1987, 1988 and 1989. Plots 5 m x 25 m were marked out either side of tramlines in a randomised block design with four replicates. Each replicate included two untreated controls.

A single fungicide spray was applied through each system at full and half recommended dose rates when crops were between the flag leaf emerged and flag leaf sheath opening growth stages (GS 39-GS 47). The fungicide used was usually a mixture of propiconazole + fenpropidin applying 125 g/ha propiconazole (as 'Radar', 0.5 l/ha) with 562.5 g/ha fenpropidin (as 'Patrol', 0.75 l/ha) for the full dose treatments. At one site, where eyespot (Pseudocercosporella herpotrichoides) was present, prochloraz was used at 400 g/ha (as 'Sportak', 1.0 l/ha) with 750 g/ha fenpropimorph (as 'Mistral', 1.0 l/ha) for the full dose treatments. Details of trial crops and fungicide application are summarised in Table 2.

TABLE 2 Details of sites and fungicide application

Year	Site	Cultivar	Growth Stage at Spraying	Fungicide
1987	Bridgets	Brock	39-45	Propiconazole + fenpropidin
1987	Rosemaund	Avalon	39-45	Propiconazole + fenpropidin
1987	Dorset	Avalon	45-47	Prochloraz + fenpropimorph
1988	Bridgets	Hornet	45	Propiconazole + fenpropidin
1988	Rosemaund	Slejpner	45	Propiconazole + fenpropidin
1988	Dorset	Mercia	47	Propiconazole + fenpropidin
1989	Bridgets	Rendezvous	39	Propiconazole + fenpropidin
1989	Rosemaund	Hornet	45	Propiconazole + fenpropidin
1989	Dorset	Slejpner	43	Propiconazole + fenpropidin

Disease assessments were made at the milky ripe growth stage (GS 73-GS 77) by randomly selecting ten main tillers from each plot and assessing foliar and stem base diseases (as appropriate). Leaf diseases were assessed as the percentage leaf area affected using standard keys (MAFF, 1976) numbering the flag leaf as leaf 1. Eyespot was assessed (selected sites only) on a scale reflecting both incidence and severity of lesions and a disease index on a 0-100 scale calculated (Scott & Hollins, 1974).

Yields were measured at each site using plot combine harvesters to harvest a strip at least 2 m wide along the centre of each plot. Yields were corrected to 85% dry matter.

RESULTS

Fungicide Efficacy

In 1987, Septoria tritici was the main disease at each site. All full dose treatments and most half dose treatments significantly reduced mean levels of S.tritici on the flag leaf (Table 3). The standard 200 l/ha hydraulic nozzle at full dose was the most effective treatment, significantly better than both the Airtec 'fine' spray and the Crop Tilter at full dose.

TABLE 3 Mean levels of Septoria tritici (% leaf area affected) on leaf 1 at GS 75 over 3 sites, 1987

Method of Application	Full dose	Half dose
Untreated	36.1	
Hydraulic 200 l/ha medium	13.7	24.2
Hydraulic 200 l/ha medium + Crop Tilter	22.3	20.1
Hydraulic 100 l/ha fine	18.8	32.6
Airtec 'medium'	20.1	28.4
Airtec 'fine'	22.7	29.3
Superjet medium	15.9	20.1
LSD ($P < 0.05$)	7.42	

Eyespot developed at the Dorset site in 1987 and provided an opportunity to assess whether application systems giving better penetration of the crop canopy improved eyespot control. Although the timing of the prochloraz + fenpropimorph spray was not optimum for eyespot control, a significant reduction in eyespot severity index was given by the standard 200 l/ha hydraulic nozzle (at both doses) and by the full dose Airtec 'fine' spray (Table 4). There was no indication that the Crop Tilter improved eyespot control.

TABLE 4 Eyespot severity index (0–100 scale) at the Dorset site at GS 75, 1987

Method of application	Full dose	Half dose
Untreated	46.4	
Hydraulic 200 l/ha medium	31.3	32.5
Hydraulic 200 l/ha medium + Crop Tilter	42.5	35.0
Hydraulic 100 l/ha fine	35.8	49.2
Airtec 'medium'	46.1	45.5
Airtec 'fine'	31.2	50.4
Superjet medium	36.0	44.2
LSD ($P < 0.05$)	13.3	

In 1988, disease levels were variable and some sites were affected by drought which induced premature senescence. However, moderate levels of S.tritici were recorded at the Dorset site (Table 5).

TABLE 5 Levels of Septoria tritici (% leaf area affected) on leaf 1 at the Dorset site at GS 75, 1988

Method of application	Full dose	Half dose
Untreated	44.7	
Hydraulic 200 l/ha medium	4.5	4.9
Hydraulic 200 l/ha medium + Crop Tilter	3.1	3.4
Hydraulic 100 l/ha fine	0.8	6.4
Airtec 'medium'	2.5	18.3
Airtec 'fine'	4.1	11.2
Superjet medium	1.5	7.2
LSD ($P < 0.05$)	6.29	

All full dose treatments gave equally good control of S.tritici on leaf 1. However, at half dose, while the 200 l/ha hydraulic nozzle treatments continued to give good control, levels of S.tritici were higher in the 100 l/ha treatments. This was most noticeable in the Airtec treatments which were significantly inferior to the 200 l/ha treatments.

Disease levels in 1989 were very low and the only significant disease recorded was yellow rust (Puccinia striiformis) at the Dorset site (Table 6). Several half dose treatments (Airtec 'fine', Superjet and hydraulic 200 l/ha medium) and the full dose hydraulic 100 l/ha fine spray failed to reduce yellow rust levels significantly on leaf 2.

TABLE 6 Levels of yellow rust (% leaf area affected) on leaf 2 at the Dorset site at GS 75, 1989

Method of application	Full dose	Half dose
Untreated	16.6	
Hydraulic 200 l/ha medium	2.2	8.2
Hydraulic 200 l/ha medium + Crop Tilter	5.6	3.7
Hydraulic 100 l/ha fine	10.5	5.2
Airtec 'medium'	6.6	4.6
Airtec 'fine'	5.8	10.9
Superjet medium	2.3	7.9
LSD (P < 0.05)	9.19	

Yield

Mean yield data over the three sites are presented for each year in Table 7. In 1987, all methods of application except the hydraulic 100 l/ha fine spray increased mean yield at full fungicide dose. However, at half dose, the yields from the Airtec 'medium', the Superjet medium and the Crop Tilter treatments were significantly lower than the standard hydraulic medium spray.

All treatments, regardless of dose rate, increased mean yield significantly in 1988, with the Crop Tilter giving the greatest increases. At full dose, all methods of application gave similar yield increases, but at half dose, the Airtec 'fine' spray gave a significantly lower yield than the hydraulic 200 l/ha treatments.

Yield effects in 1989 were low and non-significant due to the very low levels of disease and the effects of the drought.

TABLE 7 Mean untreated yield of wheat and yield increases (t/ha) over three sites (Bridgets, Rosemaund and Dorset)

Method of application	1987		1988		1989	
	Full	Half	Full	Half	Full	Half
Untreated	7.07		7.47		6.98	
Hydraulic 200 l/ha medium	+0.83	+0.72	+1.29	+1.32	+0.36	+0.32
Hydraulic 200 l/ha medium + Crop Tilter	+0.93	+0.17	+1.62	+1.37	+0.29	+0.22
Hydraulic 100 l/ha fine	+0.34	+0.56	+1.54	+1.03	+0.24	+0.23
Airtec 'medium'	+0.66	+0.23	+1.39	+0.93	+0.05	+0.04
Airtec 'fine'	+0.61	+0.52	+1.19	+0.80	+0.11	+0.17
Superjet medium	+0.55	+0.10	+1.55	+0.94	+0.39	+0.03
LSD (P < 0.05)	0.39		0.41		NS	

When considering yield data meaned over all sites for the three years (Table 8) none of the application methods differed significantly from the standard 200 l/ha hydraulic nozzle at full dose but at half dose, the Airtec 'medium', Airtec 'fine' and Superjet gave significantly lower mean yield increases than the standard.

TABLE 8 Mean untreated yield of wheat and yield increases (t/ha) for all sites over three years

Method of application	Full dose	Half dose
Untreated	7.18	
Hydraulic 200 l/ha medium	+0.85	+0.78
Hydraulic 200 l/ha medium + Crop Tilter	+0.97	+0.58
Hydraulic 100 l/ha fine	+0.70	+0.58
Airtec 'medium'	+0.69	+0.41
Airtec 'fine'	+0.67	+0.49
Superjet medium	+0.83	+0.34

LSD ($P < 0.05$)	comparing treated vs untreated	0.224
	comparing application methods	0.263
	comparing dose rates	0.234

DISCUSSION

These results demonstrate that the standard 04-F110 hydraulic nozzle delivering 200 l/ha as a medium quality spray is a reliable method of fungicide application for disease control in winter wheat. Reducing volume to 100 l/ha by using the 02-F110 hydraulic nozzle results in a fine spray which has disadvantages in terms of increased drift potential. While this nozzle generally gave good disease control on the flag leaf at full dose it was less effective on leaf 2 (Tables 5 and 6). This supports observations by Herrington and Hislop (1990) that this nozzle produced good deposits on the flag leaf in samples collected at the Bridgets site.

Although the Crop Tilter has been shown to increase fungicide deposition on the top two leaves (Herrington & Hislop, 1990) it did not offer any consistent advantages in terms of disease control on these leaves, nor did it aid eyespot control.

The reduction in drift possible from the Airtec twin fluid nozzle has been well documented (Rutherford *et al.*, 1989; Western *et al.*, 1989). This, together with the ability to spray at low volumes offers advantages in terms of more timely spray application and consequently it is often suggested by farmers that pesticide dose can be reduced. This work however clearly demonstrates that at reduced fungicide doses the twin fluid nozzle is often less effective than the standard 200 l/ha hydraulic nozzle.

Similarly, while the Delavan WRW Superjet medium spray performed as well as the standard at full fungicide doses, it was occasionally less effective at half dose.

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This work was made possible by substantial co-operation between AFRC and ADAS staff at many locations and thanks are extended to all concerned. Thanks are also due to Mr B Guthrie (Lulworth Castle Farms) and Mr M Hancock (Woodsford Farms) for enabling trials to be conducted on their farms.

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GAS-TIGHTNESS OF SOIL MULCHES IN 1,3-DICHLOROPROPENE SOIL DISINFESTATION

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ABSTRACT

The use of soil fumigants, directly or generated from formulations, can result in severe losses especially through the soil mulch. This gives rise to inadequate concentration x time products with respect to nematode and clubroot control by 1,3-dichloropropene. The diffusion of (Z)- and (E)-1,3-dichloropropene through polymer emulsion spray and plastic film mulches has been studied under laboratory conditions using gas chromatography to estimate fumigant concentrations. Results demonstrate the potential use of polymer sprays and a wide range of plastic films, including "biodegradable" materials.

INTRODUCTION

A lot of plastic films have been studied for their barrier properties in methyl bromide soil disinfestation (Kolbezen & Abu-El-Haj, 1978; Huygen & van Yssel, 1982; Van Wambeke *et al.*, 1983; Ducom, 1988).

Little or no attention has been paid to mulches for 1,3-dichloropropene (1,3-D) fumigation. Alternative mulching materials are polymer emulsions sprayed *in situ* (Alphey, 1980). It has already been shown that different fumigants and diffusion-enhancing co-fumigants exhibit very different diffusion characteristics through plastic film barriers (Van Wambeke *et al.*, 1986). Both (Z)- and (E)-isomers of 1,3-D are present in commercial fumigants, and diffusion characteristics of both were investigated in this study. A few plastic films were compared, as well as polymer sprays and biodegradable plastics, the latter two with labour savings and environmental reasons respectively in mind.

METHODS AND MATERIALS

The diffusion of both 1,3-D isomers was monitored by GC measurements of 200 µl gas syringe samplings according to Van Wambeke *et al.* (1989). The plastic film to be tested or the filter paper support for the polymer emulsion studies was inserted between two ground glass chambers, the lower one containing 1,3-D and the upper provided with a 20 ml min^{-1} air stream facility. (Z)- and (E)-1,3-D (both 97 % AI) were purchased from Janssen Chimica. Relevant fumigant data are listed in Table 1.

Table 2 lists the different samples of plastic films tested. The weight per unit surface area was the mean of several samples of a large foil. The test pieces were chosen to be close to this mean.

Table 3 lists formulations of polymer emulsions which were tested on a filter paper support following a dipping treatment.

TABLE 1. Product data on 1,3-dichloropropene (Arvieu, 1980; Worthing, 1979).

	(Z)-isomer	(E)-isomer
Boiling point (°C)	112.0	104.2
Vapour pressure (mm Hg at 20°C)	25.0	18.5
Solubility in water (mole l^{-1} mm Hg^{-1} at 20°C)	$0.97.10^{-3}$	$1.35.10^{-3}$

TABLE 2. Plastic films tested for 1,3-D gas-tightness.

	Tradename	Average weight (g m^{-2})	Test temp. (°C)
'non-biodegradable'	'Eurofilm'	30.31	20
	LDPE	40.14	20
	'Lejaplast'	38.73	20
	'LMS'	34.13	20
	'Saranex'	36.09	20
	'Waloplast'	30.57	20
'biodegradable'	'Constab 1'	71.76	15
	'Ecopolym'	32.98	15,20,25
	'Multibase'	31.80	15

TABLE 3. Film forming polymer emulsions tested for (Z)-1,3-D gas-tightness.

Compound	Tradename	Product:water dilution factor	Test temperature (°C)
Polyvinyl acetate	'Curasol AE'	1:10 v/v	25
Polyvinyl proprionate	'Agrofix'	1: 4 v/v	25
Polyvinyl alcohol	'Mowiol'	1:10 v/v	25
Polyurea	'SS336'	1: 8.3 v/v	25

The experiments on non-biodegradable plastic sheets were performed using both 1,3-D isomers in a 1:1 V/V mixture at a rate of 6.3 ml m^{-2} of diffusion surface. This was a much higher 1,3-D concentration in the gas phase than is normal in a soil application. "Biodegradable" sheets were tested using the separate 1,3-D isomers except in the case of "Ecopolym", used for the study of the influence of temperature on 1,3-D diffusion.

RESULTS AND CONCLUSIONS

Figures 1 to 6 summarise the collected data. Comparison of diffusion characteristics of the two 1,3-D isomers through the plastic films (Figures 1 to 4) demonstrates the lower loss of the E-isomer, especially through biodegradable films (Figures 3 and 4). The experiment on the biodegradable film (Figure 5) at three different temperatures shows the importance of temperature on the permeability of plastic sheeting. These results however are due to the combined effect of enhanced fumigant mobility (higher vapour

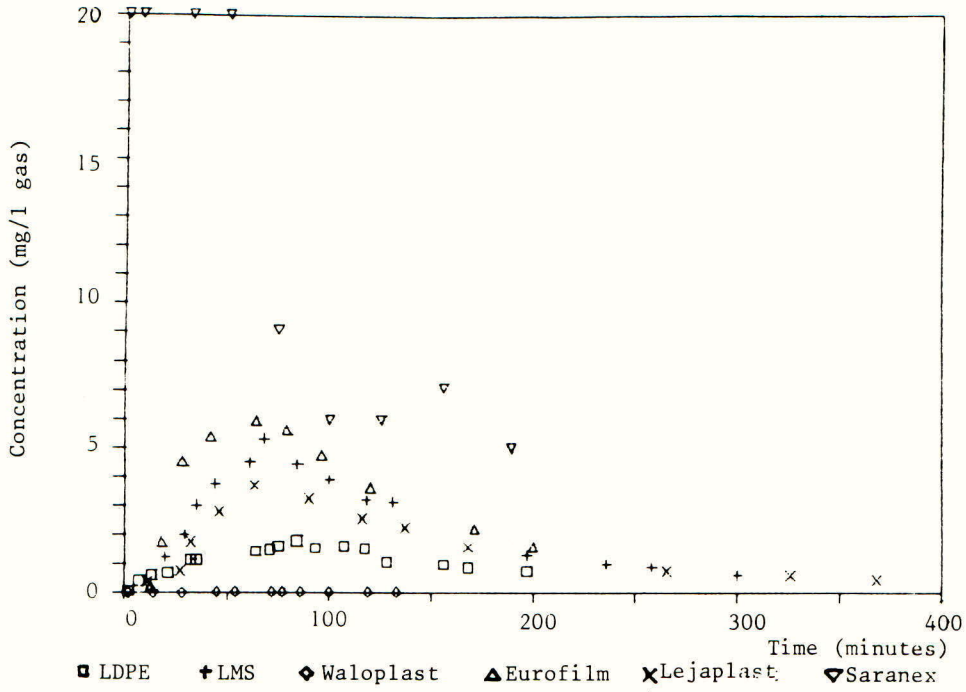

FIGURE 1. Diffusion of (Z)-1,3-dichloropropene through plastic foils (at 20°C).

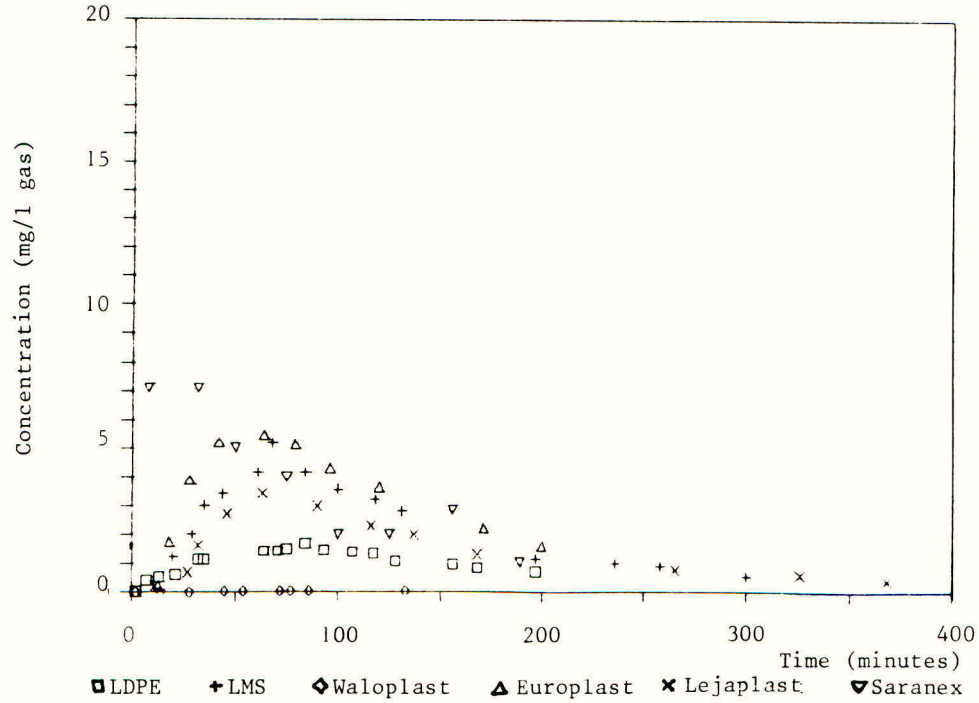

FIGURE 2. Diffusion of (E)-1,3-dichloropropene through plastic foils (at 20°C).

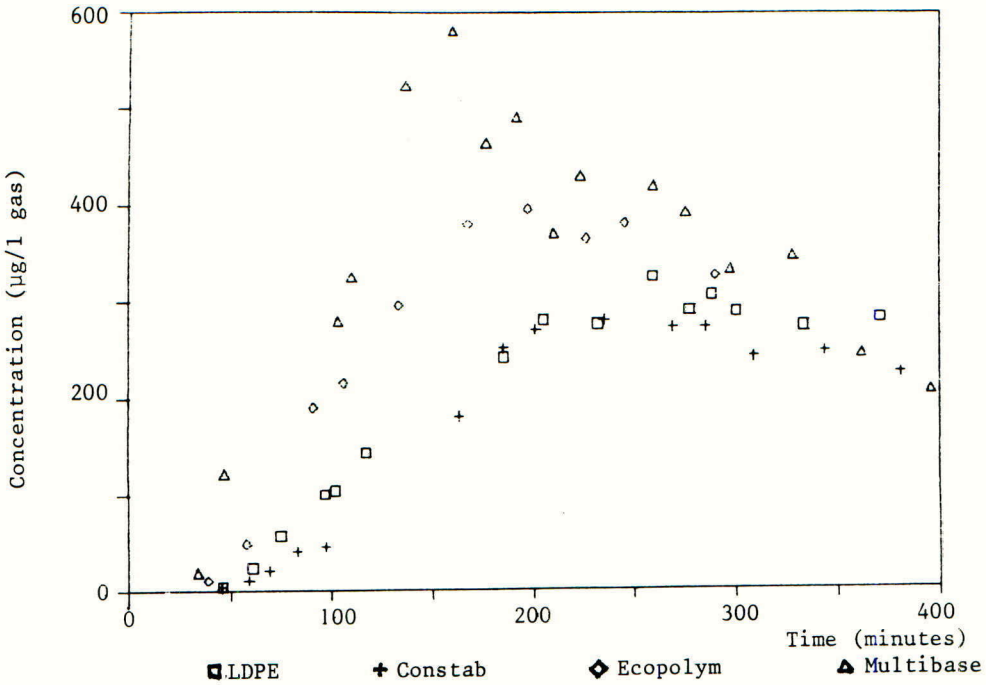

FIGURE 3. Diffusion of (Z)-1,3-dichloropropene through 3 'biodegradable' plastic foils (at 15°C; reference LDPE).

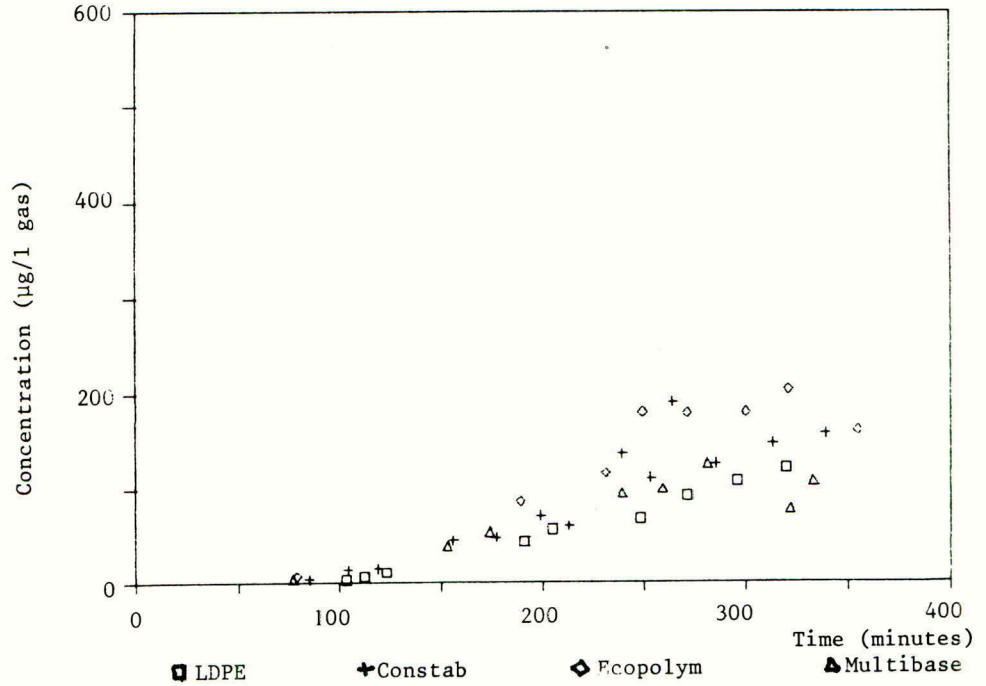

FIGURE 4. Diffusion of (E)-1,3-dichloropropene through 3 'biodegradable' plastic foils (at 15°C; reference LDPE).

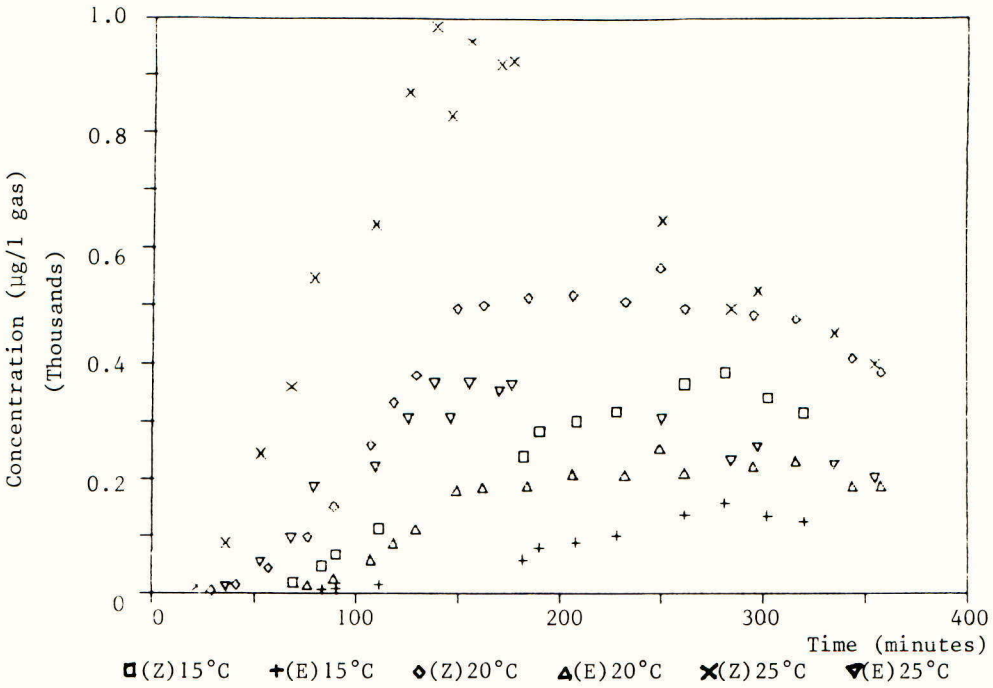

FIGURE 5. Influence of temperature on the diffusion of (Z)-and (E)-1,3-dichloropropene through Ecopolym.

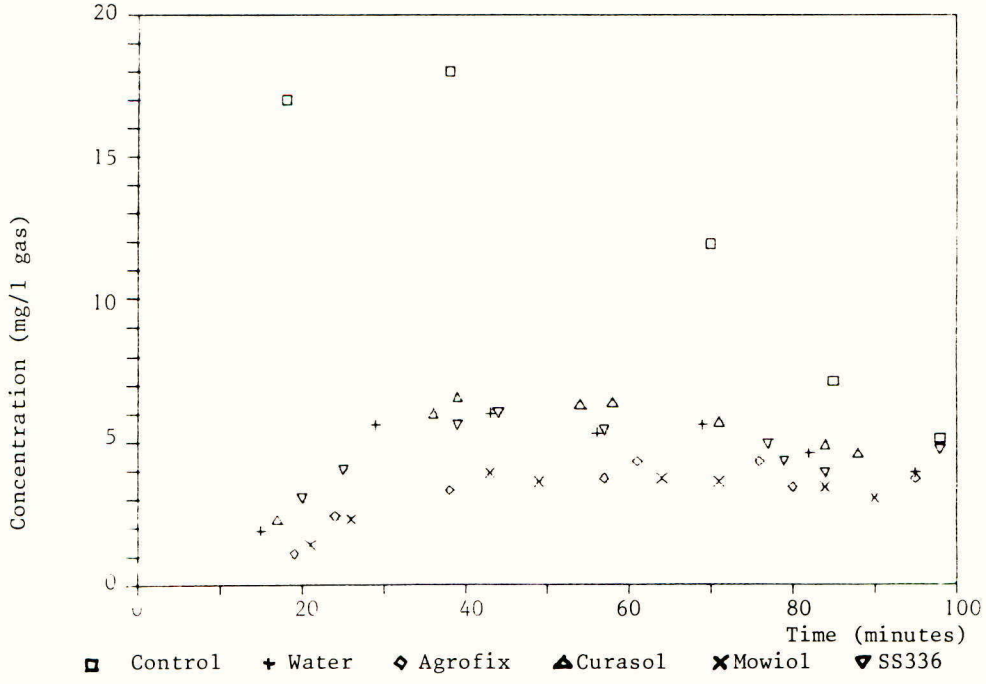

FIGURE 6. Diffusion of (Z)-1,3-dichloropropene through polymer emulsions.

pressure) and modified diffusion behaviour through the plastic barrier. The reference film for comparison of the "biodegradable" and normal plastic sheet is LDPE (Low Density PolyEthylene).

The use of polymer emulsion applications (Figure 6) as a mulching method indicates potential use for the polyvinyl proprionate (1:4 V/V) and polyvinyl alcohol (1:10 V/V) formulations. The application of water without polymer reduces fumigant losses to less than 50 % of the loss from untreated soil. In practice sprays may be applied advantageously to finely crumbled soil surfaces.

The most biologically-active (Z)-isomer apparently shows highest loss rates through plastic films. The choice of an appropriate plastic type can effectively reduce this loss. Some polymer emulsion sprays tested under laboratory conditions show comparable results to those of plastic sheets and show potential for field application.

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THE CONTROL OF BANANA CROWN ROT BY FUNGICIDE-IMPREGNATED PADS

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(NO WRITTEN SUBMISSION)

UPTAKE STUDIES OF MICROEMULSIFIED DELTAMETHRIN USING X-RAY ANALYSIS

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ABSTRACT

Monosize droplets of an oil-in-water microemulsion formulation of deltamethrin applied in vivo to the cuticular surface of American cockroaches (Periplaneta americana) were examined using scanning electron microscopy and X-ray microanalysis. Using a time course it was revealed that the distribution of detectable formulation components is random within the deposit throughout the period of intoxication. Substantial penetration of the formulants over 24 hours was shown by decreases in the amounts of deltamethrin and anionic surfactant detected, concurrent with a reduction in observable material in the deposit. A time/dose response study suggests that high (10 x LD99) doses of deltamethrin may be used to optimise detection by this technique without interference in the pharmacokinetics of the formulation.

INTRODUCTION

The phenomenon of cuticular penetration by insecticides has been of interest for some decades and several reviews on the subject have been published (for example Welling and Paterson, 1985). Conventionally, investigations have been carried out with the insecticide applied in a small volume of solvent to the cuticle of an intact insect and penetration evaluated by washing off surface residues of the insecticide with another solvent, usually acetone. Most commercial formulations are however applied using water as the diluent phase and advances in our understanding of insecticide uptake might be more facilitated if laboratory observations could be conveniently made with water-based insecticide formulations. Microemulsion formulations of insecticides are thermodynamically stable systems with a disperse phase droplet size normally between 10 and 100 nm. These formulations are water-based and it is postulated that the kinetics of insecticide penetration from these test systems would be analogous to conventional field applications of water diluted sprays.

Scanning electron microscopy (SEM) in conjunction with X-ray microanalysis has been used successfully to complement traditional techniques in the study of foliar deposits of pesticides (Hunt & Baker, 1987). Although concentrations of the formulation above field rates are required for efficient detection quantitative measurements of the elemental marker in very small areas can be made. This study uses elemental detection to determine the distribution and disappearance of the active ingredient and anionic surfactant of a microemulsion formulation of deltamethrin on intact P. americana cuticle.

MATERIALS AND METHODS

Chemicals and insects

All experiments were carried out using an oil-in-water microemulsion formulation containing 6 g/l deltamethrin (99% purity) prepared by NCH Europe. Adult males of P. americana aged between 1-4 months (mean weight 0.72 g ± 0.03 g, n = 50) were used exclusively for all the experiments.

Dose-time response study

The insecticidal potency of the formulation was assessed using a symptom progression bioassay. Groups of 5-10 cockroaches per dose were anaesthetised using a minimal amount of CO_2 and treated with single 0.3 µl droplets of the formulation applied to the ventral surface of a metathoracic femur. Dilutions from the 6 g/l formulation were made using water containing $CaCO_3$ at 150 mg/l and control insects were untreated. Subsequently the insects were confined individually in PTFE coated filter paper lined jars and incubated in the dark at 25°C and 50-60% r.h. without food or water. The time course of pyrethroid poisoning was assessed at regular intervals by recording the numbers of insects expressing knockdown, defined as the inability to walk even if righted. Results obtained from 3 replicate experiments were analysed using a probit model (GLIM release 3.77).

SEM and X-ray microanalysis

Insects were treated with a single 0.3 µl droplet containing 2 µg of deltamethrin as described in the toxicity bioassay. At various time intervals after treatment insects were reanaesthetised using CO_2 and killed by freezing their head and thorax between copper blocks held at -196°C, effectively stopping the time course. The treated leg was then removed and rapidly mounted on to a carbon stub using double-sided sticky pads. Samples to be photographed were gold-coated in a direct current sputtering device prior to examination. Specimens for X-ray analysis received no gold coating and were immediately placed in a brass holder restrained in a beaker suspended in a flask of liquid nitrogen for storage. Just prior to analysis, a hydrocarbon oil Duron(R) was painted onto the specimen whilst avoiding contamination of the deposit, thus ensuring effective earthing.

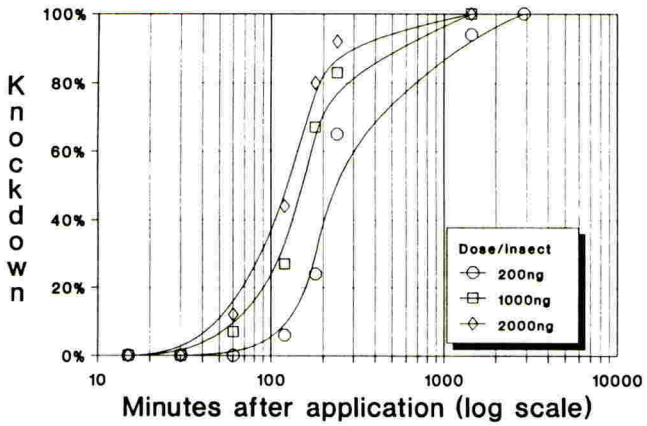
For analysis, microemulsion deposits on the cuticle surface were visualised using a STEREOSCAN 600, Cambridge instruments (UK) scanning electron microscope and the centre of the droplet located. On each sample this central site and four satellite areas 600 nm above, below and to either side of the centre were quantitatively scanned for their elemental spectrum using energy dispersive X-ray analysis. A LINK systems 860 series X-ray analyser (UK) collected the X-ray energy spectra and corrected the data against background to provide elemental counts. All scans were carried out over 100 seconds using a beam current of 25 keV at an angle of 45° and a working distance of 20 mm. At least 10 individual specimens were scanned for each point in the time course. Multiple regression analysis using GLIM (version 3.77) was used to compare elemental counts collected at each site during the time course.

RESULTS

Dose/time response study

In Figure 1, the relationship between the onset of knockdown and dose of deltamethrin is shown. At the 2 µg dose chosen for X-ray analysis the shape of this time-response curve is similar to the LD99 dose of 200 ng/insect indicating that symptom progression is dose dependent.

Figure 1. The effect of microemulsion deltamethrin dose on knockdown of P.americana



Scanning electron micrographs

Secondary electron images revealed the changing form of the microemulsion deposit during the time period of poisoning (Plate 1). 15 seconds after application (A) (mag x 200) the droplet spreads maintaining an unbroken film of smooth appearance within its perimeter. As the time course progresses many unconnected sunken areas begin to show and corresponding raised areas become connected by ridges which also radiate inwards at the droplet periphery. This appearance is pronounced after 2 hours (B) (mag x 2000). After 24 hours the surface deposit is fragmented and considerably reduced in apparent volume (C) (mag x 1000). No trace of the formulation can be seen within much of the original contaminated area and when these areas are compared to untreated cuticle (D) (mag x 2000) no surface damage is discernible.

X-ray analysis

The spectral analysis of untreated cuticle (Figure 2) reveals only low levels of cuticular chlorine and potassium. The scan for a 0.3 l droplet of a 6 g/l deltamethrin microemulsion 15 seconds after application (Figure 3) illustrates that the bromine atoms of deltamethrin and the sulphur group of the anionic surfactant are easily detected in the formulation deposit. Multiple regression analysis comparing elemental counts at each site and time demonstrated no significant difference between the elemental amounts

Fig.2. KeV spectra of untreated cuticle.

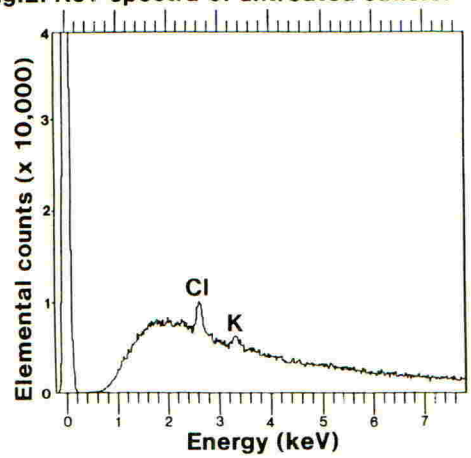


Fig.3. KeV spectra of treated cuticle.

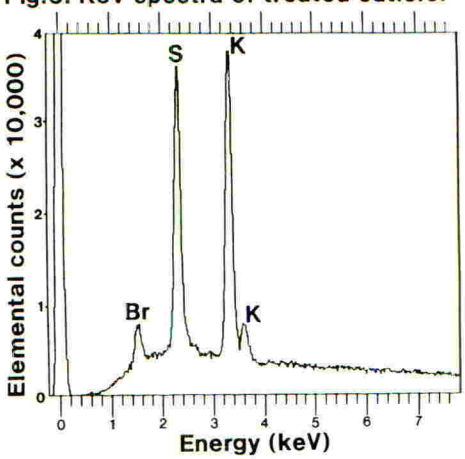
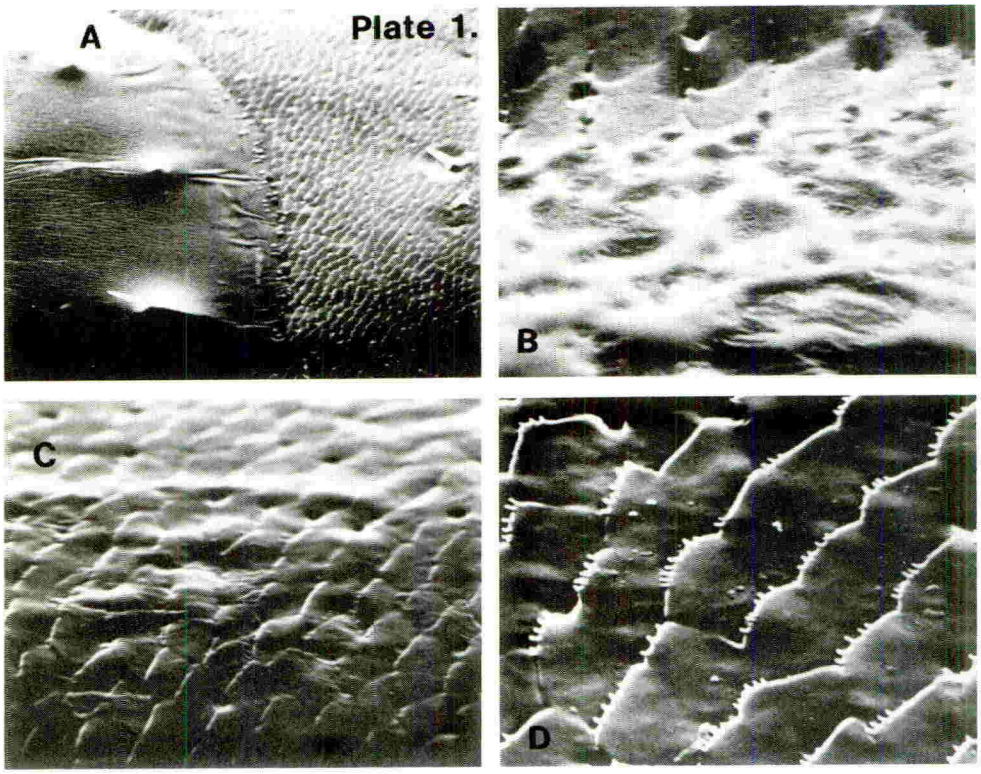
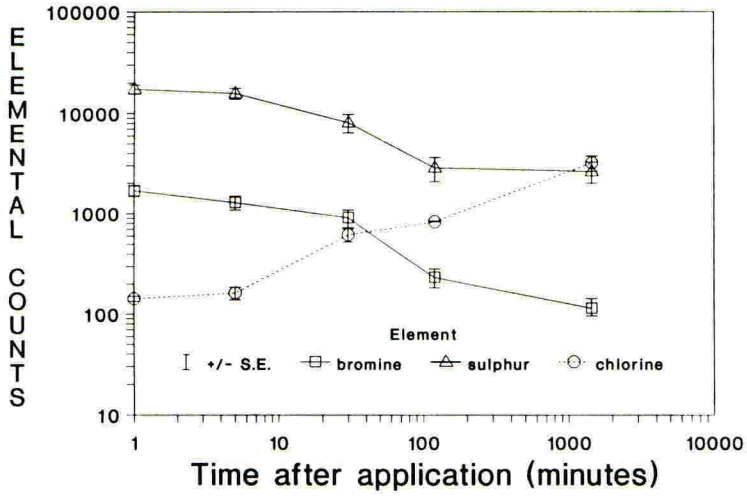


Plate 1.



detected at each site. Significant differences between each point in the time course were shown and these are illustrated in figure 4 which displays the mean elemental counts collected for bromine, sulphur and chlorine at the application site during the time course.

Figure 4. Mean element counts in aging cuticle deposits of a deltamethrin microemulsion



Disappearance of both bromine and sulphur is evident. After 30 minutes bromine detection is 54% and sulphur detection 46% of their mean 15 second counts. In contrast cuticular chlorine is initially masked by the deposit but counts rise rapidly and after 30 minutes are 3.8 times the elemental count at the time of application. This suggests a reduction in the amount of formulation on the cuticle surface as the time course progresses.

DISCUSSION

X-ray analysis is suitable for molecules containing elements which an atomic number greater than 11 and consequently most insecticides can be detected by this technique. However the range of surfactants and solvents which can be examined is more limited. In the case of the microemulsion formulation used in this study, whereas the anionic surfactant was readily quantified, the non-ionic cosurfactant was not.

Detection is also related to concentration of the elements; foliar applied emulsions have proved difficult to measure as their deposits are extremely thin (Hunt and Baker, 1987). Strong definition obtained used 10 x LD99 concentrations of this microemulsion show that for insect applications this can be overcome. Definition, in fact, remains good during uptake as there is no interference in detection of our key elements from the cuticle surface. The requirement for above lethal doses may however yield uptake data that does not reflect the pharmacokinetics of the formulation at field doses. For example the capacity of the application site for penetration and detoxification might be exceeded. Soderlund (1979) revealed that at doses increasing from LD99 to 30 x LD99 penetration rate kinetics of deltamethrin into P. americana change from first-order to linear although the fraction penetrating after 24 hours is constant.

Furthermore internal accumulation at 24 hours was directly proportional to dose only up to 10 x LD99. In this analysis the concentration relationship of knockdown throughout the time course indicates that the site of application is not overloaded. This might not be the case if other sites were investigated (Welling and Paterson, 1985).

The initial masking of cuticular chlorine and its increased detection during the time course suggests that measurements of the formulation are confined to deposits on the outside of the cuticle. The depth of penetration of the electrons into a target depends on the energy of the primary electrons and on the target material. Typically this depth in a solid target ranges from 1 to 10 μm (Heinrich, 1981). This limited and potentially measurable depth of detection is a significant advantage over solvent rinsing as a technique for evaluating penetration from the applied droplet into the cuticle. Previous studies have shown that the physicochemical properties of the rinsing solvent may affect the efficacy of recovery of the applied material and thus influence the apparent penetration rate (Welling and Paterson, 1985).

This experiment suggests that within 2 hours of application more than 90% of the deltamethrin in a topically applied microemulsion is absorbed into the cuticle together with most of the non-volatile components of the formulation. Surprisingly the anionic surfactant which has a predominantly hydrophilic nature, penetrated at a similar rate to the hydrophobic pyrethroid indicating that partition from an emulsion into the cuticle is not simply related to lipophilicity. The random distribution of formulants within the deposit suggests that surface redistribution of the insecticide relating to morphology or possible areas of preferential uptake at this application site does not occur.

The technique could also be used to study penetration within the body of the insect. No significant bromine presence has been found in P. americana (Van Rinsfelt et al), indicating that deltamethrin in the cuticle could be located and cell interactions resulting in electrolyte changes revealed.

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DUAL-COMPONENT, SOIL-APPLIED INSECTICIDE GRANULES: THE BASIS OF RATIONAL STRATEGIES TO PROTECT HORTICULTURAL BRASSICAS AGAINST ROOT-FEEDING INSECTS AND FOLIAGE APHIDS

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ABSTRACT

Field experiments with Brussels sprout plants transplanted in May or June in 1976, 1978 and 1979 evaluated the performance against the cabbage aphid (*Brevicoryne brassicae*) of aphicides in (a) single-component formulations applied in mixed or separate products with single-component formulations against the cabbage root fly (*Delia radicum*) or (b) dual-component products with insecticides against *D. radicum* formulated on the same granules. The presence of fonofos in a dual-component formulation ('Doubledown') was compatible with the performance of the aphicide disulfoton, which decreased the numbers of live aphids on buttons 24 weeks after transplanting.

INTRODUCTION

The cabbage root fly (*Delia radicum*) and aphids are the major pests of horticultural brassicas in the UK. Generally, crops are protected against *D. radicum* with granular insecticide formulations applied at drilling or planting. Although aphids can be controlled with foliar sprays in some conditions, none of the aphicides available as liquid formulations remains active for more than 2-3 weeks. Repeated applications are therefore usually necessary on long-season crops (Anon., 1973).

Research has been done at this Institute to rationalise the use of aphicides on Brussels sprout crops established in spring and early summer. This resulted in an effective strategy based on a granular formulation of disulfoton applied at drilling or planting (Suett, 1975; Suett & Padbury, 1976). This paper presents results of three years' field experiments with Brussels sprouts to evaluate the performance in granular, soil-applied treatments of simultaneous applications of insecticides applied against the cabbage root fly and cabbage aphid (*Brevicoryne brassicae*).

METHODS

An experiment was done in each of three years (1976, 1978 and 1979) on a sandy-loam at Wellesbourne. Before planting, the sites were treated with base fertiliser at 100 kg N/ha, 231 kg P_2O_5/ha and 231 kg K_2O/ha.

Crops

Bare root plants (cvs Prince Askold (1976) and Citadel (1978, 1979)) were planted with a Super Prefer$^{(R)}$ transplanter on 18 May 1976, 7 June 1978 and 8 June 1979. Single-row plots, with plants at 71 cm x 71 cm spacings, were used with 16 plants/plot in 1976. Similar spacings were used with the

five rows of 20 plants per plot in 1978 and 1979.

Insecticide treatments

Vee-belt attachments for the planter (Thompson & Wheatley, 1985) metered the granular formulations which were applied with "Leed's" coulters (Bevan & Kelly, 1975) as sub-surface soil treatments.

In 1976, carbofuran (5% AI; 'Yaltox'; Bayer) and chlorfenvinphos (10% AI; 'Birlane Granules'; Shell) were applied in single-component formulations against cabbage root fly at doses equivalent to 62.5 mg AI/m row and 70.0 mg AI/m row respectively. Single-component formulations applied in log-dose treatments (Thompson & Wheatley, 1985) against mid- and late-season aphids included: disulfoton (10% AI; 'Disyston FE-10'; Bayer) at 22.7 - 309 mg AI/m row; ethiofencarb (10% AI; 'Croneton'; Bayer) at 22.0 - 299 mg AI/m row; and thiofanox (10% AI; 'Dacamox 10G'; Diamond Shamrock) at 16.0 - 216 mg AI/m row. The aphicides were applied with the insecticides used against cabbage root fly. A dual-component formulation (Bayer) comprising carbofuran (2.5%) against root fly and early aphids + ethiofencarb (5.0%) on the same granules was evaluated over doses equivalent to 14.7 - 198 mg carbofuran/m row and 29.3 - 396 mg ethiofencarb/m row.

The treatments in 1978 were: two single component formulations, chlorfenvinphos at 70 mg AI/m row and disulfoton at 98 mg AI/ m row; a mixed product (Diamond Shamrock), comprising chlorfenvinphos (2.5%) + thiofanox (2.5%) formulated on separate granules, applied to give 50.0 mg AI/m row with each insecticide; and two dual-component formulations, 'Doubledown' (6.0% disulfoton + 4.0% fonofos; Pan Britannica Industries and Stauffer Chemical) applied to give 108 mg disulfoton/m row + 72 mg fonofos/m row and an experimental product (Shell) comprising 4.0% chlorfenvinphos + 6.0% disulfoton applied to give 70.0 mg chlorfenvinphos/m row + 105 mg disulfoton/m row.

In 1979, the 6.0% disulfoton + 4.0% fonofos formulation was applied to some plots at planting, as in 1978. In addition, a formulation of disulfoton on pumice granules (10% AI; 'Disyston P-10'; Bayer) was applied on 9 October, at doses equivalent to 99.4 mg AI/m row.

Experiment designs

Untreated 'guard' plots surrounded each experiment and, in 1978 and 1979, individual plots. In each of the six replicated blocks in 1976, two plots received the carbofuran/ethiofencarb dual-component formulation, five the single component formulations of carbofuran or chlorfenvinphos and five were not treated with insecticides against cabbage root fly. With each of the three groups of five plots, one plot was treated against mid- and late-season aphids with disulfoton, one with ethiofencarb, one with thiofanox and two did not receive aphicide.

In 1978, each of the three replicated blocks comprised two plots without insecticides and one plot assigned to each of the four insecticide treatments: two with dual-component formulations, one with the mixed product and one with the two single component formulations applied simultaneously. Each of the three replicated blocks in 1979 comprised one plot treated with the dual-component formulation applied at planting, one

with a similar treatment supplemented later with the pumice-formulation of disulfoton and a third without insecticide treatment.

Assessments of cabbage aphid infestations on Brussels sprout plants

In 1976, the % area of leaves and buttons on each plant > or < 30 cm above the soil and covered with live *B. brassicae* was categorised (0, 1-20, 21-40, 41-60, >60%) non-destructively on 24 August, 7 September, 21 September, 5 October and 25 October. On 17 October and 2 and 23 November 1978, all buttons (>1 cm diameter) on three different sets of 15, randomly-selected plants in each plot were removed; live aphids on each button were counted. In 1979, one button was removed from the upper, middle and lowest parts of 15 plants in each plot on 28 September; 5, 8, 12, 19, 26 October; and 2, 9, 16 November. Live aphids on each button were counted.

Meteorological records

For each experiment, 10 cm soil temperatures and 24 h rainfall were recorded daily at 09.00 h Greenwich Mean Time.

RESULTS

Soil temperature and moisture

In 1976, maximum mean 10 cm soil temperatures (c. 20.7°C) were recorded 4-11 weeks after transplanting; mean temperatures in 1978 (14.8°C) and 1979 (14.9°C) were lower than those in 1976 (17.6°C). The soil in 1976 and 1979 suffered prolonged periods of dryness: in 1976, the soil moisture deficit (SMD) (Greenwood & Draycott, 1989) to 20 cm depth exceeded 20 mm on 55 days 8-15 weeks after transplanting and in 1979 a similar deficit occurred on 44 days, 12-19 weeks after transplanting. A 20 mm deficit was not recorded in 1978.

Performance of insecticides against aphids

In 1976, infestations of aphids on the buttons and leaves were similar from 24 August - 25 October on untreated plants and plants treated with only carbofuran or chlorfenvinphos (Table 1); no aphicidal effect of carbofuran was apparent at this time. In August, when the SMD was high, all aphicides failed to provide three consecutive plants in the log-dose-treated plots without live aphids (Table 2). However, after rainfall in September, all aphicides became more effective; maximum effectiveness of disulfoton (in single- and dual-component formulations), ethiofencarb and thiofanox was obtained in October, 23 weeks after transplanting, with doses equivalent to <80, <90 and <40 mg AI/m row respectively. There was no evidence of carbofuran or chlorfenvinphos consistently reducing the performance of the aphicides.

Infestations of cabbage aphid on untreated plants 19-24 weeks after transplanting in 1978 were severe, live individuals being found on >96% of the buttons. The SMD during this experiment did not exceed 20 mm but none of the treatments eliminated live aphids from the buttons. Thiofanox failed to reduce the % infested buttons significantly ($P = 0.01$) compared with the levels of infestation on untreated plants, as did disulfoton (in the separate, single-component formulation, the mixed product of single

components and the two dual-component formulations) on 23 November, 24 weeks after transplanting. In all treatments, disulfoton reduced the % infested buttons significantly 19 and 21 weeks after transplanting but at least one live aphid was found on >60% of the buttons.

TABLE 1. Estimated mean % cover of buttons (B) and leaves (L) on Brussels sprout plants with live cabbage aphids (*Brevicoryne brassicae*) in 1976.

Assessment dates	Treatments applied against cabbage root fly		> 30 cm above soil		< 30 cm above soil	
	Insecticide	Dose (mg AI/m)	B	L	B	L
24 August	Nil	-	8	15	31	16
	Carbofuran	62.5	8	13	25	14
	Chlorfenvinphos	70.0	10	13	28	14
7 September	Nil	-	17	13	23	11
	Carbofuran	62.5	15	11	22	9
	Chlorfenvinphos	70.0	22	14	24	12
21 September	Nil	-	35	10	25	10
	Carbofuran	62.5	26	7	28	8
	Chlorfenvinphos	70.0	36	11	25	10
5 October	Nil	-	10	10	10	10
	Carbofuran	62.5	7	7	9	8
	Chlorfenvinphos	70.0	10	10	10	10
25 October	Nil	-	7	8	8	9
	Carbofuran	62.5	4	5	5	6
	Chlorfenvinphos	70.0	7	8	8	9

The % buttons infested with live aphids on plants in untreated plots in 1979 increased from c. 15% at the beginning of October to a maximum of c. 45% in November. Disulfoton, applied in a dual-component formulation, reduced the numbers of infested buttons 17-24 weeks after transplanting (P = 0.01), despite high SMDs on 44 days 12-19 weeks after transplanting. The foliar application, 6 weeks before the final assessments were made, of a fast-release formulation of disulfoton to complement disulfoton applied at transplanting virtually eradicated aphids.

DISCUSSION

The control of cabbage root fly and prolonged protection against aphids offered to brassicas transplanted in early summer by the results of experiments (including the 1976 experiment reported in this paper), in which combined granular root fly/aphicide treatments were evaluated, prompted the commercial production by an agrochemical company of the first dual-component granular formulation for use on vegetables in the UK (Sinclair & Purnell, 1977). A significant advantage of dual-component

TABLE 2. Lowest mean doses (mg AI/m row), for three consecutive plants in six replicates, of granular aphicides applied at transplanting in 1976 and giving Brussels sprout buttons (B) and leaves (L) without live cabbage aphids (*Brevicoryne brassicae*).

Aphicide treatments in continuous log-doses (+ insecticide treatments in uniform doses to control cabbage root fly) applied to single-row, 16-plant plots		Assessment dates											
		24 August				21 September				25 October			
		>30 cm above soil		<30 cm above soil		>30 cm above soil		<30 cm above soil		>30 cm above soil		<30 cm above soil	
Aphicide (+ insecticide)	Dose (mg AI/m row)	B	L	B	L	B	L	B	L	B	L	B	L
<u>Single-component formulations</u>													
Disulfoton	22.7 - 309	-	-	-	-	77	109	219	65	77	39	46	39
+ carbofuran	62.5	-	-	-	-	46	33	65	33	31	33	33	39
+ chlorfenvinphos	70.0	-	-	-	-	65	33	109	65	31	33	55	33
Ethiofencarb	22.0 - 299	-	-	-	-	-	177	-	177	75	75	89	75
+ carbofuran	62.5	-	-	-	-	-	149	-	177	53	53	53	53
+ chlorfenvinphos	70.0	-	-	-	-	149	-	-	211	125	125	125	125
Thiofanox	16.0 - 216	-	-	-	-	54	91	153	64	27	38	27	38
+ carbofuran	62.5	-	-	-	-	45	77	91	23	23	23	23	91
+ chlorfenvinphos	70.0	-	-	-	-	77	77	129	64	38	38	38	38
<u>Dual-component formulations</u>													
Ethiofencarb	29.3 - 396	-	-	-	-	167	99	-	118	49	70	70	140
+ carbofuran	14.7 - 198	-	-	-	-	83	49	-	59	25	35	35	70

formulations is that they obviate the need for the additional granule-metering equipment required for the simultaneous application of single-component granular products. Other dual-component granules have been developed since, formulations containing disulfoton with chlorpyrifos, fonofos or quinalphos and dimethoate with chlorpyrifos being available in 1990 (Ivens, 1990). Not only are the individual components of such formulations compatible, there is some evidence (Thompson, Percivall & Edmonds, 1979) that independent joint action between disulfoton and some of its co-formulated insecticides enhances the overall effectiveness of the products.

The chances of having new chemicals tailor-made for so-called 'minor uses' on UK vegetable crops are negligible; the financial outlay for companies developing and introducing new pesticides against even the principal pests of the major world crops has increased dramatically and incentives have dwindled to such a low-level that only a few, large, multi-national companies can afford to invest in the research needed. Thus the future control of pests in the large-scale, planned production of high-quality field vegetables in the UK depends on the availability of appropriate formulations of new insecticides introduced for the major world crops and on the better deployment of established compounds. The formulation of established insecticides in dual-component granular products to protect brassicas established in early summer against cabbage root fly and aphids provides a means for reducing the use of ill-timed and often ineffective aphicidal sprays. Opportunities to extend this approach to the control of aphids on overwintered brassica crops which have suffered greatly from aphid infestations in recent warm winters warrant exploration.

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AN ALTERNATIVE METHOD OF APPLYING COPPER TO CONTROL COCOA POD DISEASES

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ABSTRACT

Spraying of copper fungicides has been the only method of limiting losses in cocoa to black pod diseases (Phytophthora spp). Repeated applications are necessary during one season, often only achieving unreliable control. Water is required for dispersal of inoculum and infection, so disease pressure is normally highest when conditions are least favourable for retention of copper on plant surfaces. As an alternative to spraying, water-permeable reservoirs of copper fungicide were attached to cocoa stems so trunk flow and canopy drip would continuously wash copper down to pods concentrated on the lower architecture. This technique proved very successful, and data are presented from a small trial conducted in Trinidad showing the distribution of copper on bark and pods and the efficacy of the deposits against P. palmivora. Copper was distributed most efficiently when disease pressure was greatest.

INTRODUCTION

The cocoa pod pathogens Phytophthora spp. (black pod disease) can cause heavy crop losses. For many years the only economic method of limiting losses to this disease has been the spraying of protectant copper fungicides onto pods. Spraying can be costly and often unreliable, and rapid dilution of fungicide deposits by rainfall and pod growth means that effective doses have to be maintained by frequent spraying (Jollands & Jollands, 1989). Water is required for infection by the two pathogens mentioned above, and disease pressure is normally greatest during periods of high rainfall (Gregory & Maddison, 1981).

Copper fungicides readily redistribute in water (Hislop & Cox, 1970). Pereira (1985) suggested the exploitation of this property in a single application technique. This involved spraying the entire canopy once in a season, and allowing rain to redistribute fungicide onto the target pods. Conventional spray machinery is designed to provide good cover on targets that are widely dispersed through space. However cocoa pods grow directly from the main branches of trees and are concentrated in a small area. The present paper describes a field trial of an alternative technique to spraying for the application of copper fungicides to cocoa pods.

MATERIALS & METHODS

The redistribution of copper from a point source situated within the pod

growing area of the canopy was studied using absorbent collars impregnated with controlled quantities of fungicide. The collars were attached around trunks at c. 2 m above the ground, with the intention that water flowing down from the upper canopy would collect copper and carry it down to the pods lower on the same branch.

The collars consisted of non-absorbent cotton wool, in which a wettable powder (WP) formulation of cuprous oxide 'Copper Sandoz' was distributed at three rates: 5, 10 and 15 g a.i., wrapped in a man-made fibre material (Johnson & Johnson Ltd., Trinidad). A sheet of non-absorbent cotton wool 20 cm wide, 70 cm long and 0.5 cm thick, was laid flat and fungicide powder was evenly sprinkled over one surface. The sheet was folded in half lengthwise and further fungicide was sprinkled on the upper surface. The sheet was folded once more and wrapped in the outer coating material.

Three replicate trees were treated for each rate of active ingredient, plus three controls with copper-free collars. Pods were assessed at intervals during the rainy season for susceptibility to infection with Phytophthora palmivora zoospores by patch inoculations (Sreenivasan, 1984), after which total amounts of copper on pod surfaces were determined.

During the period of the collars trial, two applications of cuprous oxide by mistblower (Stihl SG17) were assessed on different sets of trees (Figure 1). The wettable powder and a suspension concentrate (SC) formulation 'Sandoz Copyflo' of cuprous oxide were sprayed at a rate of 1.12% a.i.. The machine gave a flow rate of 45 ml s^{-1}, giving approximately 5 g a.i. per tree with vmd values of 336 μm for the WP and 288 μm for the SC formulation. All trees were cleared of any diseased pods prior to spraying and random samples of immature pods were taken for copper determinations and bioassay at spraying and at two dates afterwards.

All experiments were carried out on mature cocoa trees (progeny of open pollinated TSH 1076 clone) planted at a 1.8 m x 1.8 m spacing at Centeno Field Station, Republic of Trinidad & Tobago. Rain gauges were placed in 10 positions on the ground underneath and outside the cocoa canopy and daily rainfall volume was recorded throughout the experiments. Copper determinations were carried out colorimetrically in 1M HCl extracts, by the method of Somers & Garraway (1957) adapted for use on a portable flow-injection analysis system (Heliflow - WPA Instruments Ltd., Cambridge).

RESULTS

Bioassays with P. palmivora zoospores indicated that at surface copper concentrations of 0.25 μg cm^{-2} or below, 100% infection occurred. At concentrations between 0.25 to 0.85 μg cm^{-2} and 0.85 to 1.45 μg cm^{-2}, 75 and 70% infection occurred respectively. At concentrations above 1.45 μg cm^{-2} no infection occurred on rain-eroded fungicide deposits, although some infection did occur on uneroded mistblown deposits of the WP formulation at concentrations of 1.82, 3.54 and 8.54 μg cm^{-2}. Inoculations were only carried out on the second and third harvests from collar-treated trees (Figure 1) and no successful infections were recorded except on controls.

Figure 1 :
Rain during copper application trials in Trinidad and amount of pod disease in various treatments with and without copper fungicides

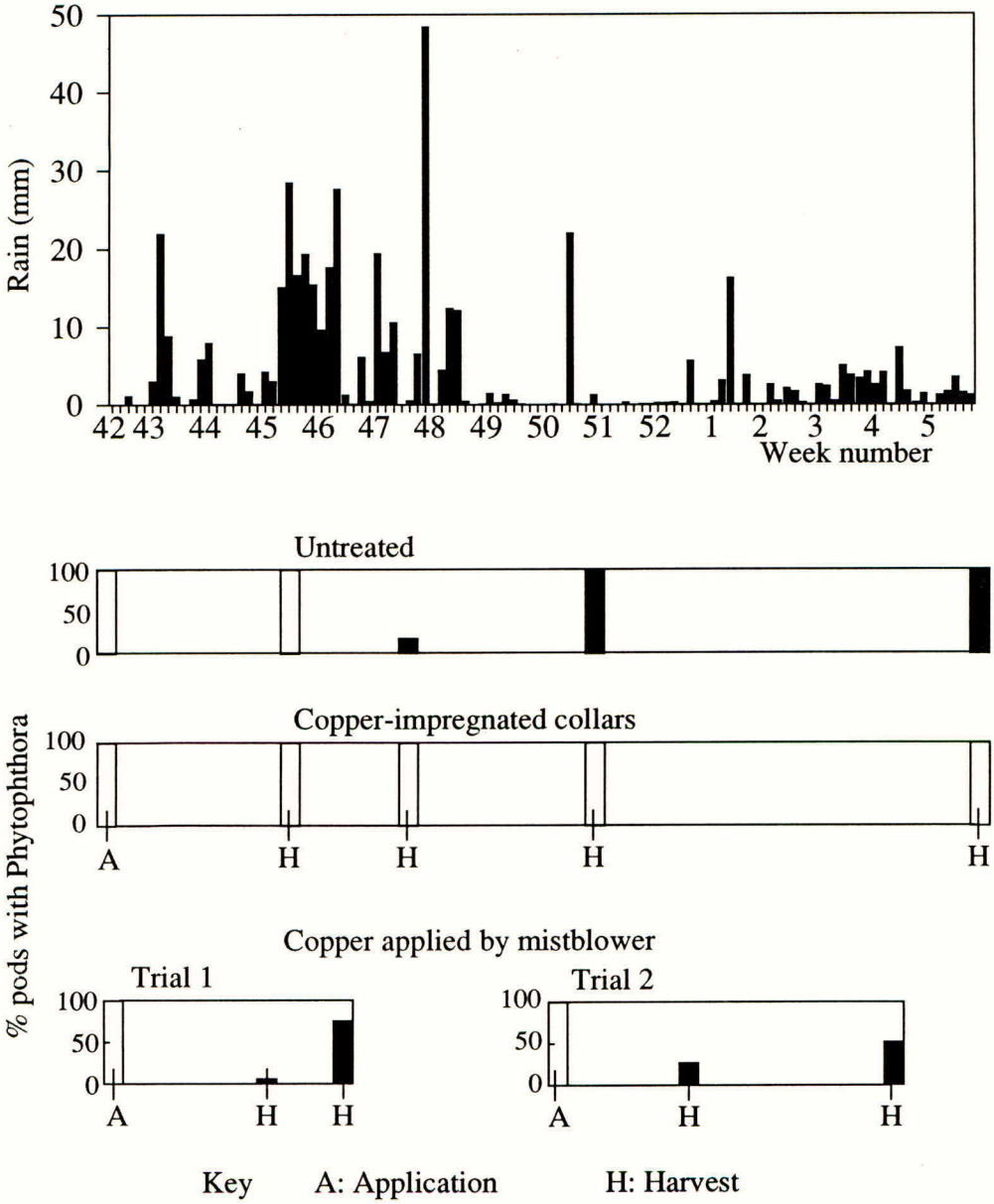

TABLE 1. Surface concentration of copper fungicide redistributed onto pods from fungicide-impregnated collars placed on main stem.

Sample time (Days)	Rain inside canopy (mm)		Amount of copper in collar (g)			Least significant difference (at P= 0.05)
	Cumulative		5	10	15	
20	23.5	23.5	0.35	3.14	0.85	1.99
35	105.9	82.4	5.40	10.23	8.27	4.18
59	153.3	47.4	5.20	6.76	5.23	N.S.
115	185.8	32.5	0.36	0.23	0.21	N.S.

The amount of copper on pod surfaces was greater when more rain fell (Table 1). The distribution of fungicide over pod surfaces was very even, in contrast to sprayed deposits where copper was more concentrated on the distal portions. Dispersal of copper was also linked with the streaming of stem-flow water. Concentrations of copper on pod surfaces generally increased with increasing distance below the collar (Table 2), although this was not reflected in the amounts detected on the bark (Table 3).

Amount of copper deposited on pods over the period of the experiment was independent of the amount of active ingredient present in the collars.

TABLE 2. Results of a destructive sample, taken on day 35 from a tree with two branches; one without a collar, the other with a 10 g a.i. collar.

Treatment	Height of pod from ground (cm)	Surface copper concentration ($\mu g\ cm^{-2}$)	No. of infections in bioassay (Counts out of 4)
Collar	78	18.97	0
	74	5.57	0
	72	7.50	0
	69	9.55	0
	55	10.87	0
	55	10.66	0
No Collar	87	0.52	4
	71	0.29	4
	71	1.05	4
	79	0.33	4
	48	2.67	3

TABLE 3. Copper concentrations on bark samples taken from collar-treated trees after 115 days.

Distance from collar (cm)	Amount of copper in collar (g)		
	5	10	15
50	23.5 (5.3)	28.1 (17.1)	21.5 (6.7)
100	16.9 (7.3)	27.7 (8.3)	18.3 (0.1)

Four samples were taken at each position, 90° from each other. Values in brackets are standard errors of the means (d.f. = 3).

TABLE 4. Copper concentrations on pod samples taken from trees sprayed with cuprous oxide formulations using a mechanical mistblower.

Trial 1				Trial 2			
Sample time (days)	Rain inside canopy (mm)	Formulation		Sample time	Rain inside Canopy (mm)	Formulation	
		WP	SC			WP	SC
0	0	12.3 (2.1)	13.6 (0.9)	0	0	14.7 (1.1)	11.3 (0.8)
17	30	0.8 (0.1)	9.6 (0.8)	20	18.2	10.2 (0.5)	8.2 (0.4)
45	98.9	0.4 (0.0)	1.1 (0.1)	29	83.3	0.6 (0.1)	1.5 (0.1)

Figures in brackets are standard errors of the means (d.f. = 19)

The dilution and loss of copper from mistblown fungicide deposits on pod surfaces was rapid and large (Table 4). Disease control broke down after 2–3 weeks in both trials (Figure 1). The flowable formulation of cuprous oxide was the best of the two compounds tested in terms of tenacity and efficacy.

Copper concentrations on pods declined towards the end of the experiment on all collar trees (Table 1). However incidence of Phytophthora was lower at this time (day 115), and copper concentrations on the bark were still quite high (Table 3). The amounts of copper remaining in the collars by day 115 were also still large (approximately 40–50% original amounts).

DISCUSSION

The concept of collar application is similar to the single application technique proposed by Pereira (1985). During the course of the field work reported here, nine impregnated collars successfully prevented disease on trees located in the middle of a serious 'black pod' epidemic, when control on sprayed trees broke down. However Pereira (1985) reported that the single application technique gave control only to a similar level to the regime of three-weekly spray intervals.

In work not reported here, a large proportion of the water incident on pods was found to be derived from trunk-flow. The movement of copper from collars was mainly in the trunk-flow water, and pods were constantly being bathed in a fungicide solution during rain events. The resulting even deposits of copper on pods gave the improved efficacy observed. Copper accumulated on pods lower down the main trunk, which are the pods at greatest risk from infection by Phytophthora from soil-splashed zoospores (Gregory & Maddison, 1981). Copper concentrations were not always above critical amounts (1.5 μg cm^{-2}) at times of low rainfall. However, there was a tendency for copper to concentrate in areas of the pod where water accumulated, which were also the most likely infection sites for Phytophthora. Continuous washing of trunks with copper may also give protection to flower cushions and prevent canker formation, thereby reducing another major source of Phytophthora 'black pod' infection.

ACKNOWLEDGEMENTS

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COST EFFECTIVE DAMAGE PREVENTION IN TRANSIT PACKAGING OF AGROCHEMICALS

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ABSTRACT

Influences on the design of transit packaging include safety considerations, transport and warehousing, and legislation. Of these safety is of prime importance and should override purely economic considerations. Use of new materials and packaging techniques can maintain or improve packaging performance and at the same time reduce costs. These cost reductions can be direct materials saving or accrue from more efficient palletisation, warehousing and transport.

INTRODUCTION

Transit packaging for chemicals is designed to protect the product including any primary packaging (bottles, cartons, sachets etc) from damage during distribution and storage. It is also designed to contain potentially hazardous products thus protecting the immediate environment and ensuring the safety of personnel handling the pack. Adoption of modern materials such as PET (polyethylene terephthalate) for primary packs or sophisticated corrugated board specifications for the transit pack can maintain safety and performance standards whilst achieving useful economies.

The major influences on transit pack design for chemicals are discussed.

MAJOR INFLUENCES

Safety

This is the primary concern and overrides purely economic factors. Safety considerations throughout the distribution chain from the filling line to the end user are of utmost importance in package design.

Legislation

National and international legislation is framed to ensure minimum standards are applied to packaging for the transport of hazardous goods. Some of the bodies specifying requirements are IMDG (for sea transport), ICAO and IATA (for air transport), ADR and RID (European road and rail). These differing sets of regulations are now adopting a unified set of performance standards. These have been produced under the auspices of the UN. They include performance tests for transit packages covering drop test, compression test, pressure and leakage tests. Performance standards are dependent upon the degree of hazard and packaging must be designed accordingly.

Legislation also calls for accurate hazard data to be displayed on the packs in the form of the 'diamond' symbols. Product identification (UN numbers), snippers name and address, emergency telephone numbers, information for the emergency services might also be called for depending upon the mode(s) of transport and country of origin and destination. It is vital that these labels remain intact and legible through normal distribution, but also in the event of an incident. New, synthetic materials are therefore being used extensively for hazard labels.

Transport and Storage

In order to make most effective use of transport, pallets are usually sized to fit vehicle sizes eg 1150 mm square pallets fit side by side across an ISO freight container. It is clearly important to design packaging to make maximum use of the deck of the pallet. It is also important not to overlook the height factor. This can be influenced either by vehicle size or by any racking system in storage areas anywhere along the distribution chain.

Apart from dimensional criteria, other factors are equally important. The height and type of stacking used throughout the distribution system will affect the compression strength requirements of the package. This might be over and above the requirement of the UN recommendation. Climatic conditions likely to be encountered must be considered. The pack should be so constructed as to protect its contents from moisture and humidity, and should be made from materials able to withstand anticipated conditions and maintain their strength properties.

New Materials and Techniques

These can often provide advantages over traditional methods from filling right through to end user. Changes to the primary packaging can have a significant effect on the transit outer package due to changes in both the level and nature of protection required.

The following case histories will illustrate whether new ideas can give economic and other advantages.

1 LITRE PESTICIDE PACKAGING

Until recently 1-litre packs for pesticides had to be made from one of a variety of materials dependent upon chemical compatability. Containers in polyethylene, tinplate, aluminium were all commonplace. Each of these materials suffered from one or more disadvantages. Polyethylene has a limited range of compatibility and is generally not suited to solvents. Tinplate is prone to leakage, it dents easily, and rusts. Aluminium bottles are expensive, easily dented, and some designs have inadequate closure systems.

The advent of PET bottles has enabled the industry to provide superior presentation without sacrificing pack performance and safety at the same time as achieving material package costs savings.

TABLE. Comparison of qualities and relative costs of 1 litre container types.

ALUMINIUM BOTTLES	PET BOTTLES
Susceptibile to impact damage.	Resilient. Do not dent easily.
Transit pack requires cushioning to protect bottles.	No cushioning required.
High compression strength.	High compression strength.
Case does not need to contribute to stacking strength.	Case does not need to contribute to stacking stength.
Difficult disposal.	Burns easily.
Poor closure features.	Easy open features.
Expensive.	Relatively inexpensive.
Typical costs:	Typical costs:
Bottle + Plug 40p x 12 = 480p Case + Fittings = 110p	Bottle + Cap 20p x 12 = 240p Case = 50p
Total 12 x 1 litre cost £5.90	Total 12 x 1 litre cost £2.90

25Kg POWDER PACKAGING

Powder products for export were traditionally packed into open top steel drums fitted with polythene liners. The drums are 356mm diameter x 610mm high. They can be stacked 3 high in a standard ISO freight container giving a full load of 270 drums. The introduction of high performance fluting media for corrugated cases has enabled the development of packs having the same capacity but smaller overall dimensions (356mm x 356mm x 480mm). This enables stacking 4 high in a container, thus increasing the payload by 25%.

The performance of the case was tailored to meet exacting demands. In addition to the use of high performance flutings in a double wall construction giving a compression stength of over 1,000kg, the outer liner of the case is a kraft/polythene/kraft sandwich to afford moisture protection.

The pack has been successfully tested to UN Group II standards. These include drop tests from 1.2 metres and static load tests to 3 metres high.

Benefits throughout the storage and distribution chain include:

- savings in storage space of empty packs. 120 boxes can be stored on one pallet where only 18 drums could previously be held;
- materials cost (drum and liner approx £4.20, case and liner approx £2.40);
- container loading (an increase of 25% in payload);
- customer convenience (the boxes can be flattened after use and disposed of by burning).

TEMPERATURE CONTROLLED PESTICIDE RELEASE SYSTEMS

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ABSTRACT

Microcapsules containing selected pesticides have been developed which utilize the unique release characteristics of Intelimer™ polymers. In response to changes in soil or air temperature, active ingredient is released at the temperature corresponding to the onset of the pest. The release point may be selected throughout the normal biological temperature range. A variety of agrichemicals including diazinon and trifluralin have been successfully microencapsulated using this technology. Greenhouse studies indicate that microencapsulating trifluralin dramatically reduces its phytotoxicity to corn and allowed a 50% reduction in the amount of active ingredient needed for weed control in cotton. In field trials, trifluralin microcapsules at an application rate of 0.55 kg AI/ha provided equivalent 90 day control of weeds to an 1.1 kg AI/ha treatment of the commercially available formulation; 'Treflan 4' emulsifiable concentrate (EC). Bioassay results have demonstrated the decrease in crop phytotoxicity.

INTRODUCTION

Interest in controlled release technology for agrichemicals is rapidly increasing. Problems associated with groundwater contamination, degradation, volatilization, and the banning of pesticides have all contributed to the need for more efficient delivery systems (Kearney, 1977). Improved economic and environmental impact can be realized by efficiently delivering lesser amounts of active ingredient while maintaining efficient pest control (Kyodonieus, 1980).

Predictable increases in soil temperature during the growing season trigger a variety of biological events such as germination, egg hatching, and pupation (Apple, 1971; Ruppel, 1984). Effective control of pest popu- lations can be obtained by delivering the pesticide at a temperature when the pest becomes active. Landec has developed a family of side chain cry- stallizable polymers (SCCP) with melting points in the 15°-30°C range, which protect agrichemicals until they are required (Stewart, 1989).

Greenhouse and field testing indicate the following advantages: 1) reduced crop phytotoxicity; 2) delayed release; 3) extended efficacy and 4) reduced application rates.

MATERIALS AND METHODS

The polymers used in this work were prepared via solution polymerization. Polymers were purified by precipitation into a non-solvent and characterized by gel permeation chromatography, infrared spectroscopy, and thermal analysis.

Technical grades of diazinon and trifluralin were supplied by Ciba Geigy and Griffin Corporation, respectively.

Microcapsules were prepared using standard emulsion processing. Diazinon release rates were conducted in water. The concentration of AI in the solution was determined using a UV spectrophotometer set at 246 nm. Trifluralin release rates were conducted in ethanol:water (1:1 V/V). The concentration of AI in the solution was determined using a UV spectrophotometer set at 279 nm.

The northern corn rootworm (Diabrotica barberi) bioassay was conducted by Midwest Research Inc., in York, Nebraska. Soil from their farm was thoroughly mixed with each formulation and put into cups. These cups were then incubated at the appropriate temperature and the efficacy against the corn rootworm was determined. This was done by adding one partially germinated corn seed to each cup and then introducing 10 corn rootworm larvae to the container which was then capped. After 48 hours, all the soil was sieved and the number of living corn rootworms was determined. Field trial chemical application were conducted according to standard farmer practice. Small trial plot were used; 4 rows of corn, 20 feet long were used for each treatment regime. Applications of the formulation were made at planting and corn root ratings were done at the end of July using the standard root rating procedure and scale (ratings of 1-5 are given with 1 being no damage and 5 being 100% damage).

The trifluralin greenhouse and field bioassays in cotton were conducted by Research for Hire, Inc., in Porterville, California. For the greenhouse bioassay, the formulations were sprayed on the soil, thoroughly mixed with it, then put into bread pans. Application rates of 0.21, 0.42, 0.83 and 0.165 kg AI/ha were used. Barnyard grass (Echinochloa crus-galli) was then planted in these pans on a monthly basis and the number of plants that germinated was counted. The greenhouse trial was run for 90 days. Field applications were made in cotton using standard grower methods for the San Joaquin Valley cotton. Application rates of 0.206, 0.412 and 0.825 kg AI/ha were used. Weed control was determined by placing a rectangular sampling template at random, in the middle three furrows of each replicate. Weed species within the rectangle were identified and counted. Three different template area ratings were made for each replicate. Weed efficacy ratings were made 30, 60 and 90 days after treatment.

The trifluralin corn field trial was conducted by Midwest Research. Field applications were made with a light incorporation (ie: to a depth of 1") using application rates of 0.55, 1.10 and 1.60 kg AI/ha. Corn phytotoxicity ratings were taken at 30, 60 and 90 days. The 30 day rating measured corn germination in number of plants germinated per 100 feet of row. The 60 and 90 day ratings were visual ratings of corn injury in per cent. Weed control ratings were done visually in per cent.

RESULTS

The primary goal of this work was to determine the potential benefits of SCCP formulated pesticides under greenhouse and field conditions. Testing was conducted with the following objectives:

A. Reduce the amount of active ingredient required to achieve economic pest control.

B. Assess the ability to reduce crop phytotoxicity.
C. Demonstrate storage stability (including temperature cycling) of SCCP microcapsules in a water based formulation.

Side chain crystallizable polymers used in encapsulation have an acrylic backbone, with a series of long chain fatty alcohols esterified to it. These fatty alcohol moieties are referred to as "side chains". It is the ability of these side chains to crystallize and then melt over a very narrow temperature range (5 degrees C), that allows us to vary the release rate of the microcapsules with temperature.

The side chain length influences the release temperature of the polymer by changing the polymer melt point(Tm). We utilized side chain lengths of 12 to 18 carbon units to achieve release temperatures from 15°C to 30°C.

Diazinon Microcapsules

A laboratory bioassay was developed with the following objectives:

1. To measure biological efficacy above and below the Tm.
2. To confirm that a lower dose of AI is required using Landec capsule suspension (CS) compared to a commercial granular formulation of diazinon '14G'.

Microcapsules with a 30°C Tm were tested. The release increased from 2.5ug/hr at 20°C to 17ug/hr at 30°C. Figure 1 illustrates the results of the biological efficacy of our formulations against the corn rootworm compared to a commercial diazinon granule 14G. This test was designed to demonstrate low efficacy at low temperature when the microcapsules are in the crystalline state and high efficacy above the Tm when the microcapsules are turned on.

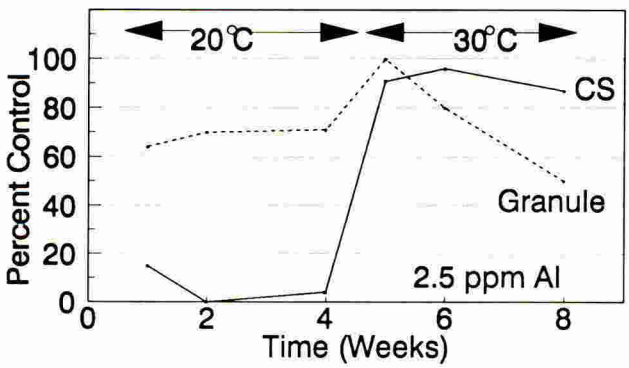

Figure 1: Diazinon corn rootworm efficacy 20°C (4 weeks) and 30°C (4 weeks).

The granule formulation exhibited the greatest efficacy at 20°C, then pest control gradually decreased after the temperature increase. This was expected since the granule releases all of its active over a short period of time. Diazinon's half life decreased rapidly with temperature as was seen during the 30°C test period. After eight weeks, the granule had the lowest efficacy at 50% control.

The CS formulation gave significantly less control at 20°C but control increased to 90% when the temperature was increased to 30°C. This higher level of control continued for 4 weeks at 30°C after which the experiment was terminated. At 2.5 ppm, this formulation was superior to the granule in its period of control. This data suggests that to achieve equivalent efficacy to

the CS formulations with the granule, more than 2.5 ppm AI would have been required.

Trifluralin Microcapsules

Microcapsules with a 30°C Tm and the release profile shown in Figure 2 were used for the cotton trials. A laboratory bioassay was developed with the following objectives:

1. To determine the period of control provided for each formulation tested.
2. To determine if the amount of AI could be reduced using SCCP formulated trifluralin.

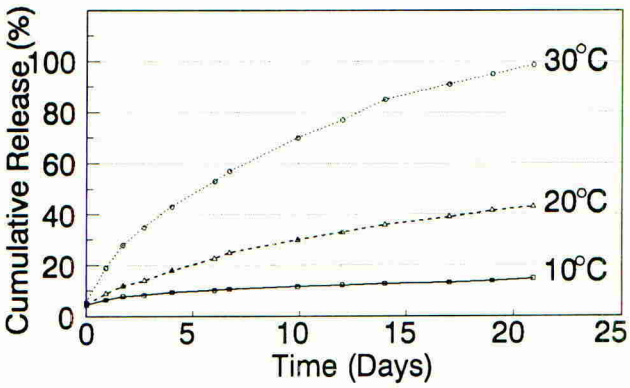

Figure 2: Release rate of trifluralin microcapsules in ethanol:water vs temperature.

Figure 3 compares the efficacy of the EC and CS formulations at 30°C, 90 days after application. At all rates tested, the CS gave superior control to the EC. Furthermore, the CS at 0.82 kg AI/ha gave better control than 1.65 kg AI/ha of the EC. In the greenhouse, microcapsules also reduced the amount of active ingredient required for economic weed control.

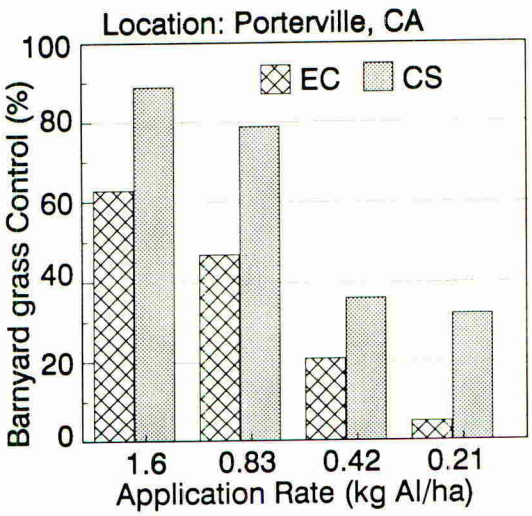

Figure 3: Trifluralin control of barnyard grass in the greenhouse at 30°C, 90 days after treatment.

Figure 4 summarizes the data for the control of pigweed at 30, 60 and 90 day intervals. At 30 days all three formulations gave equivalent control. At 60 days, the EC at 0.55 kg AI/ha was beginning to lose efficacy compared to the EC at 1.1 kg AI/ha and the CS. At 90 days the EC at 0.55 kg AI/ha exhibited a significant loss of weed control while the CS at 0.55 kg AI/ha was providing equivalent control to that of the EC at 1.1 kg AI/ha. This data illustrates quite clearly that CS formulations can reduce the application rate of trifluralin under field conditions.

Figure 5 shows the results of applying encapsulated trifluralin to corn to reduce its phytotoxicity. At the 0.55 kg AI/ha application rate, neither the EC and nor the CS show significant phytotoxicity. However, at the 1.1 kg AI/ha rate; the EC was quite phytotoxic to the corn, while the encapsulated trifluralin formula allowed greater than 90% germination. 'Dual' 8 EC (Metolachor) was used as the commercial standard and had no corn phytotoxicity at its normal use rate. All three formulations, at the rates tested, gave equivalent weed control at 30 days. This data clearly illustrates the safening ability of the CS formulations and that they may be able to both expand the safety margin of herbicides and the use of active ingredients in crops where phytotoxicity has been a problem in the past.

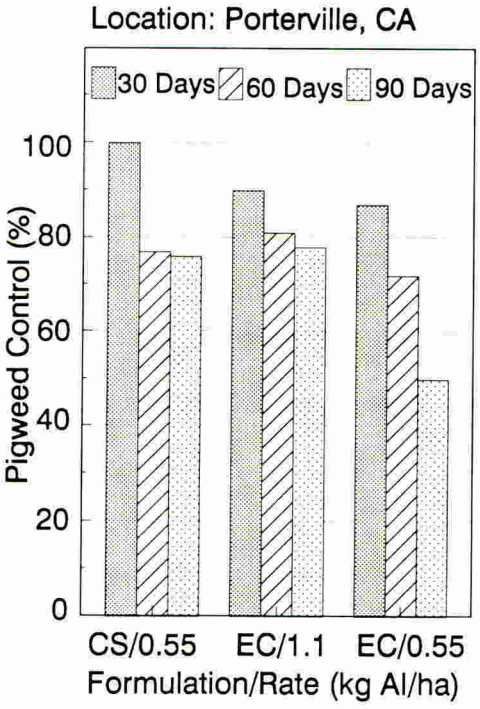

Figure 4: Trifluralin cotton field trial 30, 60 and 90 days after treatment.

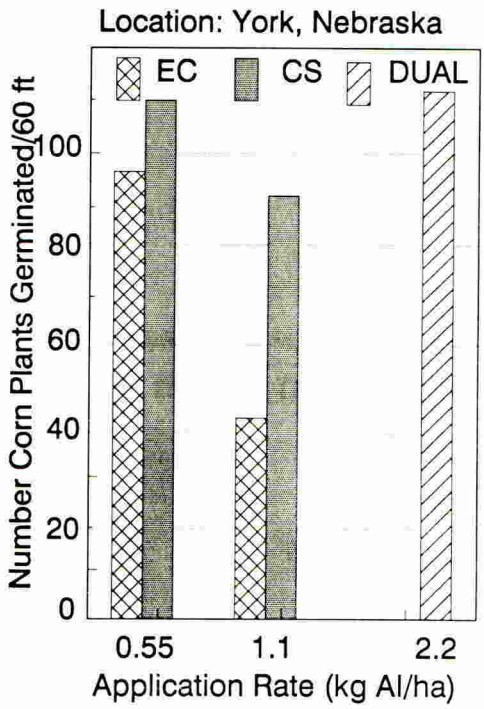

Figure 5: Corn phytotoxicity of SCCP microencapsulated trifluralin 30 days after planting.

CONCLUSIONS

Greenhouse and field trials with microcapsules of both diazinon and trifluralin gave the same general results:

1. In efficacy tests, temperature activated, delayed release of the pesticides was observed.
2. The same relative release rates were observed in soil and in vitro.
3. Efficacy can be achieved using substantially less active ingredient in an SCCP microcapsule than with conventional delivery systems.
4. Crop phytotoxicity can be significantly reduced.

Storage stable formulations have been developed that meet commercial standards. The combination of reduced field rates, high loading of active ingredient in the capsule and modest projected polymer cost indicate that the SCCP formulated pesticide system will be economically attractive.

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