SESSION 3A

CROP PROTECTION IN CHANGING AGRICULTURE

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INVITED PAPERS 3A-1 & 3A-4

SET-ASIDE - THE MANAGEMENT AND ECONOMIC FACTORS

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ABSTRACT

Rules and guidelines for a voluntary set-aside scheme were introduced into the UK in 1988. The scheme has both financial and social attractions to some farmers, but it poses many questions on how to manage land that has been set aside. This paper shows how farms should cost the implications of set-aside and raises issues that need further research.

INTRODUCTION

Speculation over whether set-aside would form part of the measures to curb cereal surpluses in the UK have been rife for several years. The publication in January 1988 of a Proposal for a European Communities Council Regulation "as regards the set-aside of agricultural land and the extensification and conversion of production" (Commission of the European Communities 1988) made it clear that set-aside was to form part of a production-cutting package throughout the European Community. This document acknowledged the need to take at least 20% of arable land out of production for at least five years; and to "maintain good husbandry conditions and lay down rules for protecting the environment." The details and levels of payment were to be left to member states to implement. While every member state was obliged to have a scheme set out by the summer of 1988, its implication was to be voluntary for farmers.

In the UK speculation came to an end when UK MInister of Agriculture, Fisheries and Food, John MacGregor, laid the government's plans before parliament on June 14 (Hansard 1988). This set the levels of payment at £200 a ha for land set aside permanently over the five year period (£180 in less favoured areas); and £180 a ha (£160 in LFAs) where the acreage set aside is rotated around the farm.

The minimum area to be set aside is 20% of the relevant arable crop acreage. Any amount of land above this minimum percentage can be taken out of production up to 100%.

As well as simply setting land aside there is the option of converting it to woodland, which will be dealt with in a later paper, and converting it to certain non-agricultural uses. In the latter case the permanent fallow payments are reduced by £50 a ha to acknowledge the income which such use would generate.

There were some unexpected parts to the proposals. For instance, the stipulation that the land set aside has to "be maintained with a green cover crop". There was also some mixed reaction to the Minister's decision not to allow extensive grazing of land. Obviously, committed livestock farmers were relieved that there would not be increased competition from "subsidised grazing land".

ECONOMICS

Initial reaction from many commentators and farming spokesmen to the proposals was that payments of £200 a ha would be insufficient to induce significant numbers of farmers to adopt set-aside. Standard farm costings show that average winter wheat crops can be expected to yield gross margins of £415 to £480 a ha (Nix 1988). However, as further details of the scheme emerged it became clear that a significant number of farmers were interested and viewing set-aside as a serious option.

Initial response had been based on straight comparisons between cereal margins and set-aside payments. This is an inadequate method of assessment. In financial terms, whole farm costings are needed to assess whether setting 20% or more of the farm aside is viable. Among the factors to be considered are the likely reduction in labour and machinery requirements. For some farmers taking some land out will allow them to cut out one unit of labour and reduce power and machinery accordingly. This will bring dramatic savings in fixed costs per unit of production. Add these savings to the set-aside payments and the discrepancy is not so large as comparisons between set-aside payments and gross margins would appear to show.

Furthermore, there is the ability to set aside field boundaries, at least 15m wide, and to rotate the land taken out around the farm. Headlands yield considerably less than the body of the field, so setting these areas aside will increase average farm yield and return on inputs.

A typical example has been supplied by Vincent Hedley Lewis, senior farms partner with chartered accountants, Spicer & Oppenheim, for his own farm in Lincolnshire. This is set out in Tables 1 and 2.

His initial calculations were based on taking certain poor yielding headlands out permanently and rotating set-aside on some heavier land. As a result of this exercise he realised that setting headlands aside was far more attractive than the option to rotate fallow around the farm - in his particular case. However, even these initial costings showed the loss in gross margin over the 315ha farmed would be £3,368 at current prices, or just £10 a ha - considerably less than the sums put forward by some opponents of the scheme.

TABLE 1

Costs and savings from permanent set-aside.

COSTS	£/ha	SAVINGS	£/ha
Fallow maintenance	30	Grant	200
Loss of margin	210	Capital interest	22
		Fuels inc drying	20
Net saving	2		
	240		242

TABLE 2

Costs and savings from rotational set-aside.

COSTS	£/ha	SAVINGS	£/ha
Fallow maintenance	37	Grant	180
Loss of margin	432	Increase yield	25
		Seed premium	49
		Fertiliser	37
		Herbicide in 1st wheat	17
		Herbicide in 2nd wheat	37
		Drying costs	25
		Interest	22
			392
		Net cost	77
	469		469

On some farms the reduction in gross margins could be more than offset by the reduction in fixed costs which the scheme allows. Savings, which have not been taken into account in the above example. For this reason it is important to consider each farm individually and to carry out detailed costings.

Another argument against the economics of set-aside is the fact that the payment is not index-linked and so any benefits on current costings will be eroded by inflation. This is not necessarily so.

Table 3 puts forward a rather gloomy projection of the likely effects on economics on the value of set-aside payments and the returns from farming. It takes a base return of £148 a ha from both options. The following assumptions for returns from conventional farming have been taken: cereal yields to continue rising by 2% pa; produce prices falling by 1% pa; and fixed costs, excluding rent and finance, rising by 5% pa. If these assumptions prove right, then set-aside could be a course for financial survival.

TABLE 3

Projected returns for set-aside and arable farming for years ending September 1989 to 1993.

Year	Set-aside £/ha	Arable farming £/ha
1989	148	148
1990	136	131
1991	124	114
1992	114	91
1993	101	70

In addition, to the straight financial considerations there are such matters as benefits in sporting and amenity values which may need to be taken into account in assessing the potential which set-aside offers an individual farmer.

MANAGING SET-ASIDE

Table 2 makes certain assumptions about yield and input requirements which are to some extent dependent on management of the set-aside land. For a number of questions and potential problems must be considered in looking at the running of set-aside land. There are a number of areas of technical, financial and environmental concern and while the questions can be identified there is little information available to provide answers.

Farmers who already operate non-agricultural enterprises such as caravan sites and pony trekking centres would point to some serious pest problems, but the handling and control of human pests is outside the remit of this paper!

The first matter which potential set-asiders must address is how to achieve green cover. The guidelines (MAFF 1988) allow cover to be achieved "by sowing or by allowing the naturally occurring vegetation to regenerate". Natural regeneration has a low-cost appeal, but it also raises considerable worries about the "Green Bridge" effect for pests and disease. The alternative, although more costly, is to establish a cover crop. Among the likely options are mustard or similar brassica or a ryegrass mixture, which are relatively inexpensive; a leguminous crop, while more expensive to establish, has attractions in the nitrogen it may supply to following crops - but this is a questionable benefit as will be discussed later.

Regenerated cover is likely to be a mix of volunteer cereals and weeds. All pose potential agronomic problems. Even if a cover crop is established, the guidelines forbid the use of pesticides (except in instances of severe weed infestation) or fertilisers so that weeds and volunteers would still thrive, although to a lesser extent given the crop competition.

Among the diseases, the main cause for concern would be the foliar diseases, especially Erysiphe graminis (powdery mildew), Puccinia recondita, P. hordei and P. striiformis (rusts), Septoria triciti and S. nodorum, and Rhynchosporium secalis. All these pose a potential threat to surrounding fields on land still in agricultural production. Such a spread could reduce yield potential and increase production costs. Also there must be doubts as to whether seed-borne diseases such as Pyrenophora teres (net blotch) would be carried over through self-seeding volunteers, given that no crop can be harvested from set-aside land.

The effect on Gaeumannomyces graminis (take-all) must also be questioned, especially in rotational fallow. The land will in theory have a year's break. However, because volunteer cereals are likely to be present in significant numbers there may be enough host tissue to prevent any effect on take-all decline. This can be used to advantage in a rotational fallow. For example, if the year before set-aside the land had been in a second year wheat, the fallow year would be likely to see take-all levels peak and the land come out of take-all decline for the first wheat sown after set-aside. However, farmers seeing a fallow year as one in which to break the take-all cycle could be disappointed through the incidence of volunteers.

Turning to pests, one can see similar difficulties being posed by set-aside. When it was thought that set-aside practice would involve bare fallowing, concern was expressed that the incidence and severity of Leptohymeia coarctata (wheat bulb fly) would increase. For permanent fallow with green cover this is no longer a risk. However, the guidelines for rotational fallow allow the land being returned to normal cropping in September to be cultivated after August 1st. Such practice will create bare land at the ideal time for egg-laying and so the question arises - will rotational set-aside increase the incidence of the pest?

Questions must also be asked about the risk of a "green bridge" being provided for aphids and the barley yellow dwarf virus they transmit. Where trash is buried in the course of establishing a cover crop it is likely that aphids on the crop residue will survive on the mulched trash. Also any living cereal crop tissue may provide a reservoir of BYDV infection for aphids feeding on buried material over winter. While this is a possibility, there is no evidence as yet of the effects.

Cover crops must be mown at least once a year, according to the guidelines. This is likely to create a mulch which will encourage slug numbers. These may pose a threat to following crops in rotational fallow, but the evidence points to weather and soil conditions at drilling being more relevant to the incidence of slug damage than populations in preceding months. Again, more work is needed to decide what implications set-aside has.

The question of mowing also raises queries about weed levels in set-aside land. Weeds are potentially the most worrying aspect of rotational fallow. The old adage that 'one year's seeds equals seven years' weeds' is worth bearing in mind.

Allowing green cover from regeneration means relying on weed cover. To prevent a severe build-up it will be important to ensure these plants do not seed. In the absence of herbicides this means relying on mowing, or topping. Farmers who calculate their financial costs of set-aside based on one pass with a mower will be caught out. Mowing trials with Alopecurus myosuroides (blackgrass) have shown that it will be necessary to mow in May when the earliest tillers come to ear, and that once mown there is rapid and vigorous re-tillering which can head in a very short time. Therefore, the implications are that a more intensive mowing regime may be needed to keep weed species under control. The higher cost of this could be avoided by investing in a vigorous smother crop such as Italian ryegrass or mustard.

As with other aspects of set-aside agronomy there is a need for much more work to done on weed population dynamics in a fallow situation.

Finally, on the subject of mowing it is worth considering the consequences for wildlife which set-aside presents. The recent work with conservation headlands where no pesticide is used on a headland strip has attracted considerable attention. It could be thought that headland set-aside would have added attractions. However, if the type of mowing regimes described above for weed control were adopted, then the wildlife would be destroyed. Guidelines for environmentally-sensitive areas in Britain and for extensification in Germany prohibit any mowing before July - cutting in May to control blackgrass and wild oats would destroy birds as they sat on their nests.

There are provisions, where an approved conservation project would be harmed by mowing, for the land to be left alone. While this may enhance chances of increased nesting and hatching, it makes no allowance for rearing young birds who depend for food on cereal crops. One suggestion, put forward by the Game Conservancy Council, is to set aside strips across large fields, so providing extra nesting sites and game bird refuges, and giving growing chicks access to cereal crops nearby.

Perhaps another answer would be to use cereals as the set-aside cover crop. This would provide the right environment for game birds and, provided the crop was not harvested, would satisfy the spirit of set-aside. However, at present the sowing of cereals on set-aside land is forbidden.

The other area of environmental concern is the issue of nitrates. The use of a green cover crop will prevent the excessive leaching which would be likely to occur under a bare fallow system, but the extent of the problem will depend on management, especially in rotational fallow. The highest risk of leaching comes in the autumn months when nitrates mineralised during summer and early autumn are washed out of the ground by autumn rains.

Allowing natural regeneration is likely to provide weak plants in the autumn with a limited nitrogen requirement. These situations are likely to lead to higher leaching rates. On the other hand a vigorous cover crop, such as a ryegrass, will have a higher nitrate demand and reduce leaching significantly. In permanent fallow situations the losses in following years are unlikely to be excessive compared with other farming systems. Where rotational fallows are returned to arable farming, good husbandry practice will demand an autumn-sown crop to mop up mineralised nitrogen reserves in the soil.

Another assumption being made is that, in rotational fallows, there will be a green manure benefit. There is little evidence to support this. Historical evidence from green manure trials showed some increase in older varieties of cereals and at rates of nitrogen below those considered commercially viable today. Where higher rates of nitrogen were applied after green manuring the response was negligible. Further work is needed with modern varieties and practices to establish if there is any benefit to be obtained from green manuring.

OVERSEAS EXPERIENCES

As yet, UK set-aside is an untried experience; no-one can predict what the likely uptake will be, or what success there will be in achieving its primary aim of cutting surplus production. Two countries have had some experience with set-aside and their experiences may be of some relevance.

In the United States set-aside programmes have been used for a number of years. Their aims have been two-fold: to control erosion and to control production. It would appear that the various programmes have been more successful in the area of erosion control. Although there has been renewed emphasis put on set-aside this year as a means of curbing production. In evidence given to the House of Lords Select Committee dealing with set-aside, US Agricultural Counsellor, R E Anderson, described how over 69 million acres of land was "idled" in 1987, the largest area ever taken out of production, with the exception of the Payment-in-Kind scheme of 1983. (House of Lords 1988).

The US experience also highlights something often referred to as slippage - the difference between the land taken out of production and the reduction in arable production. This arises because, as was seen in the example give above, it is in the farmers' interests to take the least productive land out of production. In 1986 the US had a 22.5% set-aside and achieved a decline in production of just 6.5%.

It has been argued that slippage would not be as high in the UK. In the US, slippage arises from intensification on land kept in production; UK farmers already operate a higher level of efficiency with far higher inputs and so have no room for marked increases in intensification, it is argued. However, if some of the estimates for the loss of yield on headlands are to be believed, and headland set-aside was widely practised, the slippage would be significant.

This disparity between area set aside and curbing of output was also seen in Lower Saxony in the Federal Republic of Germany, another area with some experience of set-aside. In 1986, the first year of the scheme, some 2.2% of the eligible land was set aside; grain production fell by only 1.8%.

The acceptance among the farmers of Lower Saxony was also interesting. In the first year of the scheme payments ranging from £333 to £400 a ha were offered. Only 12.6% of eligible farmers registered and, as stated above, only 2.2% of eligible land was set aside - mostly poorer sandy soils. The House of Lords Select Committee considered the Lower Saxony experience and attributed the low rate of take-up to:

- a psychological reluctance among farmers to leave land fallow;

- a relatively short deadline for registration;

- fear of disadvantage should cereal quotas be introduced;

and a survey revealed that 80% of farmers felt the payments were inadequate (House of Lords 1988). It is quite likely that a similar pattern of findings could be reported for the UK in a years' time.

In 1987, payments in Lower Saxony were raised from £446 to £533 a ha. As the application procedure was closed in mid-August, it is assumed that the government's target area and compensation budget had been reached.

CONCLUSIONS

There is good reason to believe that there are sound economic arguments for some UK farmers to consider setting a portion of their arable land aside. Although each holding needs to be considered separately and close attention paid to its cost structure. For farmers who set their land aside, there are still a great many question marks hanging over just how the land should be managed and what benefits, or risks, there are, both in the short and medium term.

State-funded trials are underway in the UK. These are being conducted jointly by the Agricultural Development and Advisory Service and the Agriculture and Food Research Council under the title, 'Fallowing Techniques and Methods'. Sites have been established on five Experimental Husbandry Farms and two AFRC locations to provide a complete spread of soil types. The trials, first laid down in autumn 1987, aim to look at various ways of managing fallow; and have had to be amended in 1988 to include the need for green cover. In the first (fallow) year, there will be intense study of the pest, weed and disease problems along with the effects of fallow on nitrate leaching. The second year sees the plots put back into cereals with a wide range of nitrogen regimes to study the fertiliser benefits which a green fallow may bring, as well as continuing to monitor nitrate effects. Pest, weed and disease monitoring continues in the second year and plant pathology studies will continue into the third year to study such phenomena as take-all decline.

That such a detailed and intensive series of investigations has been set up is to be applauded for, as this paper has shown, there are many unanswered questions on the subject of set-aside management. It is important that adequate funding is made available for such detailed studies and the Ministry must be prepared to amend its set-aside guidelines in the light of these findings.

There are also considerable social and political factors to be addressed. If any significant amount of land is set aside there will be a reduction in skills and resources in our countryside. If large tracts of the countryside are taken out of production today, it may be many years before there is the manpower or capital funding to re-establish commercial agriculture in these areas.

Also, will the public accept a countryside where fields of weeds are mown once or twice a year? Letting land revert to nature is a nice concept for the armchair countryman, but when nature is left to her own devices she is a very untidy gardener.

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IMPLICATIONS FOR DISEASES AND PESTS OF FARM DIVERSIFICATION INTO WOODLANDS, SPACED TREES AND SHORT-ROTATION COPPICE

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ABSTRACT

Implications for diseases and pests and their control are considered for farm woodlands, agroforestry (spaced trees with multiple land use) and short-rotation coppice. Diseases and pests of the tree species likely to be grown in both farm woodlands and agroforestry systems are, on average, not likely to present new or more serious problems because of the diversity of species grown and the distribution patterns of trees in these situations. Poplar is the most widely planted tree in Europe for agroforestry by virtue of substantial breeding programmes to control its major diseases (leaf spot, rust and bacterial canker); a similar approach will be necessary to deal with the major disease of cherry (bacterial canker) if cherry is to fulfil its anticipated future role in furniture manufacture. The cricket bat willow is highly profitable on a local scale but if it is to contribute to farm diversification schemes the spread of Watermark disease needs urgently to be addressed by extending the existing Watermark Disease Order.

Short-rotation coppice to produce biomass for energy, wood pulp and industrial feed stock has great potential for development on farms but because it is grown in intensive monoculture, diseases and pests are already developing which have been hitherto of little consequence to isolated trees. In collaboration with the International Energy Agency surveys are being carried out on coppice willow plantations around the world to determine the currently and potentially damaging crop protection problems. In the first year, rust diseases (*Melampsora* spp.) have been shown to be by far the most prevalent and damaging to biomass production. New research seeks to determine the scale of rust damage, the reasons for wide variations in levels of attack and the economic justification for developing suitable control measures.

INTRODUCTION

The rapid growth in agricultural productivity within the European Community has led to an embarrassing surplus of cereals, dairy and other products. It is variously estimated that between 11 and 16 million hectares will have to be taken out of food production over the next 15-20 years if agricultural surpluses are to be eliminated (Carruthers 1988).

The EEC are interested in promoting an extensification scheme

- defined in community rules as a reduction in a farmer's output of a surplus product by at least 20% without other surplus production capacity being increased (MAFF,1987). It has been estimated that one million hectares in the UK will need to be taken out of production. The implications of "set-aside" as a means of implementing this requirement are well reviewed by Dodgson (1988).

The obvious, but less simple, solution is for farmers to diversify into crops not currently in surplus production. The options available have been discussed by Carruthers (1986a) and by the National Economic Development Council (1987) and, though some crop and livestock enterprises show promise, growing trees was selected as the principal alternative to conventional agriculture.

The UK has far less forested surface area (9%) than other EEC countries (average 21%). Even in 1984 our import of forest products was over £4,500 million (National Farmers' Union, 1986).

Farm forestry enterprises could make a worthwhile contribution to import substitution. Hence, at the national level, there are good economic reasons why agricultural land surplus to requirements should be diversified into forestry. However, at the farm level, the major difficulty is a cash-flow problem imposed by the long term nature of conventional forestry - at least 50 years for yield of broadleaved trees. To help meet this problem the Government introduced the Woodland Grant Scheme in April 1988 (Forestry Commission, 1988). One of the stated objectives is to promote the contribution new woodlands can make as an alternative use of agricultural land no longer needed for production. Such land qualifies for a supplementary grant.

Other ways to alleviate the farmers' cash flow-problem are to integrate crop or animal production with forestry in so called agroforestry systems so that crops give a return in the early years whilst the wide-spaced trees are being established. Alternatively, the rotation may be reduced to a more agricultural time span of 3-8 years by growing coppice for energy, pulpwood or industrial feed stock in short rotation coppice or biomass plantations. Research and development are proceeding in agroforestry and short rotation coppice in the UK and in many other countries, and it is our view that there will be a substantial increase in varying kinds of farm and lowland woodlands. The purpose of this paper is to review the implications for pests and diseases of increases in farm forestry, agroforestry/spaced trees and short rotation coppice.

FARM FORESTRY

Farm forestry is here defined as woodlands of various types, size and purpose, planted on farms in discrete blocks, separate from the agricultural enterprises. Extensive blocks would be managed primarily for wood production, but smaller woods could be of more importance for game cover, amenity or conservation. It is estimated that approximately 300,000 ha of woodland exist on farms, mainly of mixed deciduous species (National Farmers Union, 1986). For the most part these woodlands are relatively unmanaged and unproductive but, for these very reasons, they support a wide range of pests and diseases which appear to be in equilibrium with their hosts. An increase in farm forestry is unlikely to encourage epidemics of new pathogens, particularly as the new farm forests are likely to be smaller, more dispersed and probably with a greater diversity of species than the existing conventional hardwood forestry traditionally practiced by lowland estates.

Separate mention must be made of linear belts of woodland on farms which in addition to their timber value provide shelter to neighbouring crops or livestock. In the extreme case of the single lines of trees used extensively as windbreaks in horticulture (Stott & Belcher, 1978), shelter is the main objective. In many ways hedgerows have a similar role. Because of their pronounced interaction with neighbouring crops, shelter belts, windbreaks and hedges have been considered by some authors as agroforestry (Carruthers, 1986b).

These niceties of definition need not concern us here. The new incentives favouring farm forestry are unlikely to lead to such an increase in windbreak and hedgerow planting that new problems with pests and diseases attacking them are anticipated. It is, however, prudent to note that some pests of agricultural crops have trees as alternative hosts. The bird cherry (Prunus padus) aphid, Rhopalosiphum padi, transmits barley yellow dwarf virus, black poplar Populus nigra is the alternate host of the lettuce root aphid (Pemphigus bursarius)and the spindle tree (Euonymus europaeus) of the bean aphid (Aphis fabae). Such factors must be considered when choosing trees for windbreaks, hedgerows or amenity plantings. However these sites also act as reservoirs for beneficial predators (Wratten,1988) and orchard windbreaks also have value as a source of beneficial mirid and anthocorid predators (Solomon, 1975). On balance, there appears growing acknowledgement of the value of hedges and field margins in creating a diversity of habitat (Marshall & Smith, 1987) so that there is less chance of any one pathogen or pest developing to epidemic proportions in adjacent crops (Way & Greig-Smith, 1987).

AGROFORESTY - SPACED TREES

Agroforestry has been defined as systems and practices in which trees are deliberately grown on the same land management unit as crops or pasture such that the agricultural and forestry components interact both ecologically and economically (Anon,1983). This definition encompasses a wide range of land uses throughout the world which vary in the relative importance of the agricultural and forestry components. The subject is well described in a comprehensive report published by MAFF (1986).

In Britain, interest lies essentially in systems of trees grown at or near their final density at a sufficiently wide spacing to allow cultivation and for crops to be grown between the trees or rows of trees, or for pasture to be grazed. In some situations agroforestry can be more profitable than either forestry or agriculture alone, but the main advantages foreseen for British farmers are that the crops or grazing can produce an annual income, at least in the early years, to help offset the cash flow problems imposed by the long rotations required to grow broad-leaved trees. Also, because there are few trees to the hectare, the farmer can manage them more intensively and, with pruning for example, aim to produce high value products like veneer logs of quality cabinet woods such as cherry and walnut. Other benefits to the farmer and to conservation and the environment have been detailed by Carruthers (1986b).

Since the trees are grown as widely spaced individuals for most of the rotation, it is only in the closing stages that they coalesce to become an ecological entity as a wood. Hence pests and diseases have less opportunity to spread from tree to tree and build up to damaging proportions. This is particularly true of soil borne diseases and those like the honey fungus, Armillaria mellea, which can spread by root contact. Also, because the aim is to grow high value trees like oak, ash, cherry and walnut, merchants will purchase well grown specimens in small lots of three or four, so that multiple mixtures are economically feasible, and the risk of epidemics commensurately less. Pests and diseases that have not already shown themselves to be economically damaging in existing farm woodlands are unlikely to become more important in the widely spaced environment of agroforestry systems. However, though the trees usually recommended for use on farms have relatively few serious diseases (Rose, 1988), one in particular, the wild cherry or gean (Prunus avium), can be killed by bacterial canker Pseudomonas syringae pv.mors-prunorum. In orchards this disease can be controlled by annual sprays but in timber crops these are unlikely to be economic. Hence, with tree diseases, it is important to maximise natural resistance by encouraging vigour by carefully matching species to site and, where possible, to profit from opportunities to select and breed for resistant varieties or hybrids. This has been found to be the best strategy for poplars.

Poplars (Poplus spp.) are so seriously damaged by three major diseases, leafspot (Marssonina brunnea), rust (Melampsora larici populina) and bacterial canker (Xanthomonas populi), that economic production would be restricted, or even prevented, were it not for a sustained programme of selection and breeding which has provided resistant clones (FAO,1979). The successful introduction of a range of resistant clones has allowed poplars to become the most widely planted agroforestry species in Europe.

Unlike the other trees, the aim with poplars is bulk production of low value pallet wood, or pulp, but in rotations of only 20-30 years. It is the shortness of rotation that has encouraged their widespread planting by continental farmers, often in belts or single rows round fields, but also in intercropping systems with cereals or livestock. Spaced poplars in cereals was pioneered in Britain by the Bryant and May company, working to a 25-30 year rotation with trees at 8 metre spacings, inter-row cropping with cereals for the first 8 years and then grassed down and summer grazed by sheep or cattle. Some 1500 ha were planted by 1977 and, despite a disruptive change in company policy, a few hectares still remain to demonstrate a system with many advantages for farmers seeking alternative land uses (Beaton,1987).

One of the most profitable agroforestry systems currently practised in Britain is growing cricket bat willows (Salix alba Caerulea) in single or double rows often along ditches. The unrooted sets cost £5 each and mature in 15 years to produce large trees currently worth £65 each. Unfortunately, they and other tree willows (S. alba and S. fragilis) can be killed by Watermark Disease, caused by the bacterium Erwinia salicis. So far this disease has been kept under control by the application of the Watermark Disease Orders which require that infected trees be felled and burnt. These orders presently apply only to Essex, Suffolk and Bedfordshire, the main areas of production. If farmers were to attempt widespread planting in areas where the orders cannot be enforced, then a serious epidemic could ensue, not only in bat willows, but in many thousands of white willow (S.alba) recently planted for amenity in new towns, e.g. Milton Keynes.

We still know little about the spread of this disease. No vector has been implicated. The spores can be blown on the wind, but how ingress is made is not known. Even artificial inoculations are difficult to achieve (Preece,1977). It is now thought, but not proved, that the disease is spread in infected, but symptomless sets; the symptoms rarely show in young material. Research on the disease has resumed recently and, with the help of enzyme-linked immunosorbent assay (ELISA), it is hoped that more sensitive methods of detection will facilitate the certification of disease-free set beds and certified propagating material so that plantings in new areas could be safely encouraged. Concurrently, the Watermark Disease Orders need to be extended to all lowland counties of England and the Inspectorate's resources increased, otherwise England may well suffer the fate of Holland, where the effects of the disease can be likened to those of the Dutch Elm disaster in the UK. Clearly, without the introduction of either of these measures it would be unwise for widespread diversification into growing cricket bat willows.

SHORT-ROTATION COPPICE

Coppice is one of the oldest foresty systems and depends on the ability of species, usually hardwoods, to resprout vigorously when cut down. Traditionally poles of specific sizes were produced for fencing, turnery, etc. in cutting cycles of up to 20 years; the small material was used for firewood. A more recent development is to grow coppice to be chipped for energy, pulp or industrial feed stock. Cutting cycles as short as three years have been suggested for these biomass plantations, because the size of the material is relatively unimportant. The objective is to maximize the yield of dry matter produced per hectare per year.

The economic viability of the system has been amply demonstrated by the 800,000 ha of very profitable eucalypt coppice in Portugal and Spain grown on 11-14 year cutting cycles for pulpwood and averaging 12 tonnes dry weight of harvestable biomass per hectare per year (Hummel, 1988) In Britain, similar yields have been achieved by willows and poplars. The results of research, including several projects funded by the Department of Energy, have been sufficiently encouraging for short rotation coppice for energy to be identified as potentially one of the more important alternative agricultural crops (Carruthers,1986a). In a modelling exercise some 0.8 million hectares of existing agricultural land has been identified to be more profitable under energy forestry crops. Of these, 90,000 ha (12%) would be coppice grown primarily in lowland areas (Mitchell et al., 1987). This approach has already been put into practice in Sweden where, over the last three years, farmers have planted 350 ha of willow biomass to provide wood chips for electricity generation or community heating schemes.

In the UK the concept of growing willows as short rotation coppice for biomass started in 1973 in N.Ireland (McElroy & Dawson,1986) and led to a collaborative research programme between the Horticultural Centre, Loughgall and Long Ashton Research Station, Bristol.

A review of progress up to 1986 has shown the potential of willow coppice to achieve acceptable yields of 12-15 dry tonnes/ha/yr (Stott et al.,1985),with a preference for 1 x 0.5 m spacing and a 3-5 year cutting cycle for willow, possibly longer for poplar. More recent work, much of it funded by the Department of Energy and the EEC., seeks to provide information on a wider range of species and, by large scale trials of up to 10 ha, on the costs of management and harvesting (Anon, 1987).

Willow and poplar coppice is a dense crop, growing to heights of 2-5 m commonly, and sometimes 10 m. Like other agricultural crops grown in monoculture, it carries inherent risks for pest and disease attacks. In the first 2 years of establishment, and in the next 2 years of a new production cycle, it would be possible to spray pesticides, but it is not known if chemical control would be economic and doubtful if, in some cases, it would be environmentally acceptable. Hence, unlike agroforestry systems, pests and diseases have very serious implications for the success of biomass plantations.

During many years of research on willows we have been aware of the threat posed by xylophagous insects which by burrowing in the willow stools accelerate their degeneration. However, serious infestations of willow weevil, Cryptorrhynchus lapathi, Goat moth (Cossus cossus) or the Clearwing moths (Aegeridae) have so far failed to materialise. Nor have the Chrysomelid leaf beetles been any more of a problem in biomass plantations than they are in native willows or in basket willow beds. On the other hand, Aphrophora salicis, a plant sucker hitherto extremely rare in British willow beds, has this year at Long Ashton caused serious damage to young growth in one of our main clones, S. burjatica Korso. The young shoots wilt and then break off where the stem is ringed by feeding punctures. In the same planting an eriophyid mite, invading young leaves of the shoot tips, developed to such an extent that growth of many shoots became severely stunted.

The history of rust diseases is not dissimilar. For 30 years S. burjatica Korso appeared to be immune to prevailing forms of rust (Melampsora) of which there are many species that attack willows (Hubbes, 1983). However, in 1986, outbreaks occurred in Eire, N.Ireland, Somerset and at Long Ashton and each year the attacks have begun earlier and severity has increased. In 1987, and more so in 1988, rust attacks have been noted for the first time on another important selection, S. viminalis Bowles Hybrid. In contrast, attacks by the less specialised pathogen, Chondrostereum purpureum, which causes the common silver leaf disease of plums, have not been delayed; at Long Ashton the pathogen invaded stools of Eucalyptus archerii immediately after coppicing, killing over half within 8 years.

Diseases pose a serious threat to biomass productivity and it is prudent to attempt to investigate those prevalent, whether or not they appear to be currently severe. For this reason we have been collaborating with scientists from the Swedish Energy Forest Programme and, in 1987, we started an annual survey for the International Energy Agency to identify and monitor the spread of major diseases present in willow biomass trials and plantations throughout the world. We see this as a major requirement for any new crop poised between research and commercial exploitation.

In the first year of the survey (Hunter et al.,1988), which embraced seven countries in Europe and N.America, by far the most widespread and severe disease was rust, with several species and formae speciales of Melampsora probably implicated. In plantings of some clones, notably S. burjatica Korso, attacks were very severe and led to premature defoliation, weakened growth and apparent yield reductions in both young and established plantations. Of 120 clonal plantings examined in the UK and Eire, 75% contained rust, 30% at levels causing appreciable early defoliation. Disease prevalence and severity varied markedly between plantations and different clonal selections and within the same plantation even in the same clone, due to many interacting factors that for the present we do not understand (Royle et al. 1988).

Other major diseases identified by the survey caused mostly local damage involving death of terminal shoots, stem cankers and girdling, and pruning wound invasions due to such pathogens as Fusicladium saliciperdum, a relative of the apple scab fungus, Glomerella cingulata, the apple canker pathogen, Marssonina salicicola, which also attacks foliage, and Chondrostereum purpureum. All these enter the plant through openings, usually lenticels or wounds, and the first three are rainsplash dispersed, prevail in wet conditions and cause most damage on 3 to 5-year old shoots.

Diseases can be expected to deplete willow biomass yields mainly by altering plant form and structure, causing early defoliation leading to reduced lignification and shoot growth, predisposing to frost damage and competition from weeds, and by causing shoot, root and stool death. Concern about yield losses can only be justified, however, if biomass productivity can be shown to be influenced by diseases in economic terms. So far we have only impressions that rusts can cause serious damage; a priority in our investigations is to quantify and understand the disease-loss relationship with respect to timing and severity of attacks.

Many agricultural crops have faced similar problems which have been overcome by research and modifications to their husbandry. The year the coppice is cut down offers opportunities to break the life cycle of some pests and diseases. High clearance tractors could straddle the first, and possibly second, year's growth of a new production cycle. Alleyways could be left for later access. Aerial spraying may be effective, but perhaps not acceptable on environmental grounds. Since the product is likely to be wood chips, it would be possible to introduce host diversity by growing clonal mixtures of the same species, or of different species, or even different genera if, like willow and poplar, their site requirements were similar. This could help to reduce the impact of pathogens in a similar way to variety mixtures in cereals (Wolfe,1985). Considerable scope exists for selection and breeding and for encouraging vigorous growth by meeting the site and nutritional requirements of species and clones.

Short rotation coppice presents an exciting challenge as an alternative crop and there is no compelling reason why researchers and practitioners should be any less successful than they have been in introducing other new crops, for instance oilseed rape.

CONCLUSIONS

Farm woodlands are not likely to be attacked more severely than existing forests. No special implications for pests and diseases are foreseen.

Agroforestry systems, by their very dispersed and spaced nature are in general likely to be less troubled by pests and diseases, though for some specific trees like cricket bat willow and perhaps cherry there is cause for concern.

Short rotation coppice plantations for biomass are likely to be seriously threatened by pests and diseases and will need all the expertise of breeding and evolving husbandry employed in agricultural crops if they are to succeed.

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ORGANIC FARMING - CROP PROTECTION IMPLICATIONS

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ABSTRACT

Organic farming in the United Kingdom, though only a small part of the overall farming establishment, is better organised today than at any time in the past. Interest in alternative farming practices is increasing. It is likely that new standards for organic produce introduced by the United Kingdom Register of Organic Farming Standards (UKROFS) will further consolidate the movement. Whereas soil fertility and its maintainance in the absence of soluble synthetic fertilisers is a central factor,the avoidance of synthetic pesticides gives rise to immediate crop protection implications. However,longer term advantages could be implicit. Longer and more diverse rotations, the use of composted organic manures and the effects of altered cultivation practices may indeed reduce pressures from pests, diseases and weeds. Various strategies of crop protection present themselves to organic growers. These include site selection, cultural and biological control and even the limited use of 'acceptable' pesticides. Underpinning the future success of any environmentally balanced system, must be the availability of adequate crop intelligence, improved knowledge of crop pests and the development of new technology which would be of equal value to existing conventional farming systems.

INTRODUCTION

Systems of agricultural production in the United Kingdom range widely from the very high utilisation of agrochemicals through to rough grazing systems with virtually no chemical input. In those systems which over the past forty years have been subject to intensive technological advance and increased productivity, there have developed both technical , social and economic constraints which have led to interest in alternative production systems and strategies. Among these have been the increased attention drawn to organic farming. There have been various attempts to define the organic farming system which, however thorough, have left even protagonists with different conceptions depending on whether the individual or organisation is promoting a philosophy or science, justifying an existing farming system, or aiming to market a product. For the purposes of this paper organic farming is loosely defined as a whole-farm approach that seeks some form of environmental equilibrium and which on principle avoids reliance upon synthetic fertilisers and pesticides. Organic farming is not, by definition, either low input or low technology.

In the United Kingdom there are many groups within the organic farming movement and a good deal of crosslinking and exchange exists between them due to common activities or to certain active people. Principal among these organisations are the Soil Association (SA), British Organic Farmers (BOF) and Organic Growers Association (OGA), the Elm Farm Research Centre (EFRC), the Organic Advisory Service (OAS) as well as Organic Farmers and Growers (OFG). Standards followed by these various movements are in essence the same as those of the International Federation of Organic Agricultural Movements (IFOAM). In October 1987, the United Kingdom Register of Organic Food Standards (UKROFS) was established by Food From Britain and supported by the Ministry of Agriculture, Fisheries and Food with the object, as its name implies, of establishing UK standards, devising a symbol, maintaining a register and running an independent regulation scheme. So far, commercial outlets have included co-operative farming ventures, supermarkets, millers and local retailers. A review of various market surveys (Stopes and Woodward 1988) leaves no doubt that there is at present a modest but unsatisfied market for producers of organic products in the UK. Any reduction in the overall usage of synthetic fertilisers and pesticides in the farming community as a whole, consequent on the adoption of organic farming systems is likely to be small. However, it should be made clear that the principles behind this farming system, require not only the re-examination of older established husbandry techniques but also new knowledge and new technology.

CROP PROTECTION IMPLICATIONS

There are crop protection implications which are directly consequent upon the adoption of an organic farming system, some of which have an immediate effect, others which will become operative in the short to medium term. They include:-

a) Avoidance of the use of pesticides. Recommendations by organic farming organisations for pest, weed and disease control are based on the principles of rotational cropping, cultivations and the long-term creation of balanced eco-systems. Standards required by the Soil Association (Soil Association 1987),for example, prohibit the use of synthetic pesticides together with other biocides such as nicotine, formaldehyde and mercurial seed treatments. Herbal sprays, homeopathic and biodynamic preparations and certain other natural products are permitted. Specified plant-derived insecticides and mineral elements can have restricted use subject to approval by the Association. All chemical and hormone-based herbicides are prohibited.

Longer-term soil pesticide residues from a previous conventional farming systems in land under conversion could pose problems to organic farming systems.

b) Conversion. Generally a period of two years is required to 'convert' from a conventional farming system to an organic system. On farms under conversion to organic systems, where there has been a previous reliance on the use of pesticides, immediate-term pest problems are to be expected. For example leaf pathogens such as powdery mildews, rusts and leaf blotches require little time in which to build up. However, it is quite possible, especially where crops are being grown new to that land and in that area, for pest, disease and weed problems to remain latent. In such cases the 'honeymoon' period can be misleading. The true environmental balance, if indeed attainable, is acheived in the longer term.

c) Effects of alternative nitrogen sources. In an organic farming system, where the use of synthetic soluble forms of nitrogen are generally prohibited, alternative nitrogen sources rely on the regular inclusion of nitrogen-fixing crops in the rotation and the use of composted animal manures. There are associations of increased fungal pathogenicity to aphids as well as inreases in aphid parasites in winter wheat crops under-sown to clover (Seibt 1984; Wipperfurth 1983; Grobe Wichtrup 1984). There is also evidence that applications at high rates of mineral nitrogen can increase plant susceptibilty to diseases (Darwinkel 1980), as can both its form (Huber and Watson 1974; Trolldenier 1981) and rapidity of its uptake (El Titi 1986). High nitrogen rates have disturbed the equilibrium among wild plant species, allowing the dominance of crop aggressive species. The use of weedseed-sterile composted organic manures in organic systems reduces the weed presure in its own right.

d) Effects of altered rotational systems. Rotational practices in an organic farming system are oriented towards the creation, maintainance and conservation of soil fertility, specifically that of nitrogen. Rotations that include nitrogen-fixing crops and green manures and catch-crops etc. are consequently more diverse and often of longer duration than those in conventional systems. Organic farming rotations with the wider separation of potential host crops can contribute to the reduction of crop pest, disease and weed pressures. Foot and root diseases of cereals such as take-all (Gaeumannomyces graminis) and eyespot (Pseudocercosporella herpotrichoides) are obvious examples. Nevertheless, some pathogens, such as white rot (Sclerotium cepivorum) of onions, have very long-lived resting spores and wider rotations are unlikely to reduce disease pressure. Consequent upon altered rotational systems will be a greater variety of sowing dates and the more frequent inclusion of grass breaks. Both factors will contribute substantially to the control of weeds.

e) Effects of altered cultivation practices. Closely associated with soil fertility is the maintainance of soil structure. To this end, cultivation techniques in organic farming systems aim to minimise the disruption of soil surface layers although enabling deeper loosening of the soil and avoiding practices which damage existing structure by timeliness of operations. The avoidance of deep ploughing and the reliance on lighter soil operations can assist the cultural management of weeds by reducing the potential build-up of seed banks. Reductions in tillage have also led to advantages such as the suppression of wheat bulb fly (Delia coarctata) (Raw 1955) and a reduction in diseases such as eyespot (Yarham 1979), take-all (Yarham and Hirst 1975; Yarham 1979),and septoria blotch (Septoria tritici)(Brokenshire 1975).

CROP PROTECTION STRATEGIES

Once an organic farming system has been adopted in principle, there are subsequent implications for crop protection stategies; these strategies, in a farming system that aims at an ecological balance, must by definition be holistic in nature (Widdowson 1987). Therefore, the following techniques must be regarded only as components in an overall strategem. Protection of organically grown crops will depend largely on cultural techniques but must also rely to an increasing extent upon natural/biological processes. Some 'chemical' controls are still acceptable but they are of limited importance. The importance of and the reliance upon crop pest and disease intelligence is self evident.

Site Selection:

Within the UK accumulated data on pest distribution can facilitate the safer geographical location of specific crops, thus emphasising the importance of national monitoring of both pests and diseases. At a local level, soil type and crop history will influence the on-farm siting of crops. For example, nematodes are more damaging on sandy soils and peats, whereas slugs are favoured by wetter heavier soils. Wireworm-susceptible crops, such as potatoes, are best avoided on land following long-term grass. The spread of crop diseases can be minimised by siting similar successive-season crops as far apart as possible.

Cultural Control:

Seed: the use of seed in which pest and disease levels are low is an obvious starting point. Seed, where appropriate, should be tested for nematodes and important diseases.

Variety: the availability of crop varieties resistant to particular diseases or pests presents an ideal solution. Although complete resistance is a rare phenomenon, plant breeding, even for partial resistance, is of considerable value. Varieties may be utilised in a different way, eg by mixing them within a crop or by diversifying them within the farm and thereby 'spreading' the pressure from pests and diseases. A good example of the former being the varietal diversification schemes relevant to cereal diseases where seed mixtures of spring barleys can be provided to reduce pressure from mildew, and mixtures of winter wheat varieties to combat mildew (Erisyphe graminis) and yellow rust (Puccinia striiformis). In this case consideration should be given to the possible marketing problems of mixed bread wheats.

Sowing and Harvesting: attacks by carrot fly (Psila rosae), wheat bulb fly (Delia coarctata), frit fly (Oscinella frit), slugs and aphids can be minimised by either sowing or harvesting the crop at times of minimum pest activity. For example if the drilling of winter cereals is delayed until the end of October the risk of aphid vectors of barley yellow dwarf virus migrating into the crop can be reduced. Such timing depends, again, on efficient pest monitoring. Adjustments to seed rates and crop spacing are strategies by which compensations can be made for pest attacks or by which disease-prone environments can be avoided, as well as facilitating the use of mechanical hoes. Where seedbeds have become 'stale' weeds can be controlled by shallow working.

Cultivations: in terms of weed control, techniques include the use of weedseed-sterile composted manures, green manures incorporated into the soil before seeding, and pre- and post-emergence mechanical operations. The latter can include row-crop hoes, whole-crop weeders and flame weeders. 'Rogueing' by hand is not an unusual practice even among conventional growers. The avoidance of deep ploughing, however, will create problems for trash disposal and could lead to potential pest and disease problems. For this reason, in vegetable production, crop residues are best removed from the field for composting.

Rotations: rotations can be used to minimise weed problems especially where land constraints do not limit the use of summer fallows in which weed flushes can be removed by surface cultivations. Inter-row cultivations in subsequent wide-spaced crops can reduce weed populations further, leaving a relatively clean site for a denser third-season crop. In terms of pest and disease control, rotations are generally only effective for soil dwelling and relatively innactive organisms.

Irrigation: the use of irrigation can be both advantageous and disadvantageous. In an organic vegetable enterprise the use of irrigation can greatly reduce the risk of attack by cutworms (eg Agrotis segetum), the early instars of which are vulnerable to rain showers. However, damp and humid environments will favour many diseases which affect the aerial parts of plants. In these cases trickle or seep irrigation might be preferable.

Barriers: physical barriers, such as the collars used on brassicas to prevent cabbage root fly (Delia radicum) damage, are effective but practical only on a small scale. Polythene mulches are used effectively as weed suppressants and are permitted by most organic organisations. Such mulches may have potential as a means of pest control.

Biological Control:

The longer-term development of a balanced ecosystem must include a concerted attempt to preserve and encourage the naturally occuring parasites, predators and pathogens of pest species. Avoidance of synthetic pesticides will be a primary step but the retention of hedges and shelter belts together with the encouragement of a diverse headland and hedgerow flora and fauna (Vereijken et al 1986)is a necessary corollary. Floral diversification will also help to counter the dominance of crop-aggressive weeds. The use of pheromones, either to measure or monitor pest activity or to 'modify' pest behaviour, and the use of biological pesticides (Lisansky 1981) of fungal, bacterial or viral origin, are all possible and under active development. Their use in an organic farming system would of course depend on their meeting the requirements of a natural and balanced system. The possibilities of exploiting allelopathy (Lovett and Levitt 1981), that is natural biochemical reactions between plants and other organisms, to reduce the vulnerability of crop plants to pest, disease and weed competition could be a profitable area of research.

Chemical Control:

Most pesticides permitted in organic farming systems are only moderately effective and of short persistence compared with conventional products. Although of 'fire brigade' importance in the early stages of organic farming establishment, 'chemical' control will always take second place to whole-farm cultural management.

CONCLUSION

1. A primary aim of organic farming is to create an environmentally stable system centred on the maintainance of soil fertility and structure without recourse to synthetic soluble fertilisers.

2. The adoption of an organic farming system will require the utilisation of alternative 'natural' sources of nitrogen and will incorporate the use of composted organic manures, green manures, nitrogen-fixing crops and longer and more diverse rotations. These factors may well reduce the need for crop protection measures.

3. The avoidance of synthetic pesticides may give rise to pest problems in the short to medium term and, unless alternative crop protection techniques are developed, could lead to certain pests and diseases becoming limiting factors on certain crops.

4. Crop pest intelligence, ie the monitoring of insect pests and diseases both on a local and national scale, is essential if sound advice on crop protection strategies is to be given.

5. Improved knowledge: there would be, for similar reasons, a greater need for detailed studies of pest behaviour and of disease and of weed interactions with crop plants.

6. New technology: research into physical methods of pest control such as mechanical weeders, soil cultivation and plastic mulching; biological control (including pheromones, allelopathogens and microbial biocides); plant varieties and alternative crops would serve the interests of conventional and integrated farming as well as those of organic farming systems.

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CROP PRODUCTION REALISM : THE ROLE OF AGROCHEMICALS

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ABSTRACT

Agrochemicals have played an integral part in ensuring the reliable production of food at an acceptable cost. That role will continue in the foreseeable future but emphasis will be focussed more on quality than quantity, at least in Europe. Alternative crops and land use aimed at reducing surpluses and developments in bio-control and biotechnology will modify the demand for agrochemicals but not replace them. Forecasts and threshold analysis will help to improve the efficiency of agrochemical inputs, but much further work is required. Over the past decade the agrochemical industry has made significant progress in the development of products and recommendations safer to the user, consumer and the environment. These aspects are briefly reviewed against the background of 'natural' hazards and the reassuringly low impact on most non-target organisms seen in "The Boxworth Project" in the UK.

INTRODUCTION

Prior to the Second World War, "conventional agriculture" in Europe was dominated by organic farming. Set-aside, then called fallowing, was used to clean the land and build up fertility for the benefit of future cropping. The requirement for food by a burgeoning population was such that this type of farming became totally inadequate and demand for improved technology, including agrochemicals, grew rapidly. The application of this technology, particularly over the last 30 years, has been so successful that farming using modern agrochemical and fertiliser aids is now described as "conventional" and the methods which failed to provide reliable food production in the past are being considered as means of constraint. This complete reversal of terms is a testimony to the enormous contribution that agrochemicals, as an essential part of this technology, have made to the improvement in our standard of living.

The agrochemical industry exists only because its products are in demand. That demand has been created by the inadequacies of alternatives. It is a realistic premise that the demand for food will continue to increase and that the ravages of weeds, pests and diseases will need to be contained. However, the market is beginning to mature and agrochemical companies must be realistic about the factors impacting on future demand for their products and some of these are reviewed below. Equally, the benefits of agrochemicals and the continuing need for them deserve recognition and the exaggerated claims of potential dangers seen in perspective.

THE WORLD NEED FOR FOOD AND EUROPEAN SURPLUSES

World population is increasing by 200,000 daily (UN statistics). This, combined with the need for an equitable amount of food per person, suggests that food supply must be increased by 75% by the year 2000 (Blaxter 1986). Assuming a world population of 5 billion in 1980 and 6 billion in 2000, the approximate amount of land available per person is indicated in Table 1.

TABLE 1

Actual and potential cropped land per person

	M hectares*	Area per person (ha) 1980	2000
Actual cropped land (1980)	1448	0.29	0.24
Potential Crop land	3087	0.62	0.52

* Blaxter (1986)

It is estimated that the amount of land required per capita in the year 2000 to produce animal and vegetable products will be approximately 0.5 ha (North 1988 personal communication). In reality, the amount of land available per person for food production will be significantly less than indicated in the table as no account has been taken of the need to grow non-food crops, the unsuitability of much of the permanent grass area and the need for land for urban development, recreation and other uses. The area per person can only become smaller, and continued intensive production on suitable land is obviously necessary. Even with current use of agrochemicals, it is estimated that 20-40% of potential food production is lost world-wide annually (FAO 1981). Without them, adequate food could not be produced and food shortages would occur on a massive scale.

Technical progress in crop production, including use of agrochemicals, has helped significantly to improve European self sufficiency and reduce the cost of food. In the UK, for example, home production of the foods we are able to grow increased from 63% in 1971 to 81% in 1984 and expenditure on food, as a percentage of income, declined from 30% in 1962 to 20% in 1984. (Campbell-Platt 1986). Nevertheless, the agrochemical industry must accept that the reliable production of food can now be achieved at levels above requirement in Europe. This is particularly true for the dominant crop, cereals. It has been predicted that demand for cereals in UK by the early part of next century can be met by less than 2.0m ha compared with approximately 4m ha currently (North 1986). Such a change, if it occurs, will have a major effect on agrochemical demand as the cereal crops account for approximately 75p in every £ sterling spent on crop protection in the UK. The demand for agrochemicals however is unlikely to decrease in proportion to the area because it is probable that the more marginal land will go out of production. Those farms suitable for cereal growing will continue to use high technology and indeed most individual farmers will need to do so, in order to remain competitive and especially to ensure production of the required quality.

As support for cereal prices is reduced, cost per tonne of output will become increasingly important. Against this background it is unreasonable to expect farmers to forego the possibility of exploiting more of the genetic potential of varieties by the use of appropriate agrochemicals. For example, the average yield benefit from the application of fungicides to winter wheat and winter barley in the UK, even on varieties with good levels of inbred resistance to disease, is more than 15%. Furthermore, the newer varieties show no diminution in response to fungicides. (NIAB 1988).

Organic production of cereals, which offers little opportunity for the agrochemical industry, could be considered as a means of reducing output and the evidence shows a significantly lower yield of wheat (as for most other crops).However, even if all growers were as efficient as the best, organic cereal production would soon lead to a deficit in the UK (Tyson 1988). This does not seem to be a realistic option on a large scale.

Technological progress will continue to improve yields year on year and, therefore, the political need to reduce cereal production in Europe is understandable. Nevertheless, the relative fragility of the surplus/ shortage equation should not be ignored. Following the drought, US wheat production estimates in mid-July 1988 of 43.3 million m.t indicate a carry over stock of 6.4 million m.t or just over half their 'safe level' strategic reserve (Agra Europe 1988). The EEC enters the 1988-89 trading season with intervention stocks of 3.8 million m.t of wheat compared with a peak of 10.3 in 1984/85. (Home Grown Cereals Authority personal communication). Catastrophies, like the drought in the USA and locust problems in Africa, have a habit of recurring with regular frequency. It does seem anomalous that we are cutting back cereal production in Europe, an area particularly suited for soil and climatic reasons, despite the predicted world demand for food.

ALTERNATIVE CROPS AND LAND USE

Many of the crops being considered as alternatives to cereals are already treated with agrochemicals in Europe. Although the market opportunity on some could increase, eg legumes (including navy beans) and sunflowers (especially in the UK if suitable varieties are developed) all other proposals offer only 'niche markets' for agrochemicals. Apart from sunflowers, none could justify specific product development and, therefore, any use will be as an extension from a major crop. Similar arguments apply to recreational areas.

Although land 'set aside' will need to be managed to be acceptable environmentally, it too will offer little opportunity for use of agrochemicals. Control of pernicious annual and perennial weeds can be achieved with herbicides if required. Pest and disease build-up will need to be monitored before returning the land to cropping and some increased use of agrochemicals may be necessary in the first year, compared to fields in continuous cropping. Possible problems include wireworms (*Agriotes* spp.) and frit fly (*Oscinella frit*) after grass, brassica pests and slugs after a green cover crop and diseases if cereal stubble is left as the 'cover crop' in rotational set aside. None of these opportunities, however, will compensate for the market lost in cereals.

THE INCREASING NEED FOR QUALITY

Apart from producing an adequate quantity of food, agriculture, as in any other producer industry, must continually strive to improve quality. Yield and quality are closely linked and there is an increasing need to meet the tighter specifications dictated by the processor/retailer. This creates a demand for very technical end products in most markets and achievement demands modern technical aids, increasing the market for agrochemicals.

This is highlighted by just two examples, cereals and vegetables. In the former, increasingly tight quality requirements are being defined by the Miller, Maltster and Intervention Boards. Fungicide use, apart from giving outstanding yield benefits, also improves most quality parameters of grain. Without their use, even on varieties with claimed genetic resistance to diseases, quality standards would fall below acceptability in most cases. This is particularly true under UK climatic conditions.

In the case of vegetables, quality is the paramount requirement. Customers demand reliable quality on a continuous basis and at a competitive price. The retailer reflects that demand just as for any other product on his shelf and no amount of packaging, advertising etc can compensate for poor quality. There is an increasing demand for attractive blemish-free products and this will not change however the crop is produced. (Hilborn 1986; Martyn 1986).

Without the use of agrochemicals, the required quality can only be obtained by rigid grading out. The more this is necessary, the higher the premium needed to compensate and this significantly affects the overall profitability of the enterprise. In the potato crop, even where agrochemicals are used, only between a third and one half of ware potato production in the UK is suitable for pre-packing. The control of skin blemishing diseases eg black scurf (Rhizoctonia solani) and silver scurf (Helminthosporium solani) is increasingly required in this expanding market and further opportunities exist for agrochemicals. The obvious impact of potato blight (Phytophthora infestans) and pests like wireworm, cutworm (Agrotis spp.) and slugs on quality, needs no emphasis. Similar comments on the need to control pests and diseases could be made for other vegetable crops.

Organically produced vegetables have a place in the market owing to the public perception that such foods taste better and are healthier, despite the fact that evidence to support these claims is lacking. However, it is predicted that organic vegetable production is unlikely to exceed 5-7% of the total potential market (Martyn 1986). Indeed the premium prices needed to make such production profitable can only be sustained while the market remains small. For the rest, we are likely to see increased concentration of production in the hands of specialists and such intensive growing helps to keep costs down to the purchaser. It is not realistic to believe that adequate food of the right quality can be produced in the foreseeable future without agrochemicals.

ALTERNATIVES TO AGROCHEMICALS

Most farmers already integrate agrochemical use with physical, mechanical and other husbandry methods of control available to them. Although rotation and cultivation techniques have a clear place in reducing weed, pest and disease incidence, reliable control is seldom achieved under practical farming conditions. Furthermore, there is frequently a conflict between cultivations aimed at control and what is desirable agronomically. Weed control is a major challenge for organic growers in most seasons and the wet conditions in the UK in spring 1988 emphasised the problem. Increased labour input, if available, could be used as a means of weed control in some crops but this would be totally ineffective against diseases and pests, with implications on the yield and quality of the end product, as already discussed. This is a particular problem for "organic" farmers. It could also worry "conventional" farmers as potential disease foci. Apart from being less efficient and less reliable, non-chemical means of controlling weeds, pests and diseases even if achievable, are almost always more costly.

Biological control of unwanted species is obviously attractive. Although there are several highly successful examples used on glasshouse crops, little commercial progress has been made in agriculture to date in Europe. Bio-control on its own is likely to be too specific for general use but obvious opportunities do exist, eg the control of bracken (Pteridium aquilinum). Apart from specificity, the reliability of bio-control is likely to be at least as dependent on suitable environmental conditions as agrochemicals and therefore work on integrating both methods of control should be encouraged.

Varietal resistance to pests and diseases will continue to be important but it is unlikely, even with increasing use of genetic engineering, that resistance to all the potential pests and diseases can be included in any one crop variety without significantly affecting other desirable agronomic characters like yield and quality. Much exciting work is in progress with transformed plants, insects and fungi etc but commercial success is likely to be limited before the turn of the century. Apart from the great care needed to introduce genetically engineered organisms into commercial practice, it is probable that they will suffer similar problems to agrochemicals in terms of specificity, phytotoxicity, persistence and development of resistance.

Although the advancement of the above could have some impact on agrochemical demand in the future, they are likely to be used as a supplement rather than a replacement for agrochemicals in most situations. Furthermore, it should not be assumed that the products of this new technology will reduce input costs relative to agrochemicals.

INCREASED USE OF THRESHOLDS AND FORECASTS

Thresholds and forecasts should help to increase the efficiency of agrochemical treatments and such aids will become increasingly important as the farmers' profit per unit area declines. However, most need very considerable refinement before their full potential can be realised. The vast majority of thresholds are defined for individual pests, diseases or weeds and this limits their practical usefulness. Despite the enormous number of combinations of species and the variability in growth and competitiveness, recent work aimed at defining economic optimum thresholds for weeds based on "crop equivalents" is a major step forward and should be continued. Similarly, diseases and pests seldom occur in isolation but little if any work on multiple disease or pest thresholds appears to be taking place. Thresholds will require constant review as practices and varieties change and therefore an integrated approach to these studies is desirable. Moreover, the cost of management time demanded for regular monitoring to detect when a threshold level has been exceeded should not be ignored. Nor should the need for suitable weather to apply the chemical after the threshold level has been reached.

With high value horticultural crops, the acceptable pest and disease level is almost zero and therefore the value of threshold analysis very dubious. The reliable benefits obtained with agrochemicals for a relatively low input cost in this high output market will continue to sustain demand.

General forecasts of pest and disease incidence, because of particular climatic conditions, can be very helpful, but specific warnings are often too late. For example, most potato growers have already applied their first blight spray before warnings are issued. It may be that farmers occasionally overestimate the likelihood of problems but it is borne out of the consequences of failing to act, rather than philanthropy towards the agrochemical industry.

Developments in computerised systems, to predict required inputs, will be made but they are unlikely to be totally effective even on an individual farm. Similarly, diagnostic techniques will help confirm the presence of a particular pathogen or pest but by then a curative treatment will be needed and, in some cases, this might require more agrochemical input than prior use of a prophylactic treatment. Clearly, much more work needs to be done to prescribe the conditions for optimum agrochemical use more accurately before farmers could be advised to change their current practices significantly.

FUTURE AGROCHEMICAL MARKET IN EUROPE

In the 1960's and 70's the world market for agrochemicals showed real growth in excess of 6% per annum but this declined to less than 3% over the past 5 years and in 1987 showed a slight decrease. In contrast, estimated sales in Western Europe increased from $4405m in 1986 to $5670m in 1987 and represented approximately 28% of the world market. (Wood Mackenzie 1987 & 1988). Sales in the UK, at approximately 3% of the world market in 1987, have plateaued. It is likely that cereals will occupy a less dominant position in Europe and especially in the UK in the near future and this will have the biggest impact on agrochemical demand. The actual reduction in area cannot yet be predicted with accuracy but the European market for agrochemicals looks set to become more homogeneous. Lost opportunities in cereals will be balanced at least to some extent by compensating increases in other crops. Overall, the market for agrochemicals is likely to remain static in real terms but there is no doubt that it will continue to survive for the reasons indicated in this paper. That being the case it is important to comment on the safety of agrochemicals in general and the continuous progress being made in their correct and responsible use.

THE IMPROVING SAFETY PROFILE OF AGROCHEMICALS

Thanks to the continuous attention of the media in recent years, the public is increasingly aware of agrochemicals but its information is generally unbalanced. The general perception is that agrochemicals are overused, misused and dangerous to users, consumers and the environment. Furthermore, the increased legislation surrounding the registration and use of agrochemicals has tended to emphasise the negative aspects of the industry.

It would be futile to deny that some agrochemicals can be harmful if not used correctly. Industry acknowledges and supports the need for continuing improvement and much progress has been made during the past two decades. Public concern over the use of agrochemicals, however misplaced, is real and the industry accepts the challenge to correct this imbalance. Several excellent reviews are available in the literature (eg Hessayon 1983; Ames 1983 and 1987). Space only permits a brief comment in this paper.

The relative toxicity of agrochemicals

It is widely acknowledged that there is no such thing as a harmless chemical but only a harmless dose. This applies equally to natural or synthetic chemicals. Agrochemicals are designed to kill weeds, pests and diseases but it should not be inferred that they are general poisons.

The rat LD50 range for the active ingredients in the top 10 products used in the UK in 1986/1987 (Farmstat (UK) 1988 personal communication) was 1517 to 8000 mg/kg. The relative toxicity of the 3 most widely used herbicides fungicides and insecticides and other common substances is given in Table 2.

TABLE 2

Relative toxicity to rats

Commodity	active ingredient	LD50 mg/kg
Herbicides	isoproturon, metsulfuron-methyl, triallate	1675-5000
Fungicides	propiconazole, prochloraz, fenpropimorph	1517-3515
Insecticides	dimethoate, deltamethrin, pirimicarb	135-5000
Tobacco	nicotine	50
Toothpaste	various fluorides	52-570
Shampoo	zinc pyrithione	177-207
Bleaching powder	calcium hypochlorite	850

Source : The Pesticide Manual 1987; Registry of toxic effects of chemical substances

This of course is only one measure of a chemical's toxicity but it is helpful for comparison. Acute effects from the correct use of agrochemicals in Europe are rare and for example there has not been a human death in the UK for the past 15 years. Over the same period, approximately 1000 deaths have occurred in UK Agriculture from other causes. Progress continues to be made in reducing operator exposure to agrochemicals by improved formulation and better container design. In the longer term the development of "closed fill systems" will give further improvements.

Residues in food

This is the biggest area of public concern. Residues are generally assumed to be present whereas in reality this is rarely the case. In a major survey of retail fruit and vegetables produced in the UK, more than 99% of the possible pesticide/commodity combinations tested contained no detectable residues. Approximately 98% of imported commodities were also residue free (MAFF 1986). Monitoring in Europe and elsewhere has produced similar results. This is very reassuring bearing in mind the sophistication of current analytical techniques. Where residues do occur they are generally well below the international limits of safety set by FAO. A residue per se, whether in crops or in water, is of no relevance if its toxicological impact is not considered. No death anywhere in the world has been attributed to the consumption of food treated with agrochemicals as recommended but very many people have died from eating 'natural' foods.

Concern over residues probably springs from society's extreme fear of cancer. However, naturally produced food contains a wide range of toxins synthesised by plants to protect them from pathogens and predators, few of which have received the detailed safety testing needed for an agrochemical. A ranking of possible carcinogenic hazards suggests that agrochemical residues in food or water are likely to be of minimum concern relative to the background level of natural substances. (Ames et al 1987). Proven carcinogens are found in mushrooms (hydrazines), basil (estragole), black pepper (piperine), celery and parsnips (psoralen), peanut butter (aflatoxin), mustard (allyl isothiocyanate) and many other foods which are consumed regularly. All of these, however, are of minor significance relative to smoking and alcohol. Vegetables known to be mutagenic include lettuce, paprika, rhubarb and string beans (van der Hoeven 1983). Cereals can also be an important source of fungal toxins. Sclerotia of the ergot fungus (Claviceps purpurea) are increasingly seen in home produced muesli and mycotoxins produced by Fusarium spp are also common. (Austwick 1984).

The human body is designed to cope with small amounts of a wide range of chemicals daily. Even a relatively simple lunch can contain around 10,000 different chemical substances without including 'food' molecules such as proteins and carbohydrates in the total. (Wright K Personal communication.) Whether 'good' or 'bad' the body has evolved means of metabolising or excreting them. There are innumerable examples where, over a lifetime, the equivalent of many lethal doses of many chemicals will have been ingested without harm provided they are taken into the body in small doses (CAST 1987). After considering the various constituents in food, including possible agrochemical residues, the British Medical Association summarised the risk thus "In the developed world we should count ourselves lucky that the main risk to health we face from food is eating too much of it". (BMA 1987).

Effect on wildlife and the environment

Adverse effects of agrochemicals on wildlife were caused, in particular, by overuse of the persistent organochlorine insecticides in the 1950's and 60's. Since approval for most recommendations was revoked, the situation has improved markedly. Insecticides constitute a relatively small proportion of the European agrochemical market (11.8%) and current evidence suggests that use of this group of chemicals, as well as agrochemicals in general, is not having a serious effect on wildlife populations, even if occasional members of a population are affected.

A very extensive monitoring of the environmental consequences of intensive crop protection inputs has been undertaken in "The Boxworth Project" in the UK. Results to date show no clear chemically related impact on small mammals, birds and field margin flora but some effects on certain invertebrate species in the "Full Insurance" areas. (MAFF 1987 & 1988). The agrochemical inputs under this regime, compared with the actual national average applied by farmers over the same period is given in Table 3.

TABLE 3

Mean number of agrochemical inputs to winter wheat 1983/84 - 1986/87

	Boxworth Full Insurance*	National Average**
1983/84	19.8	6.12
1984/85	18.7	6.36
1985/86	15.0	5.26
1986/87	17.0	5.78

Source * Greig-Smith 1988 ** Farmstat(UK) 1988 Both personal communications

Many of the above treatments at Boxworth were applied, as planned for experimental purposes, even in the absence of threshold levels of relevant problems. They include an average of 4.1 insecticide and one molluscicide treatments per annum, compared with the national average over the same period of 0.98 and 0.16 respectively. The results are therefore most reassuring in view of the economic constraints which would prevent the "Full Insurance" regime being adopted to any extent in practice.

Despite this very encouraging picture, crop protection sympathetic to the environment should remain a priority. Insecticides will always be needed when suitable weather conditions produce epidemics beyond the capacity of natural predators. In such circumstances the products capable of controlling the pest with least effect on beneficial insects should be chosen. The adoption by farmers of conservation headlands will help ensure improved habitats and refuge for wildlife even in areas of intensive farming.

Those who work in the agrochemical industry have at least as much concern for the environment as the general public and probably more so because of their specialist knowledge. Continuous monitoring and vigilance to ensure this very positive situation continues is therefore welcomed. Apart from registration requirements, industry aims first of all to satisfy itself on the safety of its products and there are many examples where development has been stopped because of undesirable effects on wildlife, especially birds. As a perspective, it is worth noting that some 20-70 million birds and 0.7-1.3 million hedgehogs are estimated to be killed on UK roads every year. (Observer 1986).

Operator training to improve the efficiency and accuracy of application is an increasing trend and the recent certification of operators and application machinery in some countries will ensure much greater awareness. The development of more specific products to match the more specific advice that will be required in future is also likely. This will allow growers to combine suitable materials in tank mixes at appropriate doses to control or leave the species desired without adding unnecessary ingredients into the environment.

The role of industry in improving and communicating the safe and effective use of its product is often overlooked. An enormous effort goes into educating users in the identification of problems, suitable control measures and care for the environment. Booklets like those produced by my own company "Working with Pesticides" and "Farm Conservation" are widely used. The funding of research projects like "TERF" and the development of "Pocket Parks", "Farm Trails" and "Conservation Competitions" all heighten awareness and help to ensure that intensive farming and a beautiful environment remain compatible.

CONCLUSION

Despite the general antipathetic attitude towards agrochemicals, there is little doubt that they are among the most valuable tools man has ever discovered in his fight for food. It is only because we have been successful in ensuring reliable food production that we can afford to consider the topics covered by the other three papers in this session. Society takes the reliability of good harvests for granted and needs reminding that food actually comes from the farmer and not the supermarket. Although future demand will be for increased quality rather than increased volume, the continued use of high technology will be needed to ensure a plentiful supply of cheap food.

The beauty of the UK countryside is an elegant witness to the concern farmers have for their environment and how this has been successfully integrated within their normal farming practices. But the countryside has to be managed and farmers can only afford to do so if they are profitable. Their financial viability is, therefore, likely to have the biggest environmental impact. Agrochemicals help farmer profitability without evidence of significant deleterious effects on the environment.

The agrochemical industry has been transformed over the past two decades with safer products, better targeting, more precise application and a better understanding and concern for environmental impact. These great strides need to be acknowledged.

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SESSION 3B

THE FATE OF PESTICIDES IN THE ENVIRONMENT – METHODS

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LABORATORY AUTOMATION IN PESTICIDE RESIDUE ANALYSIS

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ABSTRACT

The subject of laboratory automation is receiving an increasing amount of attention. This growth in interest can be attributed to several factors including rising workloads for many laboratories together with the rapidly developing capabilities of computing systems and automated equipment.

This paper provides a review of two years work at ICI Agrochemicals, Jealott's Hill with Zymark Laboratory Robotics applied to Residue Chemistry.

During this period, the equipment has been both reliable and flexible, processing approximately 1700 residue samples covering four different analytes.

The successful development of the system reported previously has now been augmented by the addition of on-line chromatographic analysis and the application of laboratory robotics to other tasks within our laboratory.

INTRODUCTION

The development and sale of agrochemicals is, in common with many industries, becoming more heavily regulated and controlled by Governments around the world. While it is recognised that legal control over the sale of pesticides is necessary, the increasing complexity and detail requested by the regulators has placed growing demands on laboratories responsible both for the development of new products and for enforcement once registration is granted. The growing requests for re-registration of established compounds has placed further strain on those managing environmental science groups in industry.

Faced with a large increase in workload, making the most efficient use of available resources is clearly an important target. Two approaches to achieving this aim were identified at ICI Agrochemicals Research Station at Jealott's Hill, Berkshire during 1985 : firstly a greater use of automation within existing techniques and secondly adoption of new techniques for use in pesticide analysis and metabolism studies.

Within the Residue Group at Jealott's Hill the number of samples received for analysis has doubled in the past five years. Against this background of the rising requirement for analysis a number of technical advances, employed in many laboratories, have increased the efficiency of operation of the laboratory. These changes include the introduction of autosamplers and injection systems for chromatographic instruments, enabling out of hours operation, and more recently the introduction of computer data capture and analysis systems, capable of providing an efficient route to the final analytical result. Prior to 1985 however, no attempt had been made to tackle the most labour intensive component of residue analysis, sample preparation and clean-up.

This paper summarizes the development of an automation system for pesticide residue sample preparation, provides data on the reliability and performance of the system since it was first described in 1987 (Ref 1), and looks at the potential applications of laboratory robotics in other areas.

SYSTEM SPECIFICATION AND DEVELOPMENT

Following discussions with other users of automated laboratory equipment, and now with hindsight from our own experience, one of the key factors in the successful automation of a complex process is to define accurately what is required. Applying this definition process to the area of clean-up procedures in residue chemistry enabled us to draw up a number of general criteria which needed to be satisfied by any automation system if it was to be successful. These criteria were then used to assess different pieces of equipment and later to draw up a detailed specification for the system chosen prior to purchase. Though the areas highlighted below were drawn up on the basis of our needs, they are typical of many possible applications for laboratory automation. The major criteria, and their justification are given below.

(a) Flexibility : All "total automation" systems seen during the evaluation of the project were expensive. It was therefore important that any system developed could be used for a large proportion of the available time. Even in our laboratories, which service a world wide need, no one analysis was required year round for several years. Any system had therefore to be able to perform a number of different analytical methods, with a minimum of "down time" between the different assays.

(b) Total Automation : Automated equipment for the laboratory is normally slower than the human analyst it is designed to replace. We also thought it undesirable to use human analysts to simply feed samples into different semi-automated pieces of equipment. To gain maximum benefit any system developed therefore needed the ability to automate a large portion of any assay and to be able to perform unattended and preferably overnight.

(c) Reliability : All of the analytical work carried out by the Residue Group is performed to often tight time deadlines. If an automated system was to be of real benefit it must also be sufficiently reliable to enable confidence in achieving results to deadlines.

(d) Accuracy and Precision : All of the work carried out within the Residue Group is performed to accepted analytical standards and complies with Good Laboratory Practice Guidelines. Any automated equipment would be expected to at least match the accuracy and precision of a human analyst.

(e) Safety : The equipment used needed to be able to work safely with organic solvents and radiochemicals. This area was considered particularly important given the need for unattended operation.

During late 1985 the above criteria were used to assess the equipment available which could potentially be used for automation of residue chemistry sample preparation. The only manufacturer who could offer a system with the potential to automate large parts of a residue assay reliably and unattended were Zymark. At that time the introduction of a robot able to "sense" objects by touch was expected to give a significant advance in the reliability of their automated systems.

Further discussion with equipment suppliers and examination of our existing residue chemistry methods led to a modular approach to the design of the system. Most of the methods in use were in or laboratories were composed of a series of common processes (extraction, partition etc) with variation between methods being different solvents, sorbents etc. A system able to perform these "generic" or common steps should be able to carry out a wide range of analytical procedures and hence provide the flexibility necessary to support a varied workload.

In March of 1986 a Zymark laboratory automation system was purchased for use in residue chemistry. The system was installed following the "generic" plan described above.

This approach resulted in the equipment needed for a particular operation being grouped as close together as possible. This provided for more efficient operation and enabled substitution in one area to be carried out without affecting any of the other operations on the robot table.

The table layout is shown in figure 1. The software written to control the system also followed a modular approach. This was done to enable large parts of software common to different assays to be transferred easily and remove the need to write complete programmes for each new assay. This approach has been effective; the first assay automated using the new system took approximately four months to programme, while a second related assay was added in one week.

This "generic" approach to system design and programming has now been used successfully on a number of different systems. Zymark have themselves taken this approach further and now market preprogrammed modules to perform some tasks. This can significantly reduce the time needed to commission a new robotics system.

EQUIPMENT PERFORMANCE

The first system purchased has now been in operation for a little under 2 years. During this period the available time has been divided between processing of samples and the transfer of new methods to the system. The modular approach to the design of the system has provided some major benefits:

(a) The initial assay took approximately 4 months to programme and test. Subsequent assays have taken significantly less time to programme by making use of portions of existing software. Typical method development time is currently 4 - 6 weeks. As the available software increases this time is expected to reduce further.

(b) During the early examination of the automation of residue methodology, it became clear that the equipment would be required to carry out a number of different assays. The use of the modular design has enabled the "downtime" between the applications to be minimised; changes include slotting new components onto bases fixed in known positions to the robot table or changing solvents to various work stations followed by loading of new software. Downtime varies between a matter of minutes (for a software change) to two days for a complex assay change and checking of the new assay.

In routine operation the system has processed 1700 samples covering residues of 4 different compounds. The reliability of the system has been excellent and after predictable early difficulties is curently greater than 95% (ie samples processed giving satisfactory analytical results relative to total sample processed). During one recent 3 month period of operation processing samples for analysis of residues of the pyrethroid insecticide "Karate" a total of 500 samples were analysed with no major equipment failures. With a minimum of out of hours input by the system operator, through put during a one month period was raised to 240 samples; approximately equivalent to two analysts effort for the same period.

RECENT DEVELOPMENTS

The robotics system originally installed in 1986 provided automation of the sample preparation phase of analysis but required human intervention for analysis of the samples. The need for this input from an analyst delayed both production of the final analytical result and information on the performance of the system during the unattended preparation of the samples. This delay between preparation and analysis of samples also prevented the system being used to automate assays for unstable analytes. To overcome these shortcomings a custom built, robot compatible, HPLC autoinjector has been added to the system while a commerically available GLC interface has also been purchased. (For details of the HPLC interface see Reference 2). Programming of the system to use the HPLC interface is currently in progress and within the next 3 months the system will be capable of automatically introducing the prepared sample into either of two chromatographic systems for final quantitative determination. This capability will provide the final link in "total" automation of some residue chemistry methods. This advance will enable an analyst to present the robotics system with a sample and by using "on line" analysis and computer data systems obtain an analytical result within a matter of hours without the need for further intervention.

CONCLUSIONS AND FUTURE PROGRESS

This paper briefly describes the automation of a higly complex laboratory process. Our experience of the equipment over the past two years demonstrates that laboratory robotics have now advanced to a point where difficult tasks can be automated in a flexible and reliable way. The performance of the first system and the experience gained has enabled a second residue chemistry system to be installed quickly, while the technology has been used to automate a further different piece of equipment within our laboratory (for details of automation of Harvey Sample Oxidizer see reference 3).

Further manual methods have already been identified as targets for automation using the robotics technology we have developed. In the coming year several methods will be added to the systems' capabilities, expanding the areas in which they can contribute. As our software library grows we expect the time taken to commission new methods to decrease still further. We also expect to use our growing knowledge and experience to automate other laboratory tasks.

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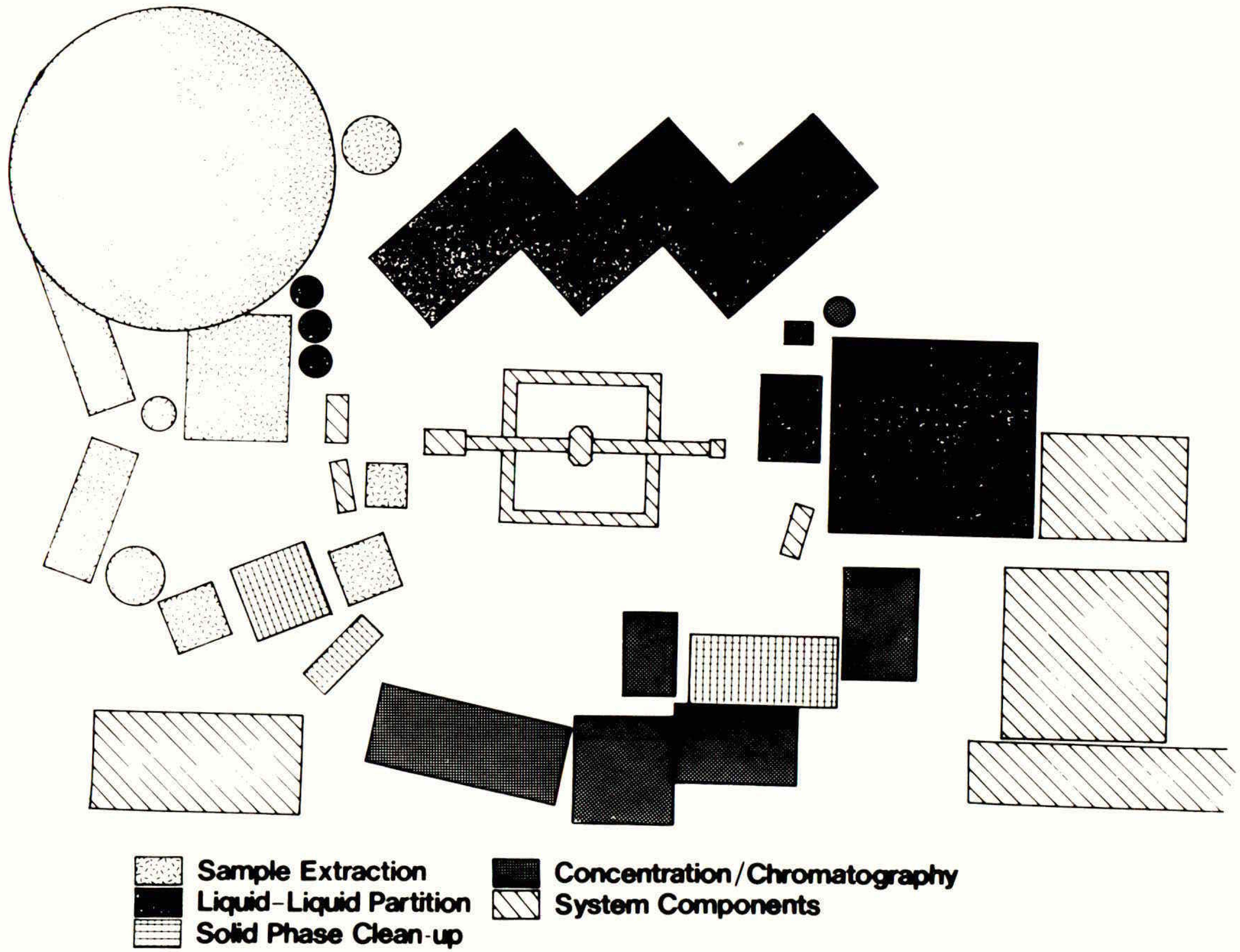
FIGURE 1 : LAYOUT OF ROBOT TABLE



IMMUNOCHEMICAL METHODS FOR PESTICIDE RESIDUE ANALYSIS

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ABSTRACT

In view of the escalating costs and demands for pesticide residue analysis, more economic alternatives are being sought, with performances comparable to existing technology. Immunoassay techniques match these requirements and have additional benefits which can fulfil the changing needs of the analyst. The speed and simplicity of immunoassay may allow the development of field environmental monitoring procedures for which conventional methods are inappropriate. There has been a steady increase in the number of pesticide immunoassays developed and several immunoassay-based agricultural diagnostic kits have been introduced.

INTRODUCTION

In recent years demands for the determination of pesticide residues have been steadily increasing. Environmental monitoring data requirements have added significantly to the sample workload necessary for product registration and support. At the same time the costs of conventional analyses have been escalating with continued advances in instrument sophistication and the necessity for many sample preparation procedures. These factors have prompted the search for low-cost, rapid and automated trace-residue detection methods.

IMMUNOCHEMICAL ANALYSIS

Immunoassay techniques may help to overcome many of the challenges currently facing residue analysts. These methods, first developed thirty years ago, are widely used as part of clinical diagnosis for the analysis of proteins, peptides, hormones and drugs. It has only been during the last ten years that immunoassays have been applied in plant disease diagnosis, food testing and environmental monitoring.

The key elements of immunochemical analyses are the antibodies, products of the vertebrate immune system. These naturally-occurring binding proteins are normally generated as part of the body's defence against foreign microorganisms and their toxins. Experience has shown that laboratory animal species can be immunised with an almost limitless variety of organic molecules resulting in the production of polyclonal antibodies which bind tightly and specifically (sometimes stereoselectively) to the immunising material. A major advance in immunology, coincidental with the interest in applying immunoassays in non-traditional areas, has been the development of hybridoma technology. This has had a major impact on the scope of immunochemical methods. The procedure has allowed the immortalisation of specific antibody-producing cells followed by their growth in tissue culture systems to generate unlimited quantities of monoclonal antibodies. Purified monoclonal antibodies can now be regarded as highly reproducible reagents which exhibit clearly defined binding affinities and specificities. These advances have permitted the development of immunoanalytical and immuno-preparative techniques which fully exploit the potential of antibodies, exhibiting high sensitivity, exquisite specificity, simplicity and universality of application.

Although immunoassays can offer these benefits to the analyst, serious consideration must be given to the level of commitment required for their successful development (Hammock *et al*, 1986). The minimum time period for the production of specific antibodies is usually six months and the assay optimisation, even without full validation, can take at least a further half-year. Although this does not represent continuous effort the overall development cost for an immunoassay is approximately equivalent to one bench-man-year. Given this level of investment, immunoassays are most suitable when the analysis of large numbers of samples over extended periods is perceived, or when simple, possibly portable, systems with low operating costs are desirable, or when a conventional alternative is unavailable.

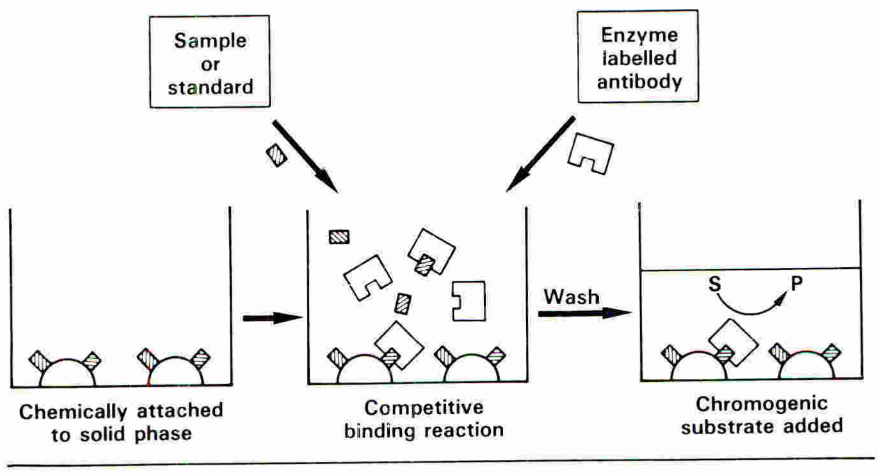
Immunoassay development

The critical element of immunoassay development is the production of a suitable antibody. When the material to be determined has a molecular weight of less than 2000 Daltons it is generally referred to as a 'hapten'. This term applies to the majority of chemicals likely to be quantified in trace pesticide residue analysis and environmental monitoring. Haptens can be bound by a specific antibody but are not intrinsically capable of stimulating the immune system. This problem is overcome by the chemical conjugation of the analyte to a suitable protein to produce an immunogen. Careful consideration must be given to the design of the chemical linkage and the reactive centres involved to facilitate the production of antibodies of the desired specificity. Furthermore, whether polyclonal antibodies from animal sera or monoclonal antibodies from cultured hybridomas are produced, the antibody assessment method requires some attention. High performance assays are only developed when the antibody screening conditions closely mimic those of the ultimate immunoassay application.

There are many immunoassay formats, with acronyms to match. When numbers of tests executed are considered the most popular is still the original procedure, radioimmunoassay (RIA). However, the current trend is to use alternatives to radioisotopes, thus avoiding possible risks to health during use and waste disposal problems. There is now available a range of assay endpoints which are capable of matching the sensitivity and precision of the radiolabel. The most widely applied of this second generation of immunoassays is the enzyme-linked immunosorbent assay (ELISA) (Fig. 1). It is most commonly carried out in plastic microwells presented in an 8×12 array. Using manual methods, with low technology and inexpensive equipment, simultaneous analysis of up to 50 samples per day is a relatively straight-forward target. A major additional advantage of an enzyme-mediated assay endpoint is that the approach can be transferred directly to field testing. Some of the best examples of these are pregnancy home-testing kits and immunoassays used in disease vector monitoring programmes in the Third World. In contrast, the simple procedures used in ELISA methods are also ideal for use with fully automatic, computer-controlled dilution and dispensing systems capable of handling many hundreds of samples daily. A further feature of ELISAs, and most other immunoassays, is their suitability for use in screening programmes. Confirmatory analysis of manageable numbers of selected samples can then be carried out using conventional methods. This approach, combined with the ease of sample replication in immunoassay, can greatly improve the statistical validity of any analytical data generated in field trials.

FIGURE 1

Competitive ELISA-limited antibody method.



S: Colourless enzyme-substrate P: Coloured product

<u>Trace pesticide immunoanalysis</u>

Applications of immunoassays for pesticide residue analysis are gradually increasing. A recent review (Mumma and Brady, 1987) cites more than 50 assays of agricultural interest, almost half of which are for pesticides (Table 1).

TABLE 1

Immunoassays for pesticides

Chemical	Detection limit	Authors
Aldicarb	5.0 ng (E)	Ferguson (1986)
Aldrin	700 pg (R)	Langone & Van Vunakis (1975)
S-Bioallethrin	0.5 ng (E)	Wing & Hammock (1979)
Dieldrin	150 pg (R)	Langone & Van Vunakis (1975)
	10 ng (E)	Ferguson (1986)
Diflubenzuron	3.9 ng (E)	Wie & Hammock (1982)
Parathion	4.0 ng (R)	Ercegovich *et al* (1981)
	2.5 ng (E)	Brady (1984)
Atrazine	1.5 pg (E)	Huber (1985)
2,4-D	13 ng (R)	Rinder & Fleeker (1981)
	2.5 ng (E)	Ferguson (1986)
Diclofop-methyl	20 ng (E)	Schwalbe *et al* (1984)
Paraquat	0.1 ng (E)	Van Emon *et al* (1986)
Surflan	– (E)	Kuniyuki & McCarthy (1986)
Terbutryn	4.8 ng (E)	Huber & Hock (1985)
2,4,5-T	3.3 ng (R)	Rinder & Fleeker (1981)
Benomyl	1.25 ng (R)	Newsome & Shields (1981)
Iprodione	0.2 ng (E)	Newsome (1986a)
Metalaxyl	63 pg (E)	Newsome (1985)
Methyl-2-benz-imadazole carbamate	125 ng (R)	Newsome & Shields (1981)
Triadimefon	1.0 ng (E)	Newsome (1986b)

E: enzymeimmunoassay R: radioimmunoassay

These data may not reflect the true depth of interest as assays are likely to be developed for in-house use by industrial research organisations. For example, Sittingbourne Research Centre has been active in immunochemistry research for over ten years and latterly has developed ELISA methods for a number of pesticides.

Cypermethrin

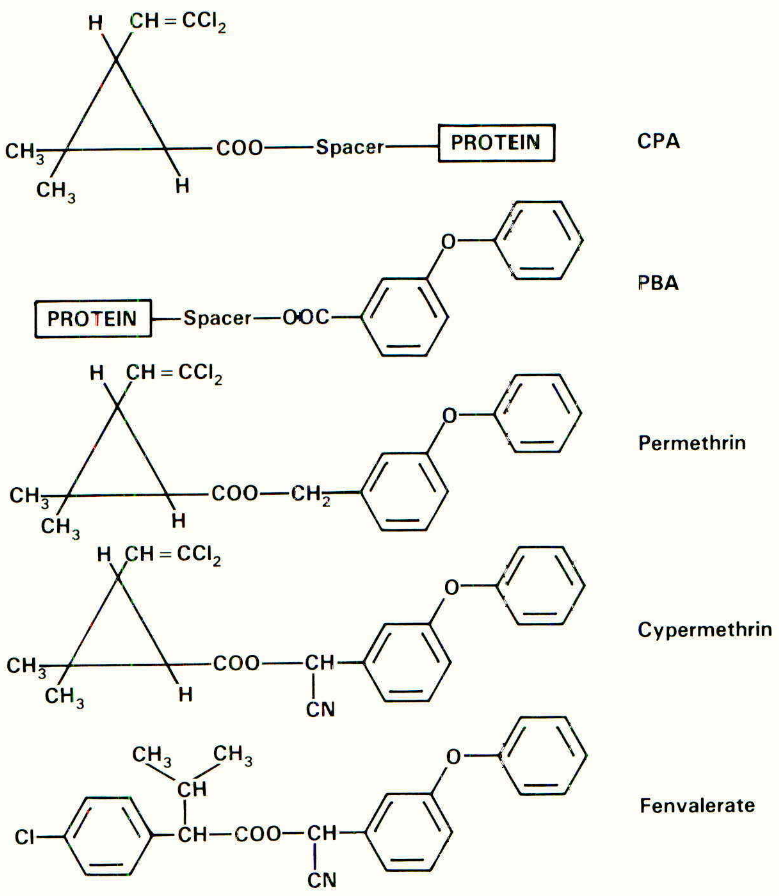
Initial studies were to develop an ELISA method using the pyrethroid insecticide cypermethrin (CYP) as a model (Wraith *et al*, 1986) (Fig. 2). The aim was to generate both polyclonal and monoclonal antibodies against the CYP plant metabolites, 3-phenoxybenzoic acid (PBA) and 3-(2′,2′-dichloro-vinyl)-2,2-dimethyl cyclopropyl carboxylic acid (CPA). Availability and ease of conjugation of these acids to proteins using carbodiimide chemistry facilitated this approach. Antibodies were produced that allowed the development of two assays. One could be used to quantify CYP (possibly also permethrin) and CPA. The second assay could quantify PBA and the class of synthetic pyrethroids containing this moiety.

In retrospect the CYP model was not an ideal model but provided good first hand experience of the pitfalls to be avoided. The principal difficulty encountered being the relative insolubility of pyrethroids in the aqueous buffers necessary for ELISA methods. However studies with water-miscible organic solvents, up to 30% v/v, indicated that antibodies can be very tolerant of conditions which would normally denature other functional proteins. The inclusion of specific solvents also had the beneficial affect of improving the gradient of the dose-response calibration curve.

An assay for PBA utilising monoclonal antibodies was developed which had a lower limit of detection of 50 ppb and an interassay coefficient of variation of 5%. In use in our laboratory this assay gave data comparable with that obtained using gc-ms and hplc techniques for the determination of PBA in water, soil and black tea. Although some sample clean-up was required, particularly for black tea, significant savings in effort were ultimately achieved.

FIGURE 2

Cypermethrin immunogens and pyrethroids with potential for cross-reaction.

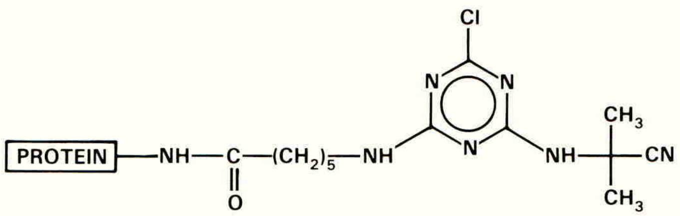


Cyanazine

An immunoassay, based on polyclonal antibodies, was developed for the triazine herbicide, cyanazine. Immunisation with desethyl cyanazine conjugated to a protein, via a 6-carbon spacer, gave highly specific antibodies (Fig. 3). Cross-reactivity of the antibodies was not observed with a wide range of other commercial triazine herbicides, including atrazine.

FIGURE 3

Cyanazine immunogen.



However, as expected, the only significant cross-reactivities found were with metabolites of cyanazine transformed in the same side chain as used for the preparation of the immunogen e.g. desethyl cyanazine. The detection limit of the assay in groundwater, without sample work-up, was 0.5 ppb. Recent work has shown that this can be improved 100-fold using C18 Bond Elut cartridges for sample clean-up and concentration. The method was validated for groundwater by analysis of samples containing various amounts of cyanazine and atrazine. The results compared favourably with data obtained using an established gc method (Table 2). The immunoassay has been used to provide data for a re-registration of the product and is currently being employed in environmental monitoring studies.

In addition to the above examples, immunoassays have been successfully developed for the rodenticide, flocoumafen (Bowler *et al*, 1984) and the acylurea insecticide, flufenoxuron (Anderson *et al*, 1986). Both of these chemicals were poorly water-soluble but the problems were overcome by applying the same approach as used for cypermethrin.

TABLE 2

Validation of cyanazine ELISA

No. Samples	Cyanazine ppb ELISA	Cyanazine ppb gc-TSD	Atrazine, ppb by gc a)
26	<0.5	<0.5	<0.5 – 2.4
1	0.69	0.68	4.3
1	1.3 b)	1.0	–
1	2.0 c)	2.0	–
1	2.2	3.0	15.0
1	1.2	1.4	8.6
1	0.68	0.42	1.2
1	5.0	4.6	9.6
1	0.92	0.46, 0.42	3.4
1	15.5	13.2, 10.7	10.2
1	3.7	2.5	9.2
1	1.7	1.9	14.2

a) Data shown to demonstrate specificity of the ELISA procedure.
b) 1.0 ppb Cyanazine added to tap water.
c) 2.0 ppb Cyanazine added to tap water.
TSD: Thermionic specific detection.

CONCLUSIONS AND PROSPECTS

When considering the application of immunoassay it is important to be aware that antibodies are unique molecules with individual properties. For example, an antibody and immunoassay produced in one laboratory may not have the same properties and performance as others developed elsewhere for the same chemical. Currently immunoassays are mainly used for specific analyses but antibodies with broader specificity or those directed against a common feature, as in the CYP example, may allow more generic applications of the technology.

The nature of the analytical technique requires a level of commitment for the development of a method greater than that usually required for conventional analysis. There should be close interaction between the development team members, the analyst, the immunologist and the synthesis chemist. However, once an in-house immunoassay has been fully optimised and validated, or if commercial kits are to be used, then the level of scientific and technical capability required for their successful application is much reduced.

Primary pesticide producers, having the range of expertise necessary, have accepted that immunoassay has the potential to provide a powerful addition to their analytical capabilities. As in conventional analysis the speed and cost is usually dependent on the degree of sample preparation. Experience to date has shown that in many cases immunoassay permits significant improvements, minimising sample clean-up requirements. However, in a few cases, only limited improvements have been achieved.

An indication of the level of acceptance of the immunoassay approach can be drawn from the increasing numbers of small immunotechnology businesses, particularly in the U.S.A., offering kits for agricultural uses. This development has led to projections that the application of immunoassay in non-traditional areas may amount to business worth $300 million within the next decade. (Klausner, 1987). However, one area of uncertainty has been how data generated by immunoassay will be viewed by the regulatory authorities. The Environmental Protection Agency is currently assessing applications of immunoassay (Vanderlaan et al., 1988) and is actively sponsoring research in the area.

During the next few years increasing numbers of immunoassays suitable for environmental monitoring will be developed. It is very likely that these tests will be produced and marketed by small independent companies in kit forms, suitable for rapid, simple and cheap field analysis. These will be available for use by producers or agencies, and, intriguingly, they may also be available to the public at large.

ACKNOWLEDGEMENT

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FATE OF PESTICIDES IN AQUATIC MESOCOSM STUDIES - AN OVERVIEW OF METHODOLOGY.

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ABSTRACT

Laboratory and field mesocosms have become more widely used for predicting the fate and effects of pesticides in aquatic systems. Laboratory mesocosms may be useful predictors of fate assessments for the field but are unlikely to be able to substitute for effect studies under field conditions. Enclosures and ponds each offer certain advantages but experimental designs must take surface-area:volume relationships into account. With pesticides of high partition coefficient, significant adsorption may take place on the sides/walls of the enclosures and give an unrealistic approximation of field dissipation rates.

INTRODUCTION

Model ecosystems, microcosms and mesocosms have been widely used as experimental tools to study the fate and effects of chemicals in the environment (Lundgren, 1985). The overall assumtion in the use of mesocosms is that they act as surrogates for the real ecosystem. Typically, the nature of the objective of mesocosm studies determine the specifics of the techniques used. These objectives are usually combinations of one or more of the following:

Prediction of the fate and effects of a pesticide in the ecosystem.
Prediction of the effects of physicochemical parameters on the fate and effects of the pesticide in the ecosystem.
Negation of the putative harmful effects of pesticides in the ecosystem.

While this paper will focus on the use of mesocosms to study the fate of pesticides in lentic systems, a large body of literature has accumulated on use of these and similar systems to study the effects of a variety of natural and synthetic substances (See reviews by Lundgren, 1985 and Crossland and Wolff, 1988). Fate- and effect-studies are often carried out simultaneously and, where appropriate, reference will be made to design considerations relevant to measuring effects as well.

As the literature lacks consistency in the use of the terms microcosm and mesocosm the former is defined for the purposes of discussion as a small system, less than 50 L capacity. The latter are larger than 50 L and include anything from a moderately sized laboratory model system to a very large and complex field enclosure. Field mesocosms are often treated separately from laboratory systems for the practical reason that they are used to study different phenomena and make use of different techniques of observation and sampling. A protocol for the use of mesocosms to study the fate and effects of pesticides in the aquatic environment has recently been proposed (U.S. EPA, 1987) and several studies for registration purposes are currently under way, however, no results from such trials have been published in the open literature. This paper will address some principles of design and construction and offer some examples of results and their interpretation.

METHODS

Design and construction of mesocosms.

The general characteristics of several mesocosm systems are illustrated in Fig. 1.

Laboratory systems

Glass aquaria and similar containers have been used for a number of studies on both fate and effects of pesticides and other chemicals (Metcalf *et al*, 1971; Franco *et al*, 1984 and Lungle, 1988). These systems are small, relatively inexpensive and their environment can be controlled with relative ease. Provision of light at realistic outdoor levels and wavelength spectrum may be difficult as the lighting systems used often are designed to maximize plant growth rather than mimic natural sunlight. Replication is easy and the systems can all be stocked with homogeneous substrate, flora and fauna selected from the field or laboratory culture. Because volumes are small, the physical and chemical properties of water and sediment may be altered to reflect the need to assess the influence of environmental factors on dissipation, movement and effects of pesticides. Perhaps the best use of such systems has been in modelling bioaccumulation and food-chain magnification of pesticides (Metcalf and Sanborn, 1975). Although such systems are generally too small to support more than two trophic levels, this drawback may be overcome through temporal manipulation of predator and prey organisms to force movement of any bioaccumulated material through the food chain (Metcalf *et al*, 1971). Although laboratory systems are generally small, some larger units have been developed. Lake column simulators, stainless steel columns, 4.5 m high and 1 m in diameter have been constructed (Millard *et al*, 1979) and used for studies on the dissipation and biomagnification of atrazine. Cooling coils allow for temperature stratification of the water column. Obviously, such structures are more expensive than aquaria and require considerable maintenance.

Outdoor tanks and ponds

Concrete, plastic and soil-lined ponds have also been used for studies on pesticides and other chemicals. The MERL tanks at the University of Rhode Island are land-based, 5 m high by 2 m diameter plastic tanks holding seawater. Sediment from marine environments can be placed in bottom trays to simulate conditions in a bay or shallow part of the ocean (Oviat *et al*, 1984). Crossland and Wolff (1988) recently reviewed the methodology and experimental design of ponds for assessing the fate and impact of pesticides. Giddings *et al*, (1984) used plastic and sediment lined ponds (18 m^3) to study the fate and effects of coal-oil and Larsen *et al*, (1986) use earth-lined ponds (470 m^3) to observe the effects of atrazine in an aquatic ecosystem.

Most pond studies use systems open to the atmosphere but make use of a local source of water and sediments for establishment. Planktonic organisms are usually added with the water (which usually comes from a holding pond) but fish can be added from known stocks and numbers may be adjusted to the design of the study. For logistic reasons, the physicochemical and population characteristics of ponds or tanks reflect the particular hydrogeological environment in which the system is located. Manipulation of these parameters is very difficult and results obtained from such studies may be specific to a region. A further design problem in these systems is the lack of ageing of the sediments in newly constructed ponds. Sediment-water interactions may be very important in mediating both fate and effects of pesticides, particularly those which absorb strongly to organic matter.

Enclosures

Enclosures made from metal or plastic have also been used to assess the fate and effects of pesticides in aquatic ecosystems (Solomon *et al*, 1980, 1985, 1986: Shires 1983: Kaushik *et al*, 1985, 1986: Stephenson *et al*, 1986: Herman *et al*, 1986: Brazner *et al*, 1987: Lungle, 1988). These enclosures range from the 1 m^3 stainless steel enclosures (Shires, 1983) to 1000 m^3 plastic

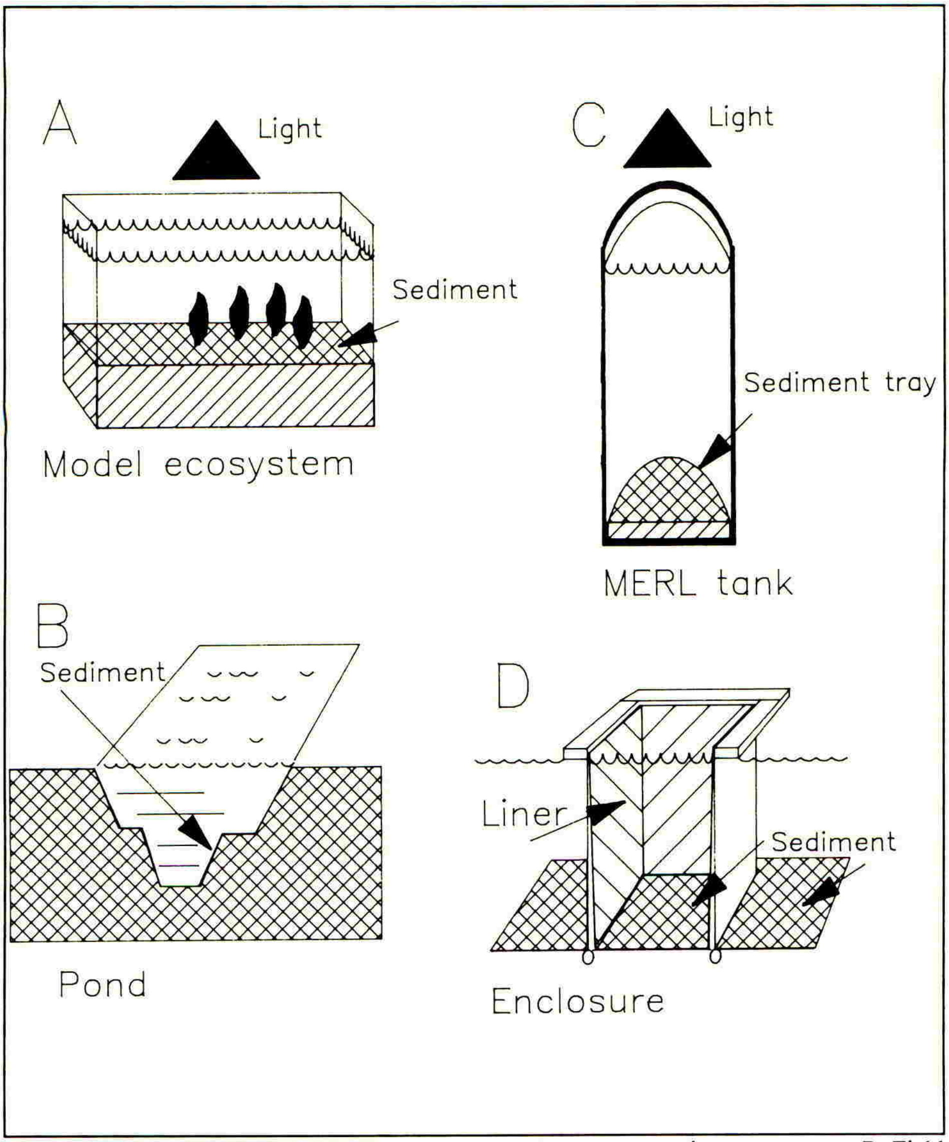

Figure 1 Construction details of mesocosms. **A:** Laboratory aquarium mesocosm. **B:** Field pond mesocosm. **C:** Field tank mesocosm. **D:** Field enclosure or limnocorral.

enclosures (Stephenson *et al*, 1984, Solomon *et al*, 1988b). By design, enclosures are open ended tubes, square or circular in cross-section with one end embedded into the sediments of a body of water and the other open to the atmosphere. Like ponds they are thus subjected to "natural" climatological conditions, however, they are usually set up in established aquatic ecosystems where sediments have undergone biogeochemical aging and where established populations of organisms already exist. They also have the added advantage of portability, both within the body of water and to different hydrologic environments. Even though these enclosures are usually fairly large, their installation captures populations of non-randomly distributed organisms which may not be similar in replicate enclosures, even immediately after installation. This observation is particularly relevant to fish and is of greatest importance where biological effects are being measured. In our own work we have been able to remove fish from enclosures with a resulting decrease in the variability of biological parameters.

Sampling methods for mesocosms.

Sampling small laboratory-type mesocosms requires sophistication of experimental design because sampling for residue analysis may actually change the pesticide concentration significantly. However, laboratory systems are particularly suited to the use of radiolabelled material which increases analytical sensitivity significantly. Larger enclosures such as ponds, tanks and enclosures usually allow repeated sampling without significantly affecting the parameters of the system. However, these systems may become stratified into distinct biological compartments and it may become necessary to tailor sample collection to take this into account. Even after surface application of pesticides, mixing with the water column appeared to be rapid (Solomon *et al*, 1986) and few differences were observed in terms of rate of dissipation with depth (Solomon *et al*, 1985). Depth-integrating samplers (Solomon *et al*, 1982 and Giddings *et al*, 1984) have been used for sampling both chemical and biological parameters. Sediment samplers for use in relatively deep enclosures have also been developed (Solomon *et al*, 1988b). Some parameters such as density, growth and productivity of macrophytes are difficult to measure. Although not well tested in the literature, it is likely that, as the size and the complexity of the mesocosm increases, so does the need for special considerations in the design of the sampling program. This has certainly been our observation in assessing the effects of methoxychlor in 1000 m^3 enclosures (Stephenson *et al*, 1984 and Solomon *et al*, 1988b).

Sampling techniques are essentially similar for the different types of field mesocosm with some minor variations due to site, locality or properties of the pesticide being investigated. In fate-only studies, sampling may be restricted to residues of pesticide and metabolites in water, sediments, wall material and biota. Other parameters which may be determined (Table 1) are physical and chemical parameters of the system and, in the case of fate-and-effect studies, biological parameters. The U.S. EPA mesocosm protocol (EPA, 1987) calls for quite extensive monitoring of water chemistry parameters, however, many of these parameters are strongly correlated and extensive sampling may be redundant. As an example, rates of photosynthesis may be measured by use of a dissolved oxygen probe (Solomon *et al*, 1988a), by diurnal measurements of dissolved oxygen (respiration/production ratios) and chlorophyll *a* levels (Franco *et al*, 1984), or by $^{14}CO_2$ uptake, either at the community level (Larsen *et al*, 1986) or at the cellular level (Hamilton *et al*, 1987; 1988). The simple oxygen measurement can indicate depression of photosynthesis but more extensive study is needed to identify species of algae resistant to the effects of the pesticide (Hamilton, 1987). As is generally the case, such detailed information is obtained at ever increasing costs in time and labour.

TABLE 1 Possible sampling parameters for mesocosms.

Fate and dissipation	Effects studies
Residues/metabolites in water, sediments-/sides/wall, interstitial water and biota. *(Some or all of the following)* Dissolved oxygen, pH, alkalinity, major ions, particulate nitrogen, phosphorus and carbon, dissolved organic and inorganic carbon, ammonia, chlorophyll *a*.	*(Some or all of the following)* Phytoplankton, zooplankton, macrophyte and benthic species and abundance. Microbiota abundance and/or species. Productivity (from the community to the species level).

RESULTS AND DISCUSSION

Replication and statistics

The major objective of mesocosm studies from a regulatory point of view is prediction of effects in the environment. Prediction presumes knowledge of the variability in a system and, from this point of view, replication is required. However, from basic principles, the need for replication is clearly related to the objectives of the study. As has been pointed out (Crossland, 1988), factors which affect the fate and dissipation of pesticides in the aquatic ecosystem are physical and chemical in nature. Variability of these parameters is low and their distribution is normal. In our own studies (Solomon *et al*; 1985; 1986; 1988) on fate-and-effects, we have used three replicate enclosures at each concentration of pesticide. Differences in actual concentration as well as rates of dissipation between replicate enclosures were low in studies with methoxychlor (Solomon *et al*, 1986; 1988b) 2,4-D, hexazinone and triclopyr (Solomon *et al*, 1988a), in studies with tetra- and penta-chlorophenol (Fig. 2) and chlorpyrifos (Lungle, 1988, Fig. 3). These results are very similar to those of Crossland (1988) obtained with trichlorobiphenyl, parathion methyl and pentachlorophenol in ponds. The considerable number and the consistency of these observations suggest that only a single replicate will suffice if one is only interested in determining the fate of a pesticide. Similarly, water chemistry parameters have shown predictable responses in most studies published thus far. In some cases only a few of these parameters show any treatment-related effects (Solomon *et al*, 1985; 1986).

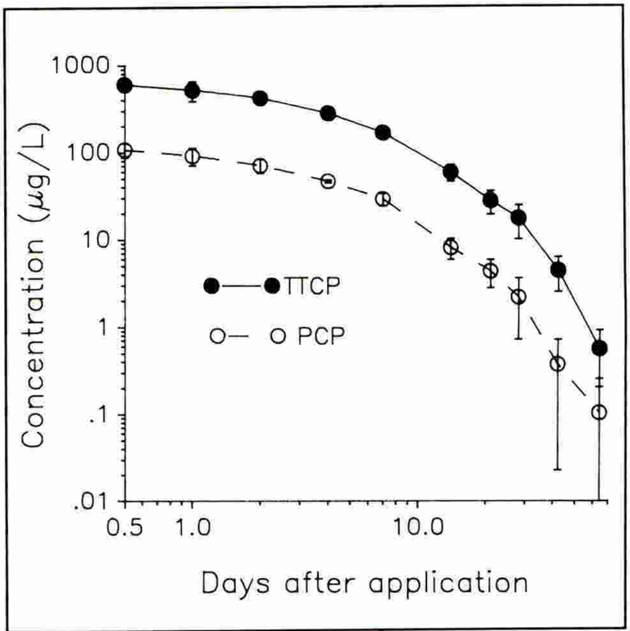

Figure 2 Dissipation of tetrachlorophenol and pentachlorophenol from treated enclosures. Vertical bars show the 95% confidence of the mean for three replicate enclosures.

From a practical point of view, biological effects as well as fate are commonly measured simultaneously and both must be considered. In measuring biological effects, replication is still a requirement to distinguish between true cause-and-effect and random or unrelated fluctuations in population levels. Zooplankton usually have non-normal distributions and statistical analysis requires, not only replication of treatments, but also the use of transformations of data or non-parametric statistical analysis. In analysis of our results we have used analysis of variance (ANOVA) of transformed data to test the significance of differences between treated and reference enclosures. While this is obviously of lesser importance in the case of clear-cut effects such as acute toxicity, it is important in identifying time of recovery and other less obvious phenomena.

A second approach is the use of a range of concentrations to determine a dose response (Giddings *et al*, 1984; Larsen *et al*, 1986 and Solomon *et al*, 1988c). From a statistical point of view, this approach makes use of regression rather than ANOVA to determine confidence intervals and deviations from control response. The dose-response approach has the additional advantage from the regulatory point of view that one can interpolate to determine environmentally acceptable concentrations or no-observable-effect-concentrations. In this case it is obviously of importance to measure residue levels at all concentrations tested.

Laboratory-Field extrapolation

The ultimate objective of mesocosm studies is prediction of environmental fate and effects. Achieving this without the necessity of field studies presents obvious advantages in time and cost. Fate of a chemical in laboratory as well as field mesocosms has not been compared often. Larsen *et al* (1986) and Giddings *et al* (1984) conducted residue analyses in field and laboratory mesocosms but did not specifically compare dissipation rates. Lungle (1988), working with chlorpyrifos, measured dissipation in glass aquaria containing 67 L of water and 40 L of sediment from a field pond and held at 16 h light (25°C) 8 h dark (20°C). These results (Fig. 3) suggest reasonable prediction of field results although adsorption to the sides was not measured in the laboratory mesocosms. This may, however, be a special case where hydrolysis is a major determinant of dissipation and, pH and alkalinity of the water being almost the same, good correlations would be expected. Where dissipation is more dependent on photolytic processes, the difficulty of duplicating natural sunlight in laboratory mesocosms may result in less correlation. This is certainly an area for future validation studies.

Wall effects

The bottom and side of a mesocosm may have a significant effect on the distribution and rate of dissipation of the pesticide. Pond mesocosms have sediment and/or periphyton on the bottom and sides of the system and enclosures may have a plastic wall in contact with the water column. This is of particular importance when the enclosure is relatively small and the surface-area to volume ratio is very different from the body of water for which the enclosure is acting as surrogate. This phenomenon may become significant if the pesticide being studied has a tendency to partition onto sediments or the plastic walls of an enclosure. Our experience with methoxychlor (Solomon *et al*, 1988b), indicated that dissipation (and effects) in 20 m^3 enclosures were significantly different from larger enclosures (100 and

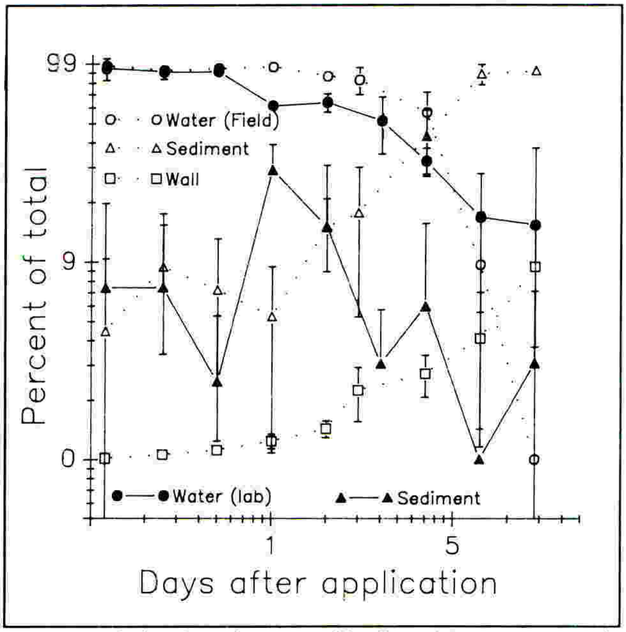

Figure 3 Dissipation of chlorpyrifos from laboratory aquaria and littoral enclosures. Vertical bars indicate 95% confidence intervals for three replicates. Redrawn from Lungle, 1988.

1000 m[3]). Lungle's (1988) work on chlorpyrifos in littoral enclosures also showed that adsorption to the plastic walls was a significant sink for the pesticide. To our knowledge, this phenomenon has not been reported in ponds of different surface-to-volume ratios but, in the worst case of a pesticide which is relatively persistent as well having a high K_{ow} or K_{oc}, this could result in erroneous or overly optimistic rates of dissipation from water. The larger the enclosure, the more closely the surface-area:volume ratio will approach that of the natural body of water. In conducting these types of studies serious consideration must be given to the possibility of surface adsorption and distortion of results. In the studies on methoxychlor (Solomon *et al*, 1988b) differences in rates of dissipation were most obvious between the 20 and 100 m^3 enclosures but different biological effects were also observed between the 100 and 1000 m^3 enclosures treated at the same concentrations.

Choice of the size of the enclosure for fate-only studies will depend on the nature of the pesticide and its physical properties. For pesticides with a low K_{ow} or K_{oc}, small mesocosms will most probably suffice while for pesticides with a high K_{ow} or K_{oc}, larger mesocosms will be required. Some guidance as to size of mesocosm is given in Fig. 4 where the effect of surface-area:volume ratio on adsorption to the sediments sides is considered through the use of a fugacity model (Mackay and Paterson, 1982)[1]. This model assumes equilibrium and no dissipation and is, as such, a worst case but the general trends are obvious. Differences between the enclosure and the "sides" of the pond as an adsorption sink for pesticides are not as great as one would expect at a K_{ow} of 100 000 and, at a K_{ow} of 1 000, adsorption the sides of a 100 m^3 enclosure is of relatively minor importance. Because of the greater organic matter content of the walls of enclosures, these are somewhat better sites for adsorption in this model system. Obviously a model such as this is very simplistic as a number of other factors such as rapid dissipation, increasing distance for diffusion become more important in larger enclosures. Since plastic enclosure walls rapidly become colonized with a periphytic community that is similar to that found on sediments and other objects in the water, adsorption, should it

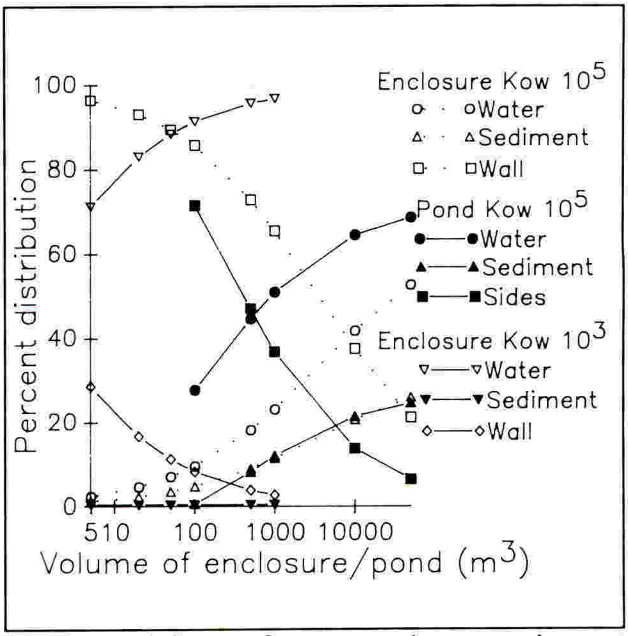

Figure 4 The influence of mesocosm volume on total amount of pesticide in water, sides and sediments.[1]

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1 Simplified level 2 fugacity model consisting of water, sediment, biota and sides/walls: Assumptions for the pesticide were: Temperature = 12°C, Molecular Weight = 200, Water solubility = 5 mg/L. Mesocosms 5 m deep, enclosures rectangular and sides of ponds slope down over a distance of 2 m. Effective thickness of sides/walls and sediment = 1 mm, organic carbon content of sediment/sides = 4%, biota and plastic wall are equivalent to 4.8 and 10 % octanol respectively in terms fugacity Z value.

occur, is probably similar in nature to that in ponds or other types of enclosures. The fact that this periphytic community changes with time of first exposure to water adds a complication which, at least in the MERL tanks, has been reduced by the constant removal of the periphytic film by regular mechanical scrubbing. This is not easily conducted in enclosures with flexible walls. However, enclosures do allow the use of relatively inexpensive disposable liners for each experiment which eliminates the possibility of carry-over from one set of tests to another. Adsorption to walls of enclosures is easily measured with the use of strips of wall material suspended in the enclosure. Measurement of this phenomenon at least allows it to be factored out in the assessment of fate studies.

CONCLUSIONS AND FUTURE DIRECTIONS

The mesocosm technique for the evaluation of fate and effects of pesticides has a number of advantages over other types of field study. These are the greater degree of control over some environmental variables, the ability to replicate studies and to carry these out in climatologically appropriate regions. Studies on the fate of pesticides could conceivably be carried out in moderately large laboratory based mesocosms provided that the mechanisms of dissipation are well known and the key parameters can be mimicked in the laboratory. However, further validation studies need to be carried out to support this approach. Replication of treatments in pesticide fate studies appear to be unnecessary but this is essential in the case of combined fate-and-effect studies where biological variation is greater. Pond and enclosure mesocosms each offer specific advantages for measuring effects of pesticides but appear to be of equal utility in fate studies. Careful consideration of design constraints is required before any form of mesocosm should be used for fate studies on pesticides with high K_{ow} partition coefficients as surface area:volume effects are likely to bias results. In the case of these compounds it is essential to measure their adsorption to the sides/walls of the enclosures.

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DEGRADATION OF PESTICIDES IN AN AQUATIC MODEL ECOSYSTEM

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ABSTRACT

The degradation behaviour of radiolabelled insecticides, fungicides and herbicides in an aquatic model ecosystem consisting of sediment and accompanying surface water is presented. For this purpose, the distribution of the radioactivity and the degradation rate in water and sediment were determined. Moreover, the intermediate products of degradation as well the rates of mineralization in relation to the incubation period were determined. The reproducibility and some parameters influencing the metabolism in the aquatic micro ecosystem are discussed using model compounds.

INTRODUCTION

Surface water contamination with plant protectants after direct application to waters or in the vicinity of waters is possible by

- wind (drift)
- water running off superficially from treated areas (run-off)
- unintended or negligent application
- movement through treated soils (leaching)

In water the active ingredient may be subject to degradation either in dissolved form, or adsorbed to the sediment or suspended particles.

Test methods according to OECD, EEC and EPA guidelines exist to examine the degradation of organic chemicals in water. As these test methods are of little use for a differentiated evaluation of the fate of plant protectants in the aquatic system, micro ecosystems with natural water and sediment content were developed by Houx & Dekker (1986), Pritchard *et al.* (1978) and Bourquin *et al.* (1977).

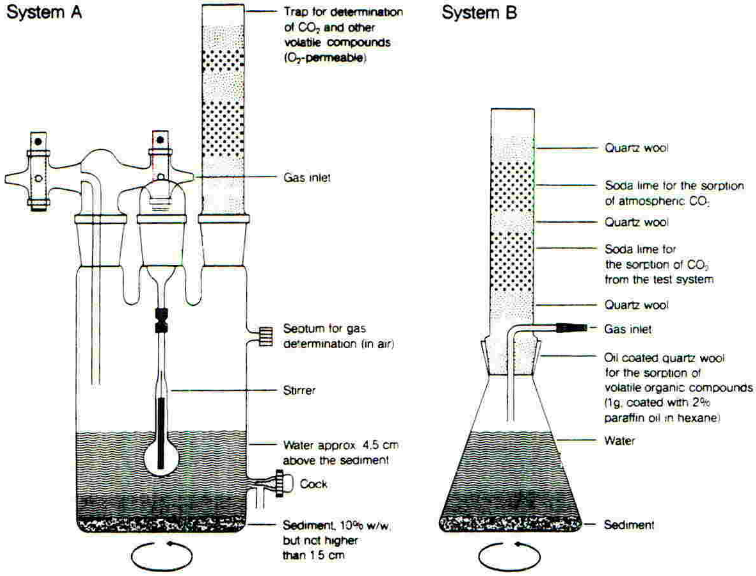
In the following, results concerning the degradation of plant protectants in two modified systems are presented.

MATERIALS AND METHODS

Two different experimental designs are used. The aqueous phase is kept in motion by means of a freely suspended stirrer in system A and by rotating the incubation vessels (50 rpm) on a mechanical shaker in system B (Figure 1). Thus the sediment phases are not disturbed.

FIGURE 1

Microecosystems for aquatic metabolism studies



The separation of sediment and water phases prevents unrealistic adsorption of the compound to the sediment and creates biological conditions favourable for microorganisms with extremely differing oxygen demand in the stagnant sediment (aerobic, aerotolerant and anaerobic microorganisms). The water-sediment ratio is 9:1.

Both systems contain trap attachments (Anderson, 1982) for the collection of volatile metabolites. Measurements of the oxygen content in the surface water during the incubation show that a free diffusion of atmospheric oxygen through the traps into the incubation vessels is guaranteed. In order to provide the possibility for aerobic microorganisms to survive, the oxygen concentration in the water should be above the 20 % saturation level.

The anaerobic experiment was conducted according to EPA-guideline § 162-3 using system B with a modified attachment.

The experiments are run in the range of the water solubility of the plant protectant (frequently at $\leq$1 mg/l). The total amount of water and sediment per incubation vessel is approximately 500 ml. This volume provides sufficient material for structure elucidation of metabolites.

In order to cover a wide range of the many inland waters, water and accompanying sediment are taken from a drainage ditch in a fruit-growing area near IJzendoorn, Netherlands (eutrophic water) and a fish pond near Lienden, Netherlands (oligotrophic water). In addition, one water and sediment batch each originate from the river Rhein at kilometer 692 and from a pond on an experimental farm in Kansas (USA).

Water and sediment characteristics can be taken from Table 1. The sediment sampling depth was 0-5 cm, approx. 50 cm below the water surface.

Table: 1

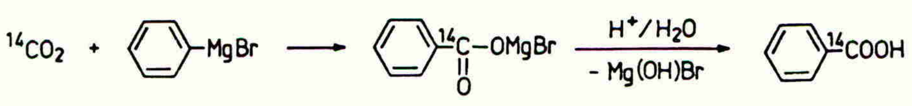
Water and sediment characteristics

Origin	IJzendoorn (NL)	Lienden (NL)	Rhein (FRG)	Kansas (USA)
Water				
pH	8.0	8.3	7.6	6.9
TOC (mg/l)	9.0	7.0	2.0	12.0
Sediment				
Organic carbon (g/100g soil)	1.9	1.5	0.3	3.2
Particle size (%)				
Sand (2000-63 µm)	57.6	75.3	94.5	13.6
Silt (63-2 µm)	26.4	18.8	3.0	58.4
Clay (<2µm)	16.0	5.9	2.5	28.0

After a 14-day-preincubation period a solution of the radio-labelled parent compound in a suitable solvent is pipetted onto the water surface. The incubation is performed at 22°C ± 2°C in the dark. At appropriate time intervals, complete incubation vessels are used for analysis in duplicate. At all processing dates important system parameters such as oxygen content, pH value, redox potential and microbial numbers as well as the radioactivity distribution between air, water and sediment are measured. The radioactivity is assigned to parent compound and metabolites.

The mineralization rate is determined on the basis of the $^{14}CO_2$ absorbed in the trap, the ^{14}C carbonic acid dissolved in the surface water and the ^{14}C carbonates which can be liberated from the sediment by means of HCl.

The formation of $^{14}CO_2$ is verified by means of the Grignard reaction:



[^{14}C] methane was detected qualitatively by radio gas chromatography. Quantitation was achieved by oxidation to $^{14}CO_2$ and subsequent liquid scintillation counting.

RESULTS AND DISCUSSION

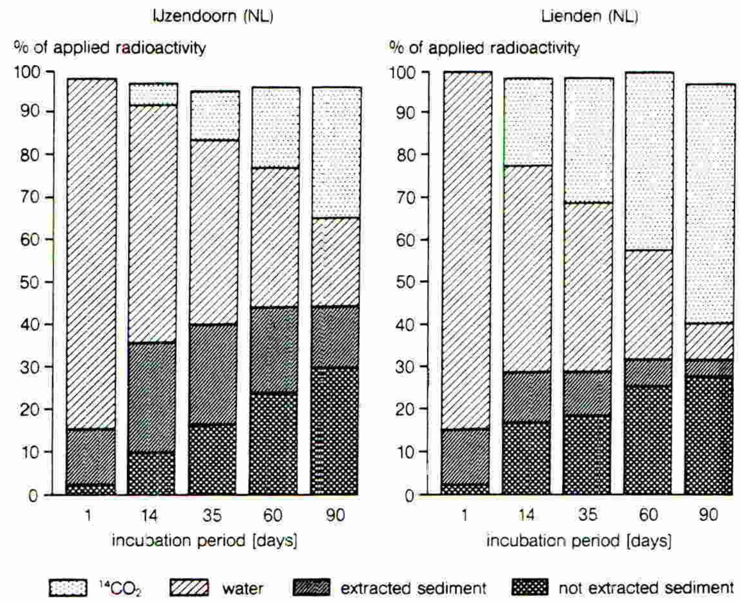
The variability of biotic and abiotic factors in the aquatic model ecosystems.

Distribution:

The herbicide metamitron was chosen as a model compound to illustrate the distribution of the radioactivity between the compartments sediment, surface water and air in an eutrophic and an oligotrophic type of water (Figure 2).

FIGURE 2

Distribution of radioactivity in the water-sediment system at various time intervals after application of [phenyl-UL-^{14}C] metamitron (3.5 mg a.i./l).

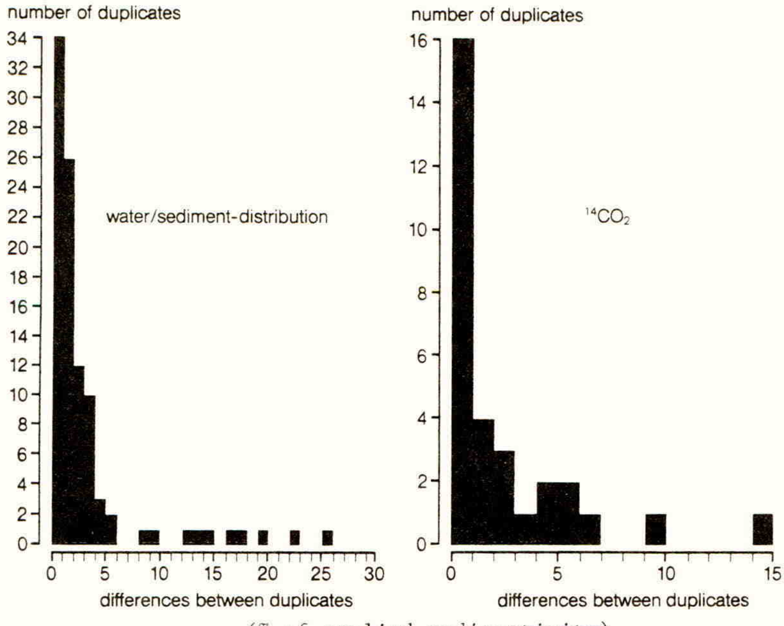


The radioactivity content in the surface water decreased continuously as the incubation period progressed. Towards the end of the experiment (90 days) approximately 22 % of the applied radioactivity remained in the water taken from IJzendoorn and approximately 12 % in the water from Lienden. The radioactivity content in the sediment increased correspondingly up to an incubation period of 60 days and remained unchanged until the end of the experiment after 90 days. A maximum of 42 % of the applied radioactivity was translocated into the sediment originally from IJzendoorn and approximately 30 % into the Lienden sediment. As the incubation period progressed, the percentage of radioactivity extracted with organic solvents decreased. In both systems the degradation of metamitron was characterized by a continuously increasing rate of $^{14}CO_2$ formation. Based on the applied radioactivity, approximately 32 % of the parent compound was mineralized in the IJzendoorn system after 90 days and approximately 55 % in the Lienden system . In contrast, considerably lower mineralization rates were found in the metabolism studies in soil (Jarczyk 1976).

Biological variation:
In several studies with different active ingredients (Table 2) it was demonstrated that the test results of duplicate samples were well in agreement with each other. The variation of the radioactivity distribution between the compartments sediment, water and air of parallel samples was presented in a frequency diagram (Figure 3) in relation to the total number of investigations. Based on the overall balance of the individual batches, more than 80 % of the parallel results varied by less than 5 %.

FIGURE 3

Variability between duplicates of six chemicals for the microecosystems A and B

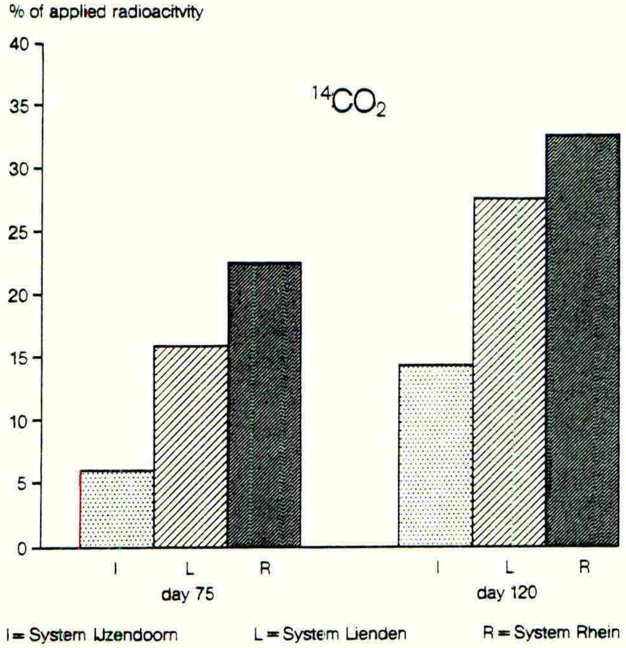


(% of applied radioactivity)

Type of water-sediment:
Comparative studies with water-sediment systems from Lienden and IJzendoorn as well as Rhein water and sediment, indicated different degradation rates as a consequence of the different types of water. Thus, the fungicide tolylfluanid showed a marked increase of the $^{14}CO_2$ formation in the order IJzendoorn, Lienden, Rhein (Figure 4).

FIGURE 4

Mineralization rates of [ring-UL-^{14}C] tolylfluanid (2.5 mg a.i./l) in different aquatic systems



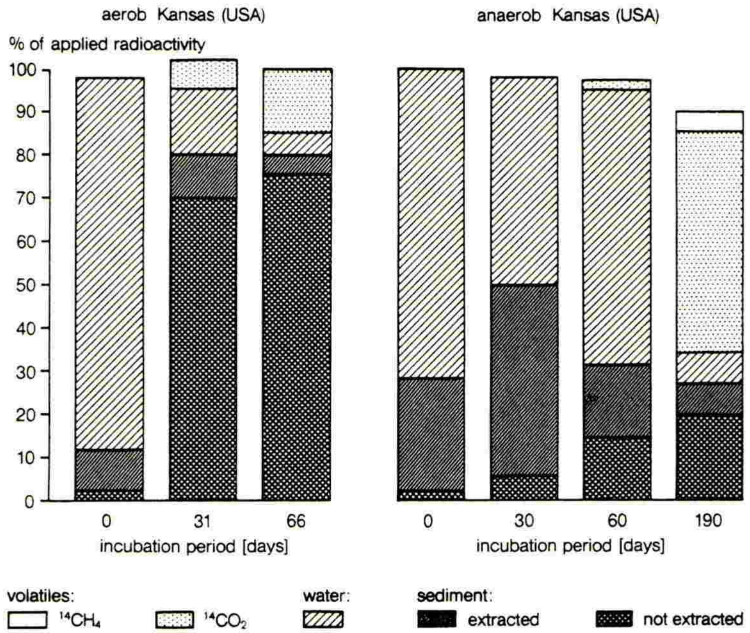
It is likely that the different rates of mineralization are due to the chemical/physical properties of water and sediment. Thus, the higher amount of organic substance and the greater clay and silt fraction in the IJzendoorn system might bind part of the parent compound and metabolites so that only smaller amounts will be available temporarily for a further mineralization. The degradation could also be due to the presence of different species and populations of microorganisms in the water-sediment systems.

Oxygen saturation:

The insecticide fenthion was used as a model compound to discuss the influence of the oxygen concentration (Figure 5). In a water-sediment system of American origin (Kansas) the parent compound was degraded to CO_2 under aerobic as well as under anaerobic conditions.

FIGURE 5

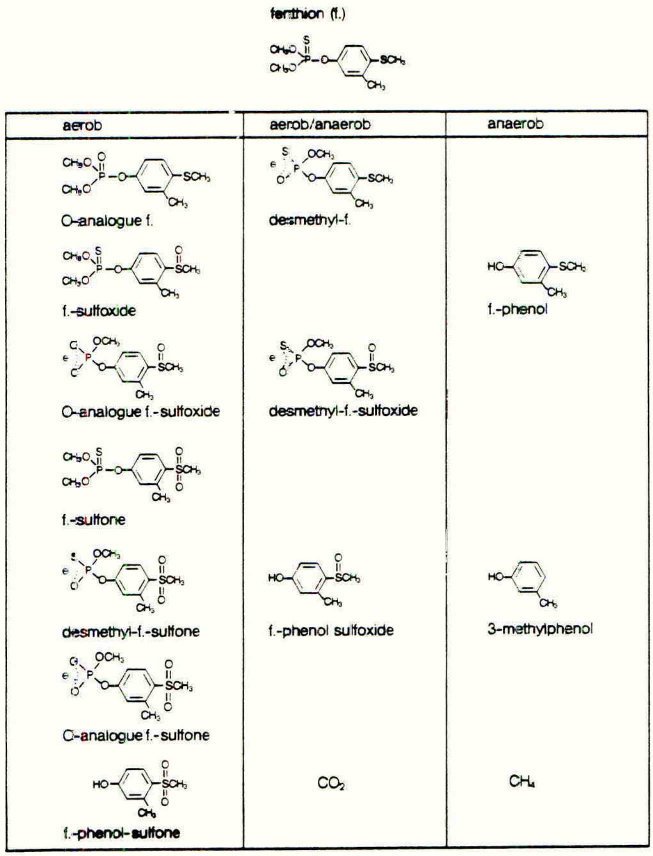
Distribution of radioactivity in the aerobic and anaerobic water-sediment system after various time intervals using [ring-1-^{14}C] fenthion (1.5 mg a.i./l) as a model compound



While $^{14}CO_2$ was produced continuously in the aerobic experiment, larger amounts of $^{14}CO_2$ were formed under anaerobic conditions only after a lag phase of 60 to 120 days. Under aerobic conditions the amount of not extracted radioactivity (bound residues) was significantly higher. This could be caused by the different nature of the microorganisms and/or different metabolism pathways. The main metabolites detected in the aerobic samples were fenthion sulfoxide and desmethyl-P=O-fenthion-sulfone. In the anaerobic experiment, the main metabolites were fenthion phenol sulfoxide and fenthion phenol (Figure 6). Further degradation products detected in the anaerobic system were 3-methyl phenol and methane. Metabolites containing the sulfone functional group were not detected.

FIGURE 6

Aquatic metabolism of [ring-1-^{14}C] fenthion (1.5 mg a.i./l) under aerobic and anaerobic conditions



<u>Organic solvents:</u>

The influence of organic solvents used for the application of the parent compound is shown in Table 2.

The parent compound is usually added to the water-sediment system in a small amount of a solvent which is miscible with water. Methanol was found to be suitable, yet it had a slightly positive influence upon the mineralization rate, when the degree of mineralization was high (> 10 %/100 days).

Despite a possible influence upon the activity of the microorganisms, it appears quite appropriate to apply active ingredients with low water solubility in small amounts of organic solvents, as a direct application of the active ingredient into the incubation vessel would be too inaccurate and would not result in a homogenous distribution in the water.

TABLE 2

$^{14}CO_2$-formation in relation to the application of the parent compound

Active ingredient (incubation time)	Origin IJzendoorn (NL)		Origin Lienden (NL)	
	active ingredient dissolved in:			
	200µl MeOH	10ml H_2O	200µl MeOH	10ml H_2O
[ring-UL-^{14}C]= tolylfluanid (day 120)	14.5	11.6	28.0	21.0
[cyclohexyl-UL-^{14}C]= azocyclotin (day 105)	16.5	13.0	17.0	15.5
[ring-UL-^{14}C]= dichlofluanid (day 120)	3.3	3.1	5.1	5.9
[benzyl-UL-^{14}C]= methabenzthiazuron (day 182)	5.0	5.0	2.5	1.5

% of applied radioactivity

CONCLUSION

The presented test systems including the selected types of waters are suitable to study the degradation of plant protectants in the aquatic system. Even after incubation periods of 1 year, the systems remain biologically active. The use of these test systems allows a statement about the fate of the parent compound and the distribution of the metabolites in the compartments sediment, surface water and air. Analysis of the complete samples allows the radioactivity balance to be determined as well as the degradation rate of the parent compound and patterns of formation and decline of the metabolites. Usually a total volume of 500 ml water-sediment is sufficient for structure elucidation of metabolites with spectroscopic and chromatographic methods.

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RADIO-GAS CHROMATOGRAPHY - MASS SPECTROSCOPY IN METABOLITE IDENTIFICATION

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ABSTRACT

Mass spectroscopy is one of the most powerful techniques available for identification of unknown compounds, but preparation of samples for mass spectroscopy is time consuming and labour intensive. By introducing the sample into the mass spectrometer via a gas chromatograph, spectra can be obtained for the individual components of quite complex mixtures, thus saving preparation time. If the chromatogram is too complex however, the spectroscopist may be unable to tell which of the peaks is of interest. By splitting the effluent of a gas chromatograph between a radiodetector and a mass spectrometer, simultaneous mass and radiodetection has been achieved. Since metabolism studies are conducted using radiolabelled material, the presence of radioactivity indicates which of the peaks in the chromatogram are derived from the test compound. As little as 5 ng of material and 150 DPM is sufficient to give a mass spectrum and a detectable radio-peak.

INTRODUCTION

Metabolite identification is an important step in the development of a new compound. The work is normally done by preparing extracts of a biological system previously treated with radiolabelled test material, then isolating and purifying the radiolabelled metabolites present in the extracts. The purification process typically involves several chromatographic procedures. It is time consuming and labour intensive and the ultimate yield of pure material is often very small.

Once the metabolites have been purified, a variety of techniques are available for determining the structures, but the most commonly used method is mass spectrometry. The mass spectrometer offers extreme sensitivity and can be readily linked to a gas chromatograph. The advantage of this is that if the sample under test is not completely pure, spectra can be obtained for the different components present because they emerge from the glc column at different times. However, if the chromatogram contains a large number of peaks, it may be impossible to determine which of the peaks are derived from the test compound and which are co-extractives from the original biological matrix.

In order to solve this problem a radio-gas detector and a bench-top mass spectrometer have been linked to a gas chromatograph so as to provide simultaneous mass and radio detection. In this paper, set up and operation of the system are described.

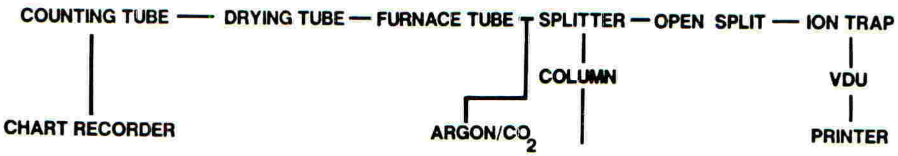
MATERIALS AND METHODS

Gas chromatograph

The gas chromatograph used was an Analytical Instruments Ai 93 fitted with an on-column injector and a 25 m SGE, BP1 bonded phase megabore column (equivalent to OV 101). A block diagram of the system is shown in Figure 1.

FIGURE 1

Block diagram of the radio-gc-ms system



At the outlet of the column, a flow splitter was installed. Initially, a variable splitter was used but better results were obtained with a fixed splitter. The splitter is a 1/16", glass-lined stainless steel capillary union with two 0.2 mm i.d. deactivated, fused silica transfer lines in a two-hole ferrule at one end. The split ratio was adjusted by varying the relative lengths of the two transfer lines. The dead volume of the splitter was very small and no make-up gas was required.

Radiodetector

An ESI Panax type 504 radiodetector was used. The detector operates by combusting the organic materials in the column effluent to CO_2 and quantifying the radioactivity in a flow through proportional counter.

Combustion takes place in a quartz tube (0.8 x 25 cm) packed with a mixture of cupric oxide wire (0.4 mm x 5 mm) and ground quartz (0.3 - 0.5 mm) in the ratio 5:1 by weight. This mixture has been shown to give efficient combustion and a low and constant back pressure over a long period of use (Rodriguez et al., 1983). The furnace tube was heated to 700°C. The wall of the furnace was mounted against the outside of the glc oven with the end of the tube protruding into the oven so that a heated interface was not necessary. The quenching gas for the proportional counter (Argon/CO_2, 95/5) was introduced at the entrance to the furnace tube. In this way the quenching gas serves as make-up gas at the point where the column flow enters the furnace tube, and the CO_2 in the mixture acts as a carrier for the small quantities of [$^{14}CO_2$] produced by combustion. When the system is not in use, a slow flow of oxygen is passed through the furnace tube to keep the packing oxidised.

From the furnace tube, the gas flow passes to a drying tube packed with magnesium perchlorate and then to the counter. The voltage on the proportional counter was adjusted to allow it to operate in the Geiger-Muller region. The exact voltage necessary depends on the composition of the gas but once set it requires no adjustment.

Mass spectrometer

A Finnigan Ion Trap was used as the mass detector. Sample was supplied to the ion trap from one arm of the flow splitter via an open slit interface mounted inside the glc oven. The temperature of the open split was controlled by the Ion Trap. The operation of the Ion Trap was controlled by an IBM PC. Tuning and calibration are performed automatically.

Gas flow settings

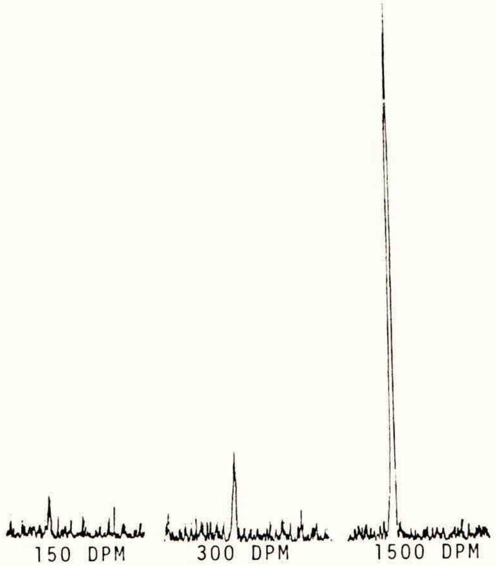
The glc column was operated at 3 to 5 ml min^{-1} which is close to the optimum flow rate with helium as carrier gas. The length of the transfer line on the splitter was adjusted to give a split ratio of approximately 3:1 in favour of the radiodetector. This was achieved with the transfer line to the Ion Trap four times the length of the line leading to the radiodetector. The total gas flow through the radiodetector was 40 ml min^{-1}.

RESULTS AND DISCUSSION

The sensitivity of the radiodetector is illustrated by Figure 2. An injection of 1500 DPM gave a full scale peak (250 mm) on the chart recorder. Peak height was proportional to activity over the range investigated, and the lower limit of detection appeared to be 150 DPM on column. Sensitivity clearly depends on peak width and on background. Background is normally less than 5 counts per minute.

FIGURE 2

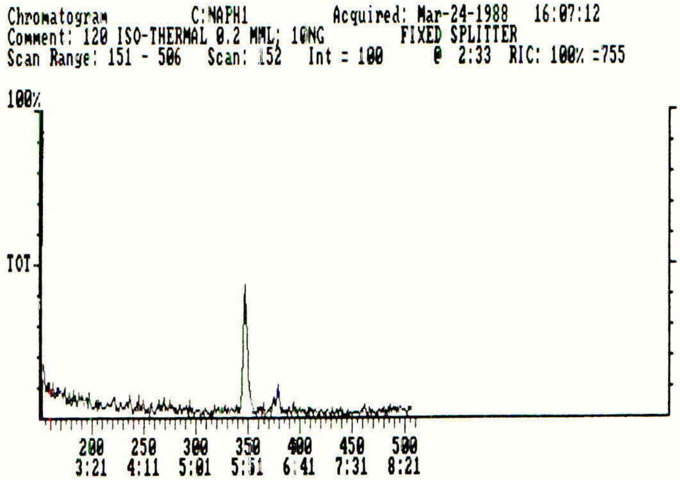
Response of radiodetector to varying amounts of radioactivity



The performance of the ITD is illustrated in Figure 3. The trace shows total ion current for an on-column injection of 10 ng of naphthalene. The signal to noise ratio is approximately 20:1 and the spectrum obtained from the peak searched successfully on the NBS library.

FIGURE 3

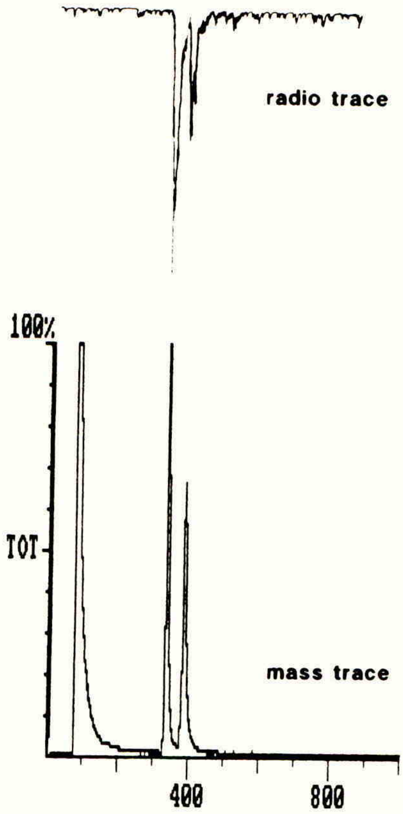
Total ion current produced by an injection of 10 ng of naphthalene



The chromatographic performance of the system is illustrated by Figure 4. The sample contained the methyl and ethyl esters of benazolin (50 ng total) and the total activity was 500 DPM. Under the conditions of this analysis, baseline resolution was not achieved. The resolution on the radiodetector was similar to that on the mass detector. The radio peaks were slightly wider than the mass peaks (12 seconds at half height as opposed to 8 seconds) probably due to dead volumes in the furnace tube.

FIGURE 4

Radio signal and mass signal for the methyl and ethyl esters of benazolin. Total compound = 50 ng, total activity 500 DPM

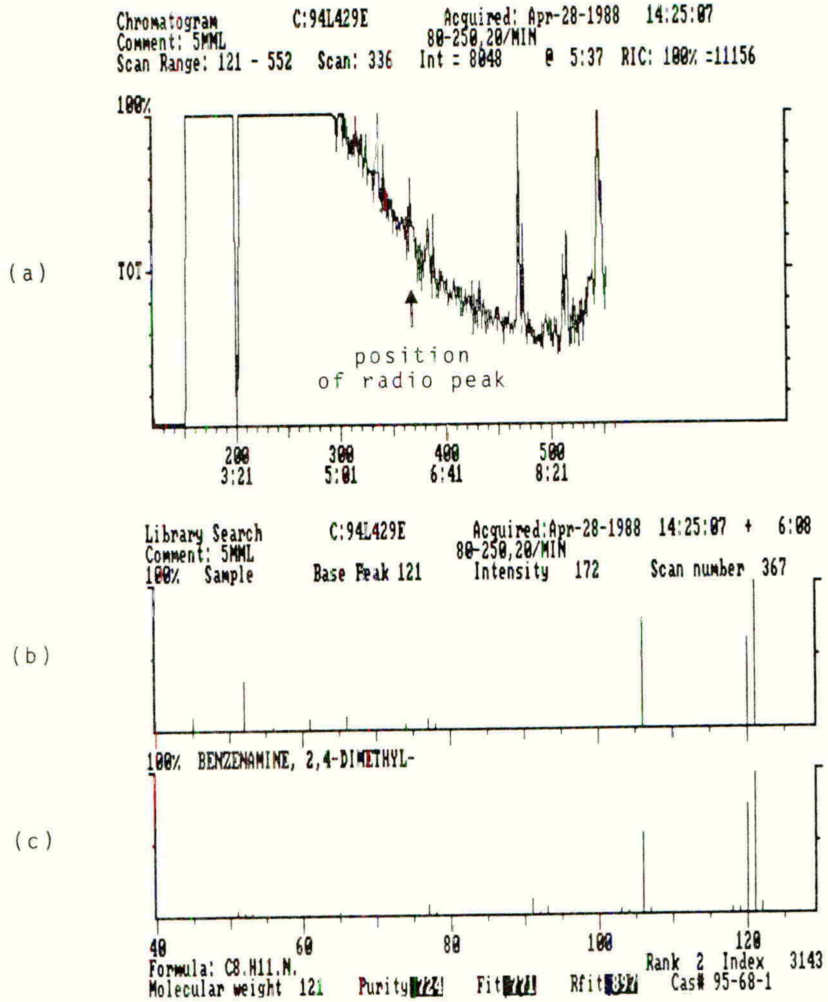


These results demonstrate that the system functions as expected with standard compounds and that the detection limits and resolution are adequate for experimental purposes. The following examples illustrate the practical use of the system.

A crop of pears were given multiple applications of [^{14}C] labelled amitraz and at harvest, a proportion of the residue was bound to the skin. Exhaustive extraction with solvents failed to remove the residue. The skin was subjected to acid hydrolysis followed by base hydrolysis. The hydrolysate was then extracted with hexane under alkaline conditions. The radioactivity in the hexane extract was partitioned into aqueous acid, the acid was made alkaline and extracted with a small volume of hexane. The hexane extract was analysed by radio-gc-ms. Figure 5 shows the total ion current trace for the hexane extract. The radiodetector revealed a single radioactive peak corresponding to the position indicated in Figure 5.

FIGURE 5

(a) Total ion current for hexane extract of hydrolysed pear skin
(b) Mass spectrum of the radioactive peak
(c) Library spectrum of 2,4-dimethylaniline



A spectrum was obtained at that point on the chromatogram and by comparing the test spectrum with spectra in the NBS library the identity of the radioactive material was confirmed as 2,4-dimethylaniline.

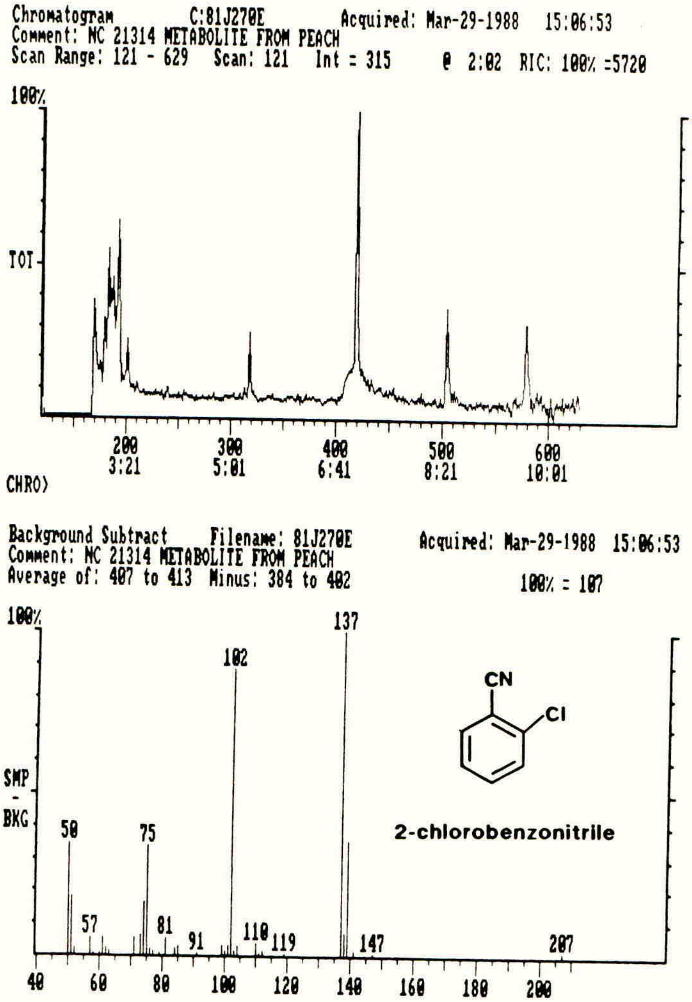
In another experiment a metabolite was extracted from peaches treated with clofentezine. Large quantities of this material were available and a sample was purified by hplc for subsequent analysis by radio-gc-ms. The total ion current for this sample is presented in Figure 6. The radiodetector revealed a single broad peak at between 400 and 450 seconds. The background-subtracted spectrum in this region of the chromatogram was identical to the spectrum of 2-chlorobenzonitrile.

FIGURE 6

(a) Total ion current trace for clofentezine metabolite purified from peach extract
(b) Background substracted mass spectrum of the radiopeak

(a)

(b)



The examples given in Figure 5 and 6 clearly indicate the advantages of the radio-gc-ms system. In the case of the amitraz hydrolysis product (Figure 5) a crude extract was successfully analysed after minimal clean-up. The mass peak corresponding to the metabolite was indistinguishable from the background and could not have been located without the radiodetector.

The clofentezine metabolite (Figure 6) illustrates the problem faced by the mass spectroscopist when given an impure sample. Although this sample was pure by hplc (UV detection at 269 nm) the gas chromatogram contained numerous peaks and the peak of interest was by no means the largest in the chromatogram.

Radio-gas chromatography is an established technique, well documented in the literature (Matucha and Smolkova, 1976). Unfortunately it has gained a reputation for being technically difficult and in recent years has been overshadowed by radio hplc. Radio-glc has however two distinct advantages. Firstly the extreme sensitivity of the gas counter compared to the solid scintillator type used in hplc (Simpson, 1968), and secondly the ease of interfacing to a mass spectrometer. A fused silica column can perform in a few minutes a separation and purification which might take weeks on the laboratory bench. Using simultaneous radiodetection to locate the peaks derived from the test compound, metabolite identification can be achieved on relatively crude samples with significant savings in time and cost.

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